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Appendix A

Abundance and Total Numbers of Chinook Salmon and Trout in the Chiwawa River basin, Washington, 2016

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January 25, 2017

TO: HCP Hatchery Committee

FROM: Tracy Hillman

Subject: Abundance and Total Numbers of Chinook Salmon and Trout in the Chiwawa River basin, Washington, 2016

The Chelan County Public Utility District (PUD) hatchery program is operated through a habitat conservation plan (HCP) that was incorporated into the PUD's license in 2004. The HCP directed the signatories to develop a monitoring and evaluation plan within one year of the effective date. This resulted in the development of the Conceptual Approach to Monitoring and Evaluating the Chelan County Public Utility District Hatchery Programs (Murdoch and Peven 2005). In 2013, the Hatchery Committees updated the hatchery monitoring and evaluation plan (Hillman et al. 2013). This study will help the Hatchery Committees determine if it is meeting Objective 2 in the updated monitoring and evaluation plan.

Objective 2: *Determine if the proportion of hatchery fish on the spawning grounds affects the freshwater productivity of supplemented stocks.*

We estimated densities and total numbers of age-0 spring Chinook salmon *Oncorhynchus tshawytscha,* trout *Oncorhynchus* sp., and char *Salvelinus* sp. in the Chiwawa River basin, Washington, in August 2016. This was the 24th year of an ongoing study to assess the freshwater productivity (juveniles/redd) of Chinook salmon in the Chiwawa River basin. We used landscape classification to stratify streams in the basin that supported juvenile Chinook salmon (Hillman and Miller 2004). Classification "explained" most of the variability in fish numbers caused by geology, land type, valley bottom type, stream state condition, and habitat type. We identified ten reaches on the lower 31 miles (50 km) of the Chiwawa River and one reach in each of Phelps, Rock, Chikamin, Big Meadow, Alder, Brush, Clear, Y, and Unnamed[1] creeks (Figure 1). Each reach consisted of several combinations of state-type and habitat-type strata. We used classification to find reference areas for reaches in the Chiwawa River. We matched Reach 3 and Reach 8 of the Chiwawa River with a moderately-confined section of Nason Creek (RM 0.62-1.70) and an unconfined area of the Little Wenatchee River (RM 4.39-8.55), respectively

[1]Unnamed tributary that drains the eastside of Chiwawa Ridge. Its confluence with the Chiwawa River is about 1 mile (1.6 km) downstream from the mouth of Phelps Creek.

(Hillman and Miller 2004). Because of the supplementation program in Nason Creek, the use of Nason Creek as a reference for the Chiwawa River is no longer valid. However, as directed by the Hatchery Committee, we continue to sample sites in Nason Creek. Following methods described in Hillman and Miller (2004), we used underwater observations to estimate numbers of fish in 187 randomly selected sites.

During sampling in August 2016, discharge in the Chiwawa River averaged 202 cubic feet per second (cfs) and ranged from 126-325 cfs (Figure 2). Stream temperatures during the study period ranged from 8.0 to 18.0ºC. Fish species observed in the Chiwawa River basin and reference areas during the 1992-2016 survey period[2] included: spring Chinook salmon, coho salmon *O. kisutch*, sockeye salmon *O. nerka*, steelhead/rainbow trout *O. mykiss* (hatchery rainbow were present only in 1992 and 1993), cutthroat trout *O. clarki lewisi*, bull trout *S. confluentus*, brook trout *S. fontinalis*, mountain whitefish *Prosopium williamsoni*, dace *Rhinichthys* sp., northern pikeminnow *Ptychocheilus oregonensis*, suckers *Catostomus* sp., and sculpin *Cottus* sp. The age-0 spring Chinook that we observed in the Chiwawa River basin during the 2016 survey were produced from 543 redds counted in the fall of 2015 (Hillman et al. 2016). Assuming a mean fecundity of 4,847 eggs per female Chinook (from females collected for broodstock), and that no female produced more than one redd (Murdoch et al. 2009), we estimated that the Chiwawa River basin was seeded with 2,631,921 eggs in 2015 (Appendix A).

In 2016, riffles made up the largest fraction of habitat types in reaches of the Chiwawa River basin (54% of the total stream surface area) (Table 1). Pools (24%), glides (6%), and multiple channels (16%) constituted the remaining 46% of the stream surface area. We found woody debris associated with most multiple-channel habitat.

Chinook Salmon Abundance

Chinook salmon were the most abundant salmonid in the Chiwawa River basin. We estimated, based on surface area, that age-0 Chinook salmon numbered 140,172 (±10% of the estimated total) in the Chiwawa River basin in August 2016 (Table 2). Extrapolating based on volume of habitat types, age-0 Chinook numbered 137,525 (±13%) in the Chiwawa River basin. About 3% of the juvenile Chinook were in tributaries to the Chiwawa River. During the 1992-2016 surveys, numbers of age-0 Chinook ranged from 5,815 to 149,563 in the Chiwawa River basin (Figure 3; Appendix A and B). Most of the difference in juvenile numbers among years resulted from different seeding (stock) levels (Figure 4). Numbers of Chinook redds in the Chiwawa River basin during 1992-2015 ranged from 13 to 1,078, resulting in seeding levels of 66,248 to 4,984,672 eggs (Appendix A).

As in most years, age-0 Chinook in 2016 were distributed contagiously among reaches in the Chiwawa River (Table 2). In the Chiwawa River, densities of age-0 Chinook were highest in the upper reaches (Reaches 7-10). The highest densities in the Chiwawa River basin were in tributaries to the Chiwawa River (Table 2). Age-0 Chinook were most abundant in multiple channels and least abundant in glides and riffles. We found the majority of the Chinook

[2] The study period 1992-2016 includes only 24 years of sampling because there was no sampling in 2000.

associated with woody debris in multiple channels (multiple channel use index = 2.83)[3]. These sites (multiple channels) made up 16% of the total surface area of the Chiwawa River basin, but they provided habitat for 56% of all the age-0 Chinook in the basin in 2016 (Appendix C). In contrast, riffles made up 54% of the total surface area, but provided habitat for only 8% of all age-0 Chinook in the Chiwawa River basin (riffle use index = 0.24). Pools made up 24% of the total surface area and provided habitat for 35% of all age-0 Chinook in the basin (pool use index = 1.59). Few Chinook used glides that lacked woody debris (glide use index = 0.25).

As noted earlier, we assumed that the Chiwawa River was seeded with 2,631,921 Chinook eggs (543 redds times 4,847 eggs/female) in fall, 2015, and that at least 140,172 of those survived to August 2016. This means that the egg-to-parr survival was at least 5.3% (95% confidence bound 4.8-5.9%). During 1992-2016, egg-to-parr survival averaged 8.0% (range 2.7-19.1%) in the Chiwawa River basin (Appendix A). This survival rate comports with those from other streams. For example, Mullan et al. (1992) estimated an egg-to-parr survival rate of 9.8% for spring Chinook salmon in Icicle Creek, a tributary of the Wenatchee River. Using a Beverton and Holt model, Hubble (1993) estimated that egg-to-parr survival of Chinook in the Chewuck River, a tributary to the Methow River, ranged between 13% and 32%, depending on percent seeding level in the basin. Kiefer and Forster (1991) estimated a mean egg-to-parr survival rate of 5.5% (range 5.1-6.7%) for naturally-spawning spring Chinook salmon in the entire upper Salmon River. They also noted that egg-to-parr survival of natural spawners and adult outplants in the headwater streams of the upper Salmon River averaged 24.4% (range 16.1-32.0%). Petrosky (1990) reported an egg-to-parr survival range of 1.2-29.0% for Chinook in the upper Salmon River, Idaho. Konopacky et al. (1986) estimated egg-to-parr survival of Chinook in Bear Valley Creek, Idaho, as 8.1-9.4%. Work by Richards and Cernera (1987) in Bear Valley Creek indicated an egg-to-parr survival of 2.1%.

Mean densities of age-0 Chinook salmon in two reaches of the Chiwawa River were generally less than those in corresponding reference areas (Figure 5). Within both the Chiwawa River and its reference areas, pools and multiple channels consistently had the highest densities of age-0 Chinook.

We estimated a total of 282 (±43% of the estimated total) age-1+ Chinook salmon in the Chiwawa River basin in August 2016 (Table 3). In August 1992-2016, numbers of age-1+ Chinook ranged from 5 to 967 in the Chiwawa River basin (Figure 3; Appendix B). These fish occurred throughout the Chiwawa River. We found relatively few age-1+ Chinook in tributaries; although, numbers in Big Meadow Creek were higher in 2015 than in past years. Age-1+ Chinook were most abundant in multiple channels and pools.

[3] The habitat use index was calculated as follows: Multiple channel use = $(parr_{mc}/parr_t) / (area_{mc}/area_t)$, where $parr_{mc}$ = the number of parr counted in multiple channel habitat, $parr_t$ = the total number of parr counted within all habitat types, $area_{mc}$ = the area of multiple channel habitat within the sampling frame, and $area_t$ = the total area of the sampling frame. A multiple channel use index value of 1 would indicate that parr were uniformly distributed among habitat types and exhibited no preference for multiple habitat types. Values greater than 1 indicate use of multiple channels to a greater extent than the average, while scores between 0 and 1 indicate below-average use of multiple channel habitat.

Juvenile Chinook Salmon Productivity (Fish/Redd)

Freshwater productivity of juvenile Chinook salmon was estimated as the number of parr (age-0 Chinook) per redd in the Chiwawa River basin. Theoretically, the relationship between number of parr and redds can be explained mathematically provided the relationship between the two parameters goes through the origin, increases monotonically at low spawning levels, and shows some level of density dependence at high spawning levels. We identified four alternative hypotheses that may explain the relationship between spawning level (redds) and numbers of age-0 Chinook:

1. The first hypothesis assumed that the number of juveniles increases constantly toward an asymptote as the number of redds increases. After the asymptote is reached, the number of juveniles neither increases nor decreases. The asymptote represents the maximum number of juveniles the system can support (i.e., carrying capacity for the system). This hypothesis was modeled with a Beverton-Holt curve that took the form:

$$\boldsymbol{J = \frac{(\alpha R)}{(\beta + R)}}$$

where $\boldsymbol{J}$ is the number of juvenile (age-0) Chinook, $\boldsymbol{R}$ is the number or redds, $\boldsymbol{\alpha}$ is the maximum number of juveniles produced, and $\boldsymbol{\beta}$ is the number of redds needed to produce (on average) juveniles equal to one-half the maximum number of juveniles.

2. The second hypothesis, like the first, assumed that the number of juveniles increases toward an asymptote (carrying capacity) as the number of redds increases. After the carrying capacity is reached, the number of juveniles neither increases nor decreases. The carrying capacity represents the maximum number of juveniles the system can support. This hypothesis was modeled with a smooth hockey stick function that took the form:

$$\boldsymbol{J = J_{\infty}\left(1 - e^{-\left(\frac{\alpha}{J_{\infty}}\right)R}\right)}$$

where $\boldsymbol{J}$ and $\boldsymbol{R}$ are as above, $\boldsymbol{\alpha}$ is the slope at the origin of the spawner-recruitment curve, and $\boldsymbol{J_{\infty}}$ is the carrying capacity of juveniles.

3. The third hypothesis assumed that the number of juveniles increases to a maximum and then declines as the number or redds increases. In this case, mortality rate of juveniles (or eggs) is proportional to the initial number of redds. Higher mortality rate is associated with density-dependent growth coupled with size-dependent predation. This hypothesis was modeled with a Ricker curve that took the form:

$$\boldsymbol{J = \alpha R e^{-\beta R}}$$

where $\boldsymbol{J}$ and $\boldsymbol{R}$ are as above, $\boldsymbol{\alpha}$ is the number of juveniles per redd at low spawning levels, and $\boldsymbol{\beta}$ describes how quickly the juveniles per redd drop as the number of redds increases.

4. The fourth hypothesis, like the first, assumed that the number of juveniles increases constantly, but unlike the first, the number of juveniles does not reach an asymptote. Rather, the number of juveniles increases indefinitely, but at a slowing rate of increase. This hypothesis was modeled with both a Cushing curve and a Gamma function. The

Cushing curve took the form:

$$\boldsymbol{J = \alpha R^{\gamma}}$$

where $\boldsymbol{J}$ and $\boldsymbol{R}$ are as above, $\boldsymbol{\alpha}$ is the number of juveniles per redd at low spawning levels, and $\boldsymbol{\gamma}$ describes the level of density dependence at high spawning levels. The Gamma function is a three-parameter model that has the form:

$$\boldsymbol{J = \alpha R^{\gamma} e^{-\beta R}.}$$

This is an un-normalized gamma function that is similar to the Cushing curve when $\beta = 0$.

We used Akaike's Information Criterion for small sample size (AIC_c) to determine which model(s) best explained the productivity of juvenile Chinook in the Chiwawa River basin. AIC_c was estimated as:

$$\boldsymbol{AIC_c = -2log\big(£(\theta|data)\big) + 2K + \left(\frac{2K(K+1)}{n-K-1}\right)}$$

where $\boldsymbol{log(£(\theta|data))}$ is the maximum likelihood estimate, $\boldsymbol{K}$ is the number of estimable parameters (structural parameters plus the residual variance parameter), and $\boldsymbol{n}$ is the sample size (Burnham and Anderson 2002). We used least-squares methods to estimate $\boldsymbol{log(£(\theta|data))}$, which was calculated as $\boldsymbol{log(\sigma^2)}$, where $\boldsymbol{\sigma^2}$ = residual sum of squares divided by the sample size ($\boldsymbol{\sigma^2 = RSS/n}$). AIC_c assesses model fit in relation to model complexity (number of parameters). The model with the smallest AIC_c value represents the "best approximating" model within the model set. Remaining models were ranked relative to the best model using AIC_c difference scores ($\boldsymbol{\Delta AIC_c}$), Akaike weights ($\boldsymbol{w_i}$), and evidence ratios. Models with $\boldsymbol{\Delta AIC_c}$ values less than 2 indicate that there is substantial support for these models as being the best-fitting models within the set (Burnham and Anderson 2002). Models with values greater than 2 have less support. Akaike weights are probabilities estimating the strength of the evidence supporting a particular model as being the best model within the model set. Models with small $\boldsymbol{w_i}$ values are less plausible as competing models (Burnham and Anderson 2002). If no single model could be specified as the best model, a "best subset" of competing models was identified using (1) AIC_c differences to indicate the level of empirical support each model had as being the best model, (2) evidence ratios based on Akaike weights to indicate the relative probability that any model is the best model, and (3) coefficients of determination (R^2) assessing the explanatory power of each model.

The use of AIC_c indicated that the Beverton-Holt model best approximated the information in the juveniles/redd data (Table 4; Figure 6). The estimated structural parameters for this model were:

$$Juveniles = \frac{(152{,}439 \times Redds)}{(191 + Redds)}$$

where the bootstrap estimated standard errors for the two parameters were 17,210 and 56, respectively. The adjusted $R^2 = 0.84$. The second-best model was the smooth hockey stick model, which was 1.70 AIC_c units from the best model (Table 4; Figure 6). The estimated parameters for this model were:

$$LN(Juveniles) = 11.7 + LN\left(1 - e^{-\left(\frac{715.9}{116{,}314}\right)Redds}\right)$$

where the bootstrap estimated standard errors of the two parameters were 0.1 and 391, respectively, and the $R^2 = 0.83$. The AIC_c difference scores, Akaike weights, and evidence ratios indicated that there was substantial support for both the Beverton-Holt and smooth hockey stick models (Table 4). There was less support for the remaining models (Ricker, Gamma[4], and Cushing), which were > 2 AIC_c units from the best models. This was further supported by the fact that, relative to the best models, the remaining models had evidence ratios greater than 10.

Although the Beverton-Holt, smooth hockey stick, and Ricker models have different biological assumptions, they all indicated a density-dependent relationship between spawning levels (redds) and juvenile Chinook production. This was not only evident in the best approximating models, but there was also a significant negative relationship between juveniles per redd and numbers of redds in the Chiwawa River basin (Figure 7). Although data at high seeding levels are lacking, the Beverton-Holt model estimates the population capacity[5] of juvenile Chinook in the Chiwawa River basin at about 152,000 parr. This equates to about 1,197 Chinook parr per hectare. In contrast, the smooth hockey stick model, which fit the data as well as the Beverton-Holt model, estimates the population carrying capacity for juvenile Chinook at about 116,000 parr. This equates to about 913 Chinook parr per hectare. As a comparison, Thorson et al. (2013) estimated the carrying capacity for 15 populations of juvenile Chinook in the Snake River metapopulation as 5,000 juveniles per hectare. However, those authors noted that the estimate could be biased because of imperfect detectability and estimates of spawning numbers.

Steelhead/Rainbow Abundance

Based on stream surface area, we estimated a total of 16,244 (±14% of the estimated total) age-0 steelhead/rainbow (<4 in) in reaches of the Chiwawa River basin in August 2016 (Table 5). During the 1992-2016 survey period, numbers of age-0 steelhead/rainbow ranged from 1,410 to 45,727 in the Chiwawa River basin (Figure 8; Appendix B). In 1992-2016, numbers of age-0 steelhead/rainbow varied among reaches, but were typically highest in the lower reaches of the Chiwawa River. In all years they most often used riffle and multiple channel habitats in the Chiwawa River, although we also found them associated with woody debris in pool and glide habitat. In tributaries, they were generally most abundant in small pools. Those that we observed in riffles selected stations in quiet water behind small and large boulders or occupied stations in quiet water along the stream margin. In pool and multiple-channel habitats, we found age-0 steelhead/rainbow using the same kinds of habitat as age-0 Chinook salmon.

We estimated that 4,031 (±15% of the estimated total) age-1+ steelhead/rainbow (4-8 in) lived in reaches of the Chiwawa River basin in August 2016 (Table 6). During the survey period 1992-

[4] The γ parameter in the Gamma model was greater than 0, which means that this model is nearly identical to the Ricker model.

[5] In these analyses, we are calculating "population" carrying capacity (*K*), which is defined as the maximum equilibrium population size estimated with population models. This should not be confused with "habitat" carrying capacity (*C*), which is defined as the maximum population of a given species that a particular environment can sustain.

2016, numbers of age-1+ steelhead/rainbow ranged from 754 to 22,130 (Figure 8; Appendix B). In most years, we found these fish in nearly all reaches, but they were typically most numerous in lower reaches of the Chiwawa River. We observed age-1+ steelhead/rainbow mostly in pool, riffle, and multiple-channel habitats. Those that we observed in pools were usually in deeper water than age-0 steelhead/rainbow and Chinook. Like age-0 steelhead/rainbow, age-1+ steelhead/rainbow selected stations in quiet water behind boulders in riffles, but we generally did not find the two age groups together. Age-1+ steelhead/rainbow appeared to use deeper and faster water than did age-0 steelhead/rainbow.

We estimated that steelhead/rainbow larger than 8 inches numbered 14 (±71% of the estimated total) in the Chiwawa River basin in August 2016 (Table 7). During the period 1992-2016, steelhead/rainbow numbers ranged from 8 to 1,869 (Appendix B). Steelhead/rainbow larger than 8 inches were most abundant in the lower Chiwawa River; however, in 1992 and 1993, they were most abundant near campgrounds in Reaches 8, 9, and 10 (these were mostly hatchery rainbow trout planted near the campgrounds). We found very few in tributaries. Most of the steelhead/rainbow larger than 8 inches used deep pools (>5 feet), and occupied stations near the bottom at the upstream end of pools.

Bull Trout Abundance

We estimated, based on surface area that at least 291 (±20% of the estimated total) juvenile (2-8 in) bull trout lived in reaches of the Chiwawa River basin in August 2016 (Table 8). We found most of these fish in the upper-most reaches of the Chiwawa River and in Rock and Phelps creeks. During 1992-2016, numbers of juvenile bull trout ranged from 79 to 505 (Figure 9; Appendix B). These estimates and those for adult bull trout are incomplete because we did not sample the entire range of bull trout in all tributaries. That is, we did not extend our surveys into the headwaters of the Chiwawa River because there were no juvenile Chinook there. Areas beyond the distribution of juvenile Chinook salmon are known to support bull trout, steelhead/rainbow, and cutthroat trout (USFS 1993). In addition, our estimates of bull trout abundance were based on daytime snorkel surveys, which may underestimate the actual abundance of bull trout.[6] Several studies (e.g., Goetz 1994; Thurow and Schill 1996; Hillman and Chapman 1996; Bonar et al. 1997) have found bull trout population estimates based on nighttime snorkeling to be in some cases more accurate than daytime snorkeling, especially for juvenile bull trout. Our estimates of adult bull trout numbers may be more accurate than those for juveniles.

In all years, we found most juvenile bull trout in the upstream reaches of the Chiwawa River. In 2016, they occurred primarily in Reaches 9-10 on the Chiwawa River. We found the majority of these fish in multiple channels, pools, and riffles, and few in glides. They consistently occupied stations close to the stream bottom over rubble and small boulder substrate or near woody debris. This is similar to the observation of Pratt (1984) in the upper Flathead River Basin in Montana. She found that juvenile bull trout lay close to instream cover and that they tended to conceal

[6] Because there are no estimates for probability of detecting bull trout with daytime underwater observation methods in the Chiwawa River basin, we could not adjust bull trout numbers based on detectability. Therefore, the numbers reported in this report likely underestimate the "true" number of bull trout in the survey area.

themselves. Consequently, she found it difficult to estimate accurately their numbers. Although this implies that we underestimated numbers of juvenile bull trout in the Chiwawa River, the relative distribution of juvenile bull trout is valid if we assume that we saw the same fraction of juveniles in all reaches (i.e., detection probability was the same across survey sites).

We estimated a total of 1,254 (±12% of the estimated total) adult (>8 in) bull trout in reaches of the Chiwawa River basin in August 2016 (Table 9). This was the second highest number of adult bull trout that we recorded during the more than 20-year survey period. During 1992-2016, numbers of adult bull trout ranged from 76 to 2,286 (Figure 9; Appendix B). As with juvenile bull trout, we found most of the adult bull trout upstream from Reach 6; although they were found in all reaches on the Chiwawa River. We found few adult bull trout in tributaries of the Chiwawa River. Adult bull trout primarily used pools and multiple channel habitat, although most of the smaller adults (<10 in) used riffles.

Abundance of Other Salmonids

In August 2016, we estimated that at least 66 brook trout, an exotic species closely related to the bull trout, occurred in the Chiwawa River, Chikamin Creek, Big Meadow Creek, Minnow Creek, and in the Little Wenatchee River survey areas. In both the Chiwawa and Little Wenatchee rivers, brook trout usually used multiple channels and pools. Few appeared to be bull trout/brook trout hybrids. In Chikamin, Minnow, and Big Meadow creeks, brook trout were most abundant in pools. Brook trout lengths ranged from 2-12 inches.

At least 550 westslope cutthroat trout occurred in the Chiwawa River, Phelps Creek, Rock Creek, and Little Wenatchee River survey areas in August 2016. These fish most often occurred in pools and multiple channel habitats. They ranged in size from 2-22 inches. Juvenile coho salmon were observed in Nason Creek and the Chiwawa River.

We observed both juvenile and adult mountain whitefish in the Chiwawa River, Phelps Creek, Rock Creek, Nason Creek, and the Little Wenatchee River survey areas. In sum, at least 6,031 adult and 1,454 juvenile whitefish lived in these streams in August 2016. We found few whitefish in most tributaries to the Chiwawa River.

Conclusion

This was the 24th year of a study to monitor trends in juvenile spring Chinook production in the Chiwawa River basin. As shown in Figure 3, numbers of juvenile Chinook salmon in the Chiwawa River basin have fluctuated widely over the 24-year period. Numbers of juveniles in 2001, 2002, and 2009-2016 were some of the highest recorded, while numbers in the mid-1990s were some of the lowest. Interestingly, the highest spawning escapements (highest redd numbers) resulted in the lowest egg-parr survival rates (Appendix A). This is supported by the fact that the best approximating models clearly demonstrated a density-dependent relationship between seeding levels and juvenile production. Indeed, there was a significant negative relationship between parr per redd and numbers of redds in the Chiwawa River basin. This is an important observation because some of the hypotheses in the revised monitoring and evaluation plan (Hillman et al. 2013) are only valid when the supplemented population is below its carrying capacity.

The best fitting stock-recruitment models indicate that the population capacity of the Chiwawa River basin is between 140,000 to 185,000 spring Chinook parr. This equates to an overall density of about 1,100-1,400 parr per hectare. These densities can be achieved with about 490 redds. Assuming a female Chinook produces only one redd (Murdoch et al. 2009), a spawning escapement of about 490 females is needed to fill the capacity of the Chiwawa River basin.

The proportion of hatchery-origin spawners (pHOS) within the Chiwawa River basin during the survey period has ranged from 0 to 100%. Thus, some of the variation in juvenile productivity may be related to pHOS. Although there appeared to be a negative relationship between juvenile productivity (parr/redd) and pHOS, the correlation was not significant (Figure 10). In addition, there was no relationship between juvenile productivity and pHOS after the effects of spawning escapement were removed from the analysis (Figure 10). This suggests that spawning escapement has a larger effect on juvenile productivity than does the presence of hatchery spawners.

The presence of density dependence in the early life stages of spring Chinook is not surprising. Rarely does density dependence appear in numbers of adult spring Chinook or on their spawning grounds. The Chiwawa River basin appears to have plenty of spawning habitat, as indicated by the large numbers of spawners and redds widely distributed throughout the basin during high spawning escapements. However, those large spawning escapements did not translate into large numbers of juveniles or smolts. Thus, density-dependent regulation appears to occur sometime during the early life stages of the fish, likely at the fry stage. It is possible that physical habitat (space) during higher flows when fry are emerging may limit juvenile Chinook production in the basin. Low nutrient levels and its effects on food webs may also be a limiting factor in the basin. If spawning escapements remain relatively high, marine-derived nutrients should increase in the basin, resulting in more food for juvenile Chinook salmon.

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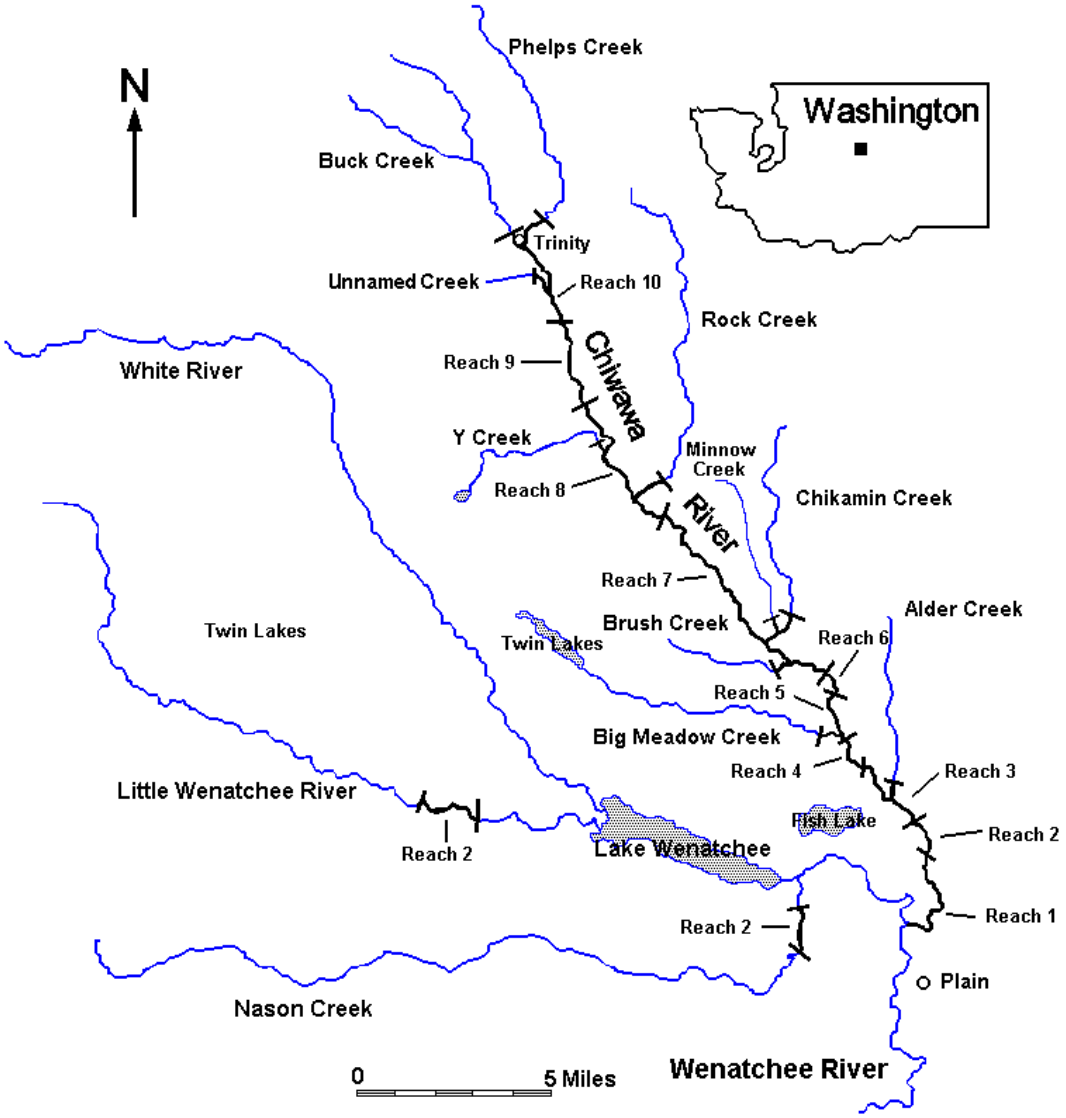

Figure 1. Location of study reaches on the Chiwawa River, and Chikamin, Rock, Big Meadow, Unnamed, Alder, Brush and Phelps creeks, Chelan County, Washington. Reach 2 on Nason Creek and Reach 2 on the Little Wenatchee River were matched with Reaches 3 and 8 on the Chiwawa River, respectively.

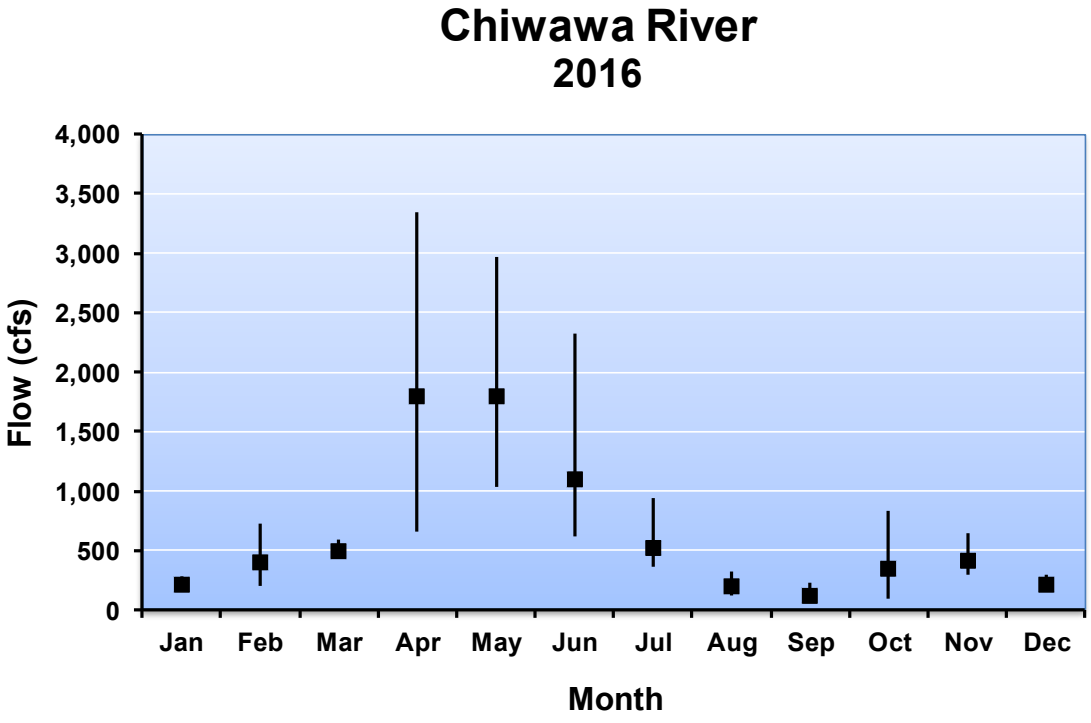

Figure 2. Mean, minimum, and maximum monthly flows in the Chiwawa River for 2016.

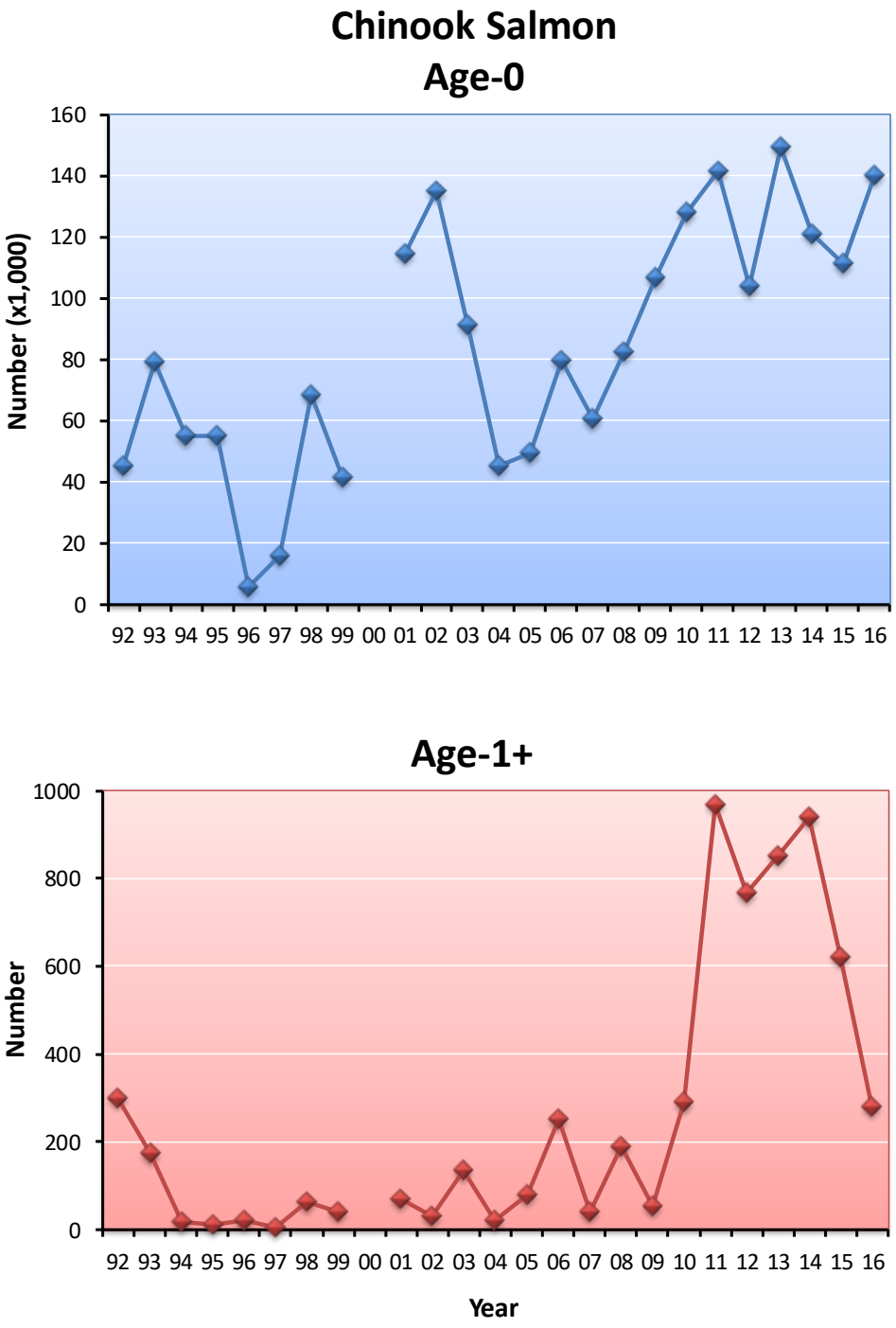

Figure 3. Numbers of age-0 and age-1+ Chinook salmon within the Chiwawa River basin in August 1992-2016; ND = no data.

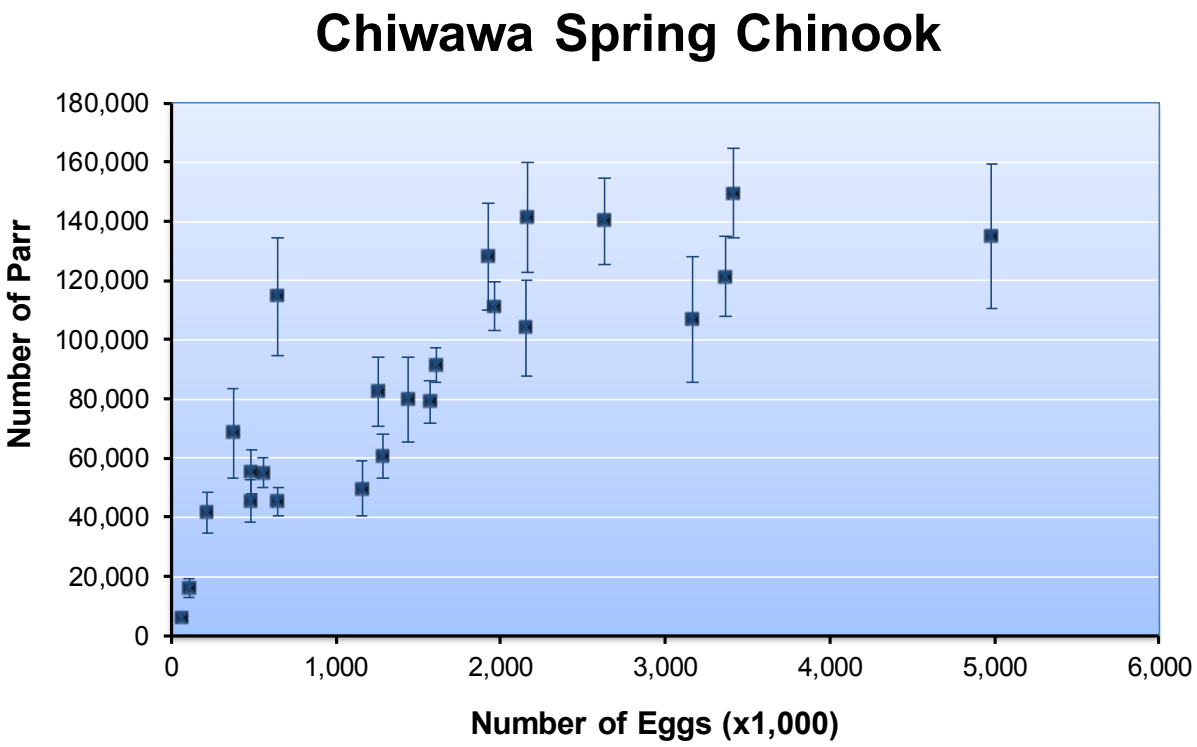

Figure 4. Relationship between total number of Chinook salmon parr counted during the summer (based on fish/ha) and number of eggs deposited in the Chiwawa River basin, 1992-2016. Vertical bars indicate 95% confidence bounds.

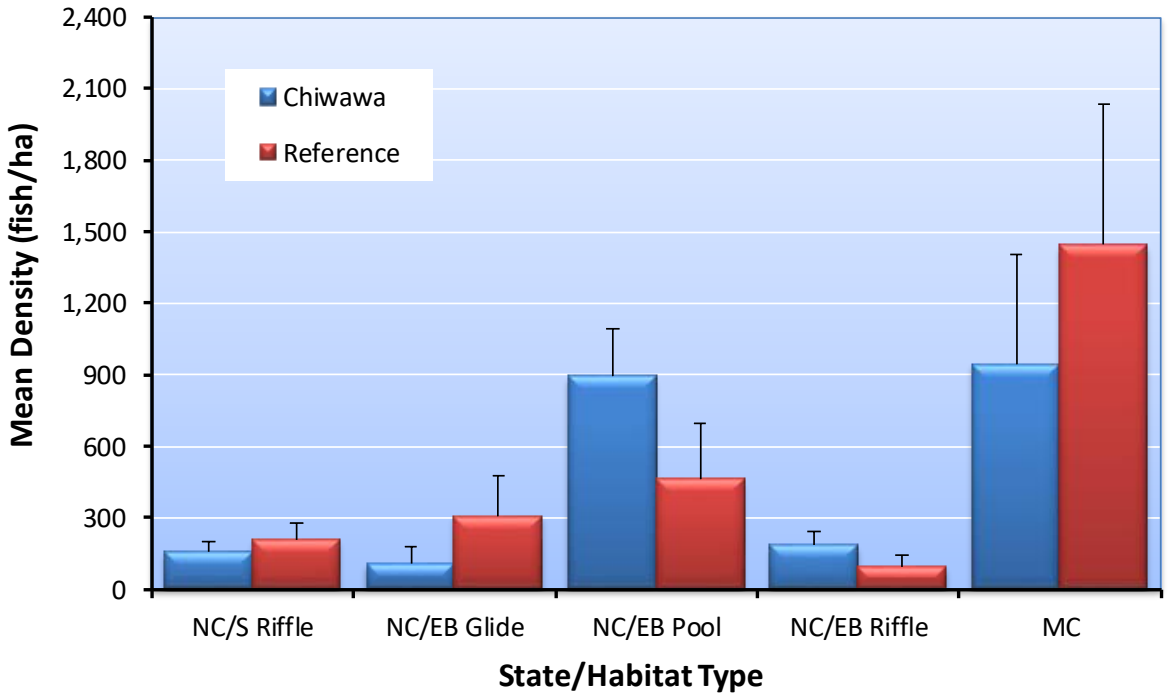

Figure 5. Comparison of the means (95% CI) of age-0 Chinook salmon densities (fish/ha) within state/habitat types in Reaches 3 and 8 of the Chiwawa River and their matched reference areas on Nason Creek and the Little Wenatchee River. There was no sampling in 2000 and no sampling in reference areas in 1992.

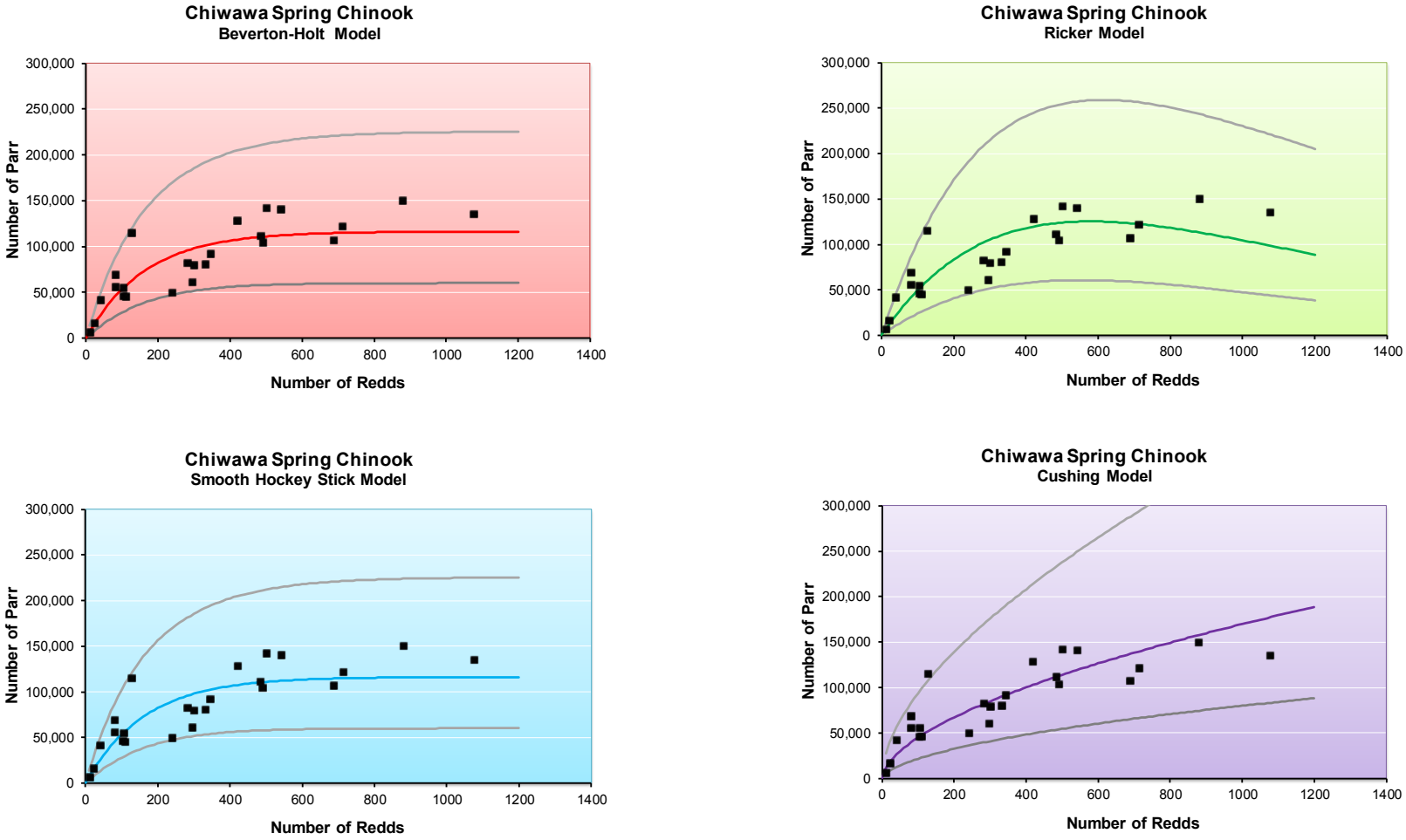

Figure 6. Relationship between numbers of juvenile (age-0) Chinook and redds in the Chiwawa River basin, 1992-2016 (no sampling occurred in 2000). Figures show the fit of the Beverton-Holt model, smooth hockey stick, Ricker model, and the Cushing model to the data. Gray lines indicate the upper and lower 95% C.B.

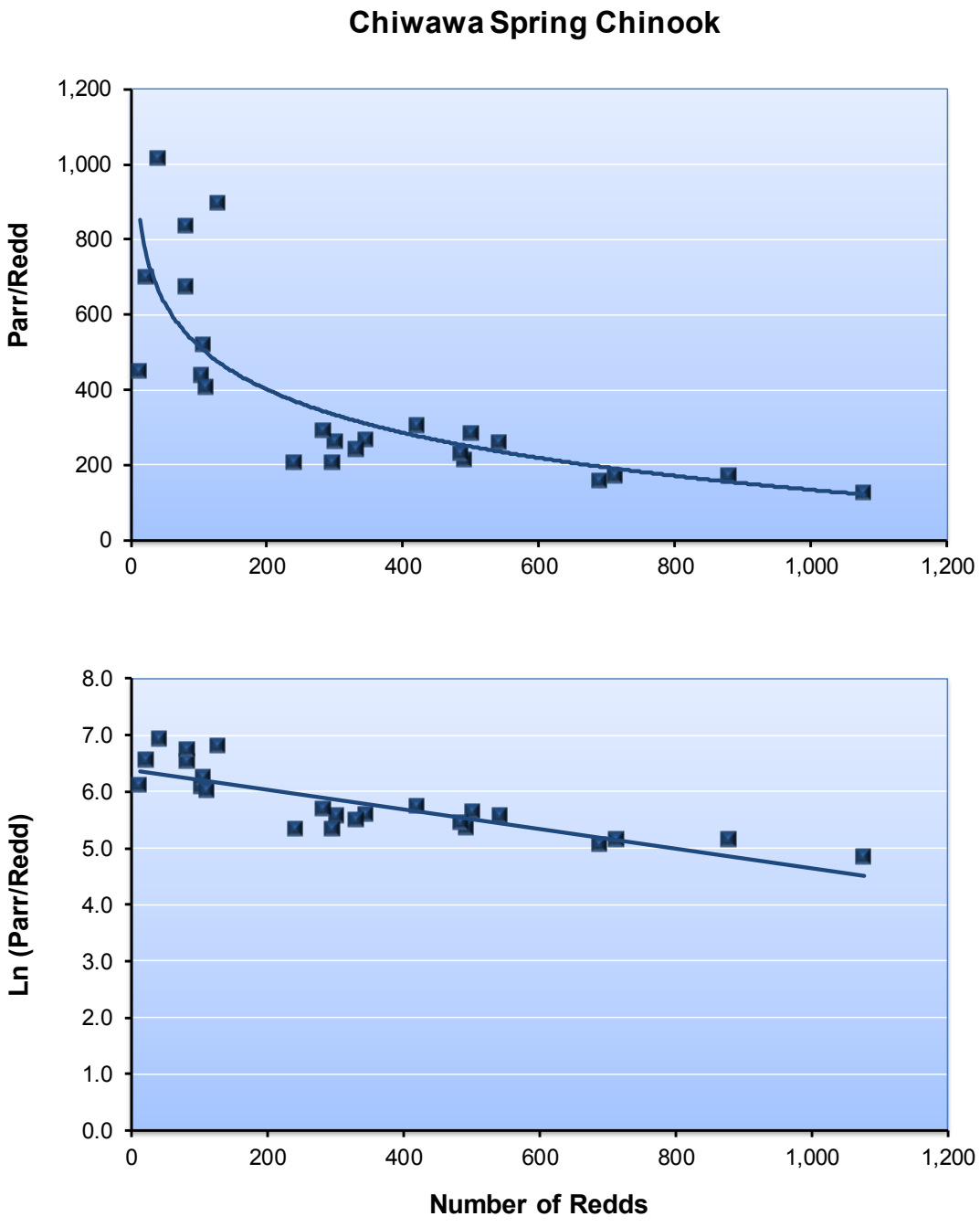

Figure 7. Relationship between parr/redd and numbers of redds (top figure) and natural log parr/redd and numbers of redds (bottom figure) in the Chiwawa River basin, 1992-2016. No sampling was conducted in 2000. Estimates for 1993-2016 included the Chiwawa River and its tributaries; the 1992 estimate included only the Chiwawa River. The linear relationship LN(P/R) = 6.38 – 0.002(Redds) was significant with P = 0.0000; R^2 = 0.690.

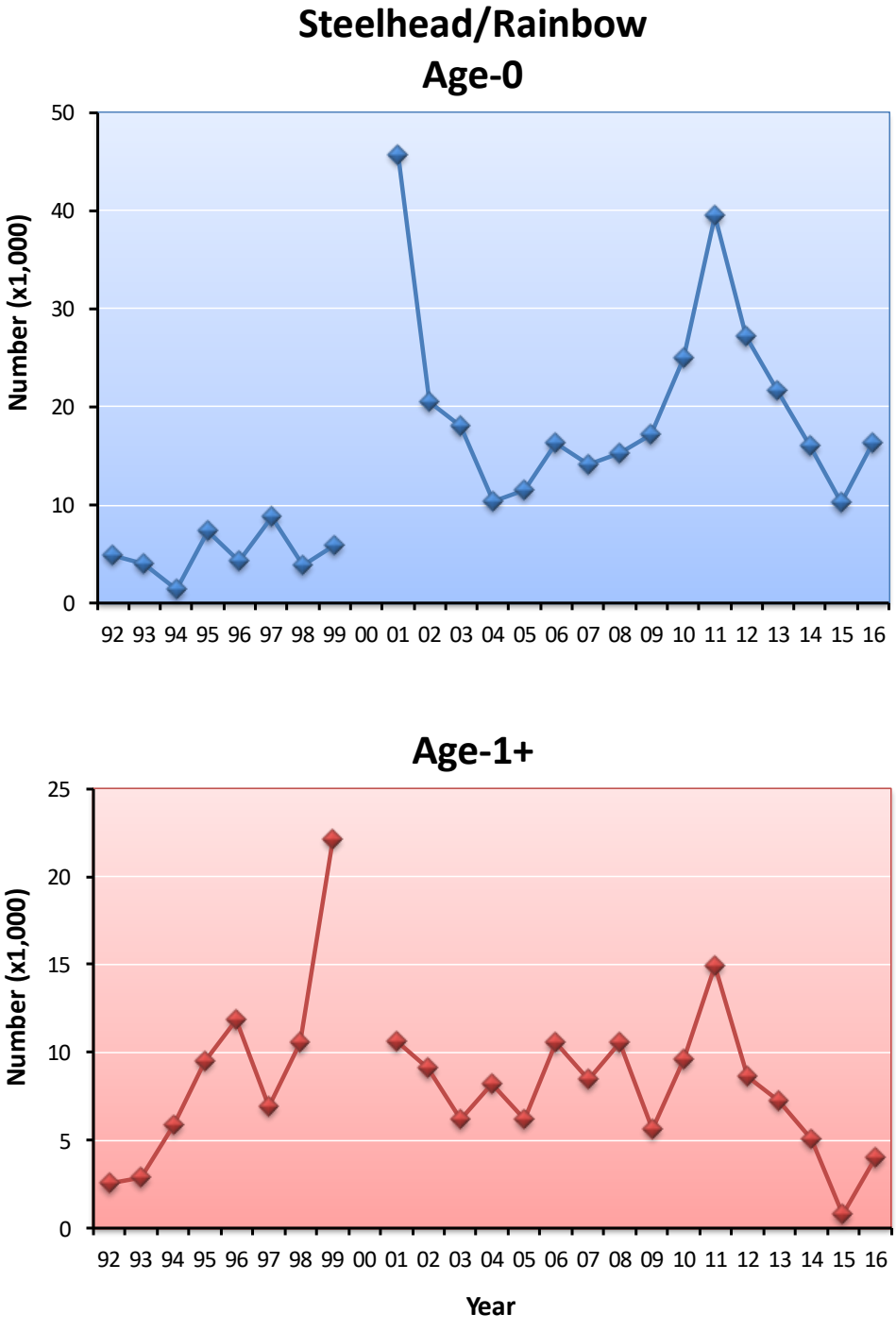

Figure 8. Numbers of age-0 (<4 in) and age-1+ (4-8 in) steelhead/rainbow within the Chiwawa River basin in August 1992-2016; ND = no data.

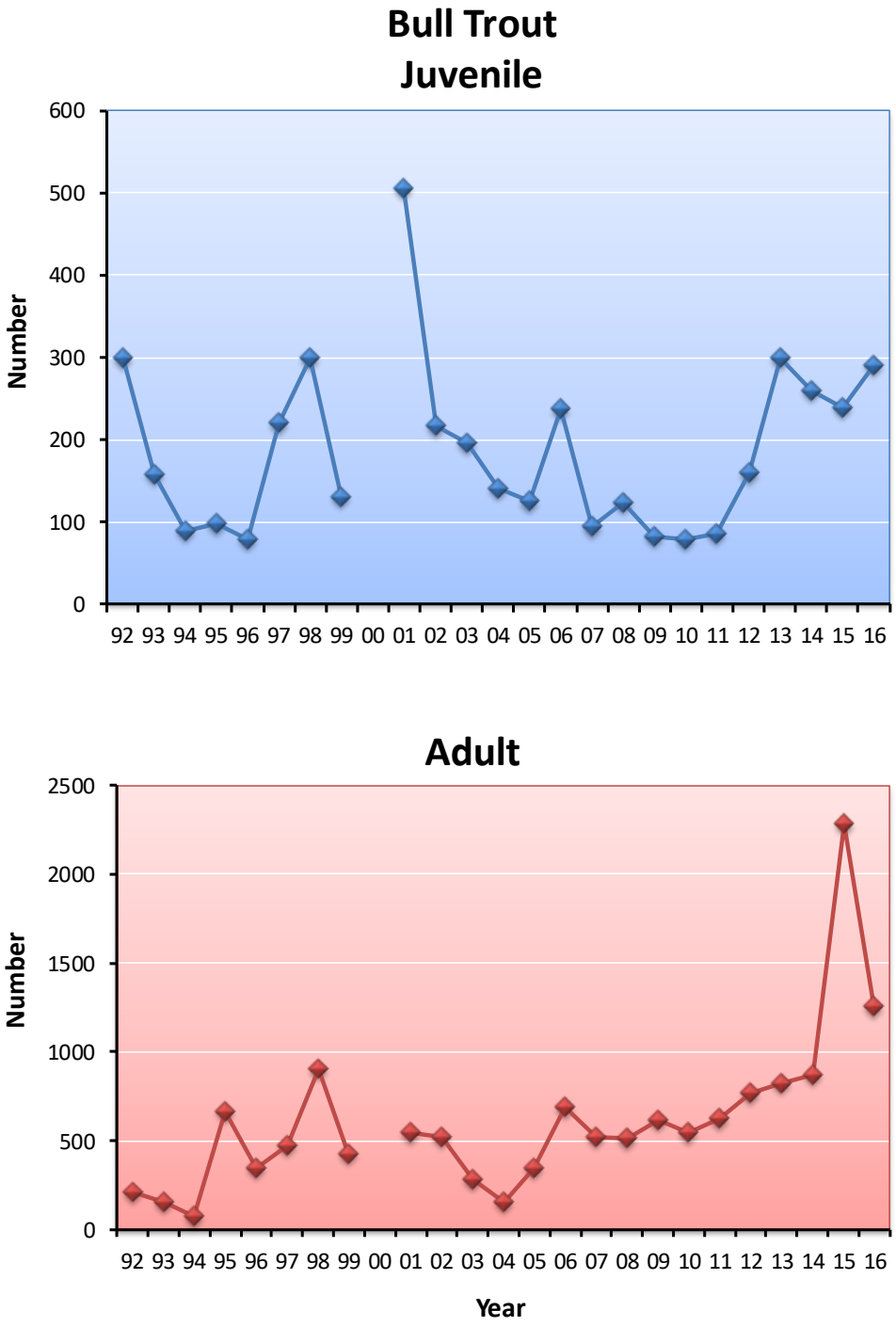

Figure 9. Numbers of juvenile (2-8 inches) and adult (>8 inches) bull trout within the Chiwawa River basin in August 1992-2016; ND = no data.

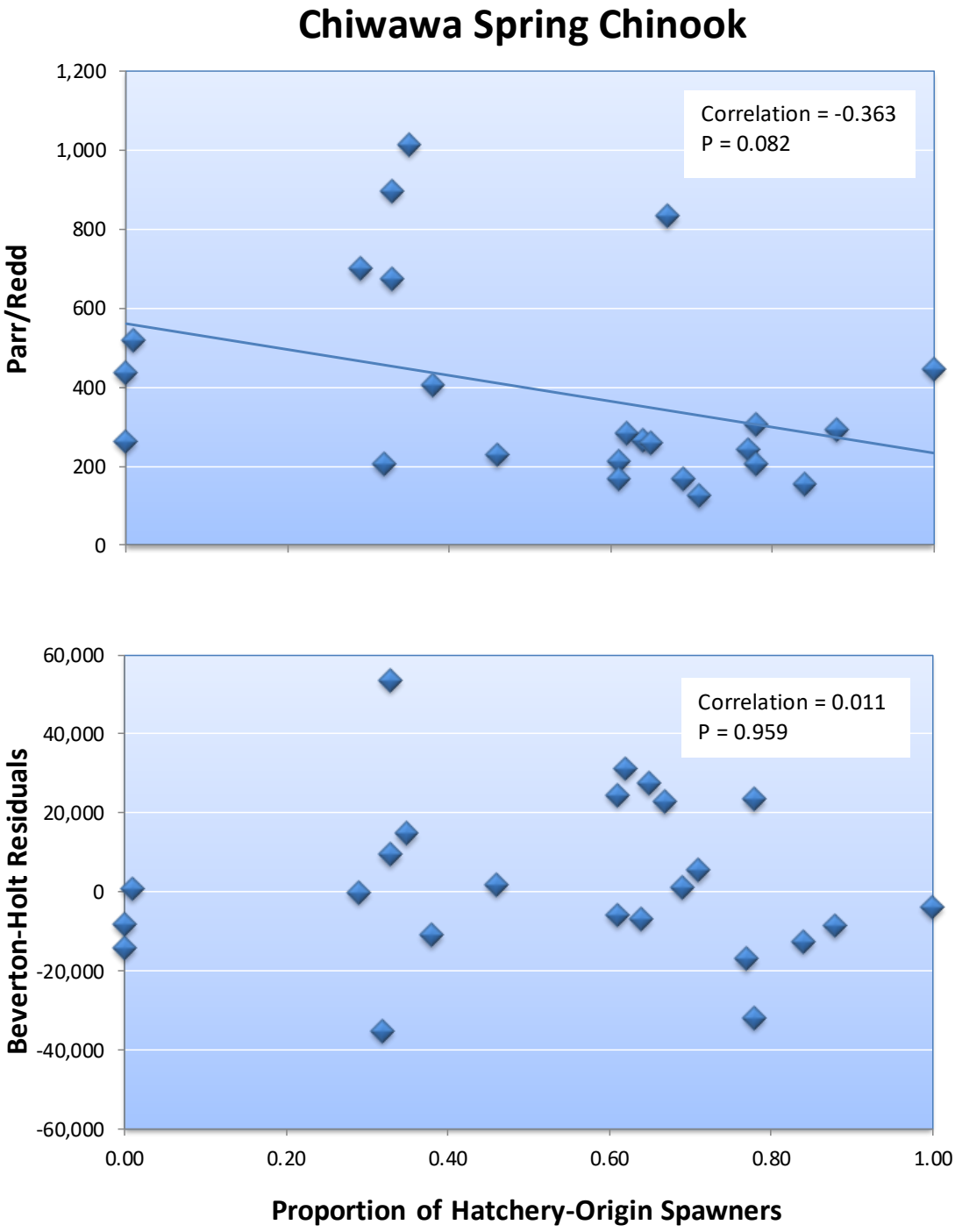

Figure 10. Relationship between juvenile productivity (parr/redd) and the proportion of hatchery-origin spawners (pHOS) (top figure) and the relationship between the residuals from the Beverton-Holt stock/recruitment relationship and pHOS (bottom figure).

Table 1. Description, location (river mile), and area (hectares) of land-class strata (reaches) used by age-0 Chinook salmon in the Chiwawa River basin, 2016. Reaches were classified according to geologic district, landtype association, valley-bottom type, stream state-type, and habitat type within the Cascade Ecoregion; MCV = moderately confined valley, CC = confined canyon, UCV = unconfined valley, NC = natural channel, EB = eroded banks, S = straight, G = glide, P = pool, R = riffle, and MC = multiple channel. See Hillman and Miller (2004) for definitions of stream state codes.

Reach	RM	Gradient	Geologic district	Landtype association	Valley bottom type	Stream state type	Habitat type	Area (ha)	
								Total	Sample
Chiwawa River									
1	0.00-3.77	0.007	Glacial Drift over Chumstick Formation	Glacial Valley	MCV Alluvial	NC/EB	G	0.60	0.60
						NC/EB	P	1.37	1.01
						NC/EB	R	16.35	1.75
2	3.77-5.51	0.010	Glacial Drift over Chumstick Formation	Glacial Canyon	CC Fluvial	NC/EB	G	0.26	0.26
						NC/EB	P	0.78	0.29
						NC/EB	R	7.21	0.67
3	5.51-7.88	0.009	Glacial Drift over Chumstick Formation	Glacial Valley	MCV Alluvial	NC/S	R	5.71	0.80
						NC/EB	G	0.13	0.13
						NC/EB	R	4.21	0.47
						MC	MC	0.32	0.32
4	7.88-8.90	0.007	Glacial Drift over Chumstick Formation	Glacial Canyon	CC Fluvial	NC/EB	P	0.39	0.27
						NC/EB	R	2.86	0.42
						MC	MC	0.44	0.44
5	8.90-10.83	0.011	Glacial Drift over Chumstick Formation	Glacial Valley	MCV Alluvial	NC/EB	P	0.13	0.13
						NC/EB	R	11.44	0.99
6	10.83-11.80	0.008	Glacial Drift over Chumstick Formation	Glacial Canyon	CC Fluvial	NC/EB	P	0.37	0.37
						NC/EB	R	3.53	0.98
						MC	MC	0.36	0.36
7	11.80-20.03	0.001	Glacial Drift over Chumstick Formation	Glacial Valley	UCV Alluvial	NC	G	2.13	0.73
						NC	P	6.52	0.70
						NC	R	0.99	0.20
						NC/EB	G	2.55	1.36
						NC/EB	P	6.89	1.84
						NC/EB	R	4.75	0.52
						MC	MC	4.30	1.65
8	20.03-25.42	0.003	Glacial Drift over Swakane Gneiss	Glacial Valley	UCV Alluvial	NC/EB	G	2.44	1.06
						NC/EB	P	7.41	2.24
						NC/EB	R	5.24	0.98
						EB	P	0.22	0.22
						EB	R	0.34	0.34
						MC	MC	7.79	2.65
9	25.42-28.81	0.007	Glacial Drift over Swakane Gneiss	Glacial Valley	MCV Alluvial	NC	P	4.52	0.51
						NC	R	2.80	0.58
						MC	MC	2.88	0.95
10	28.81-31.11	0.011	Pre-upper Jurassic Gneiss	Glacial Valley	MCV Alluvial	NC	P	0.60	0.31
						NC	R	2.24	0.49
						MC	MC	4.13	0.44

Table 1. Concluded.

Reach	RM	Gradient	Geologic district	Landtype association	Valley bottom type	Stream state type	Habitat type	Area (ha)	
								Total	Sampled
Trinity Side Channel									
10b	0.00-0.75	0.011	Pre-upper Jurassic Gneiss	Glacial Valley	MCV Alluvial	NC	P	0.39	0.09
						NC	R	0.12	0.03
						NC	MC	0.18	0.18
Phelps Creek									
1	0.00-0.35	0.043	Pre-upper Jurassic Gneiss	Glacial Valley	MCV Alluvial	NC	R	0.00	0.00
						NC	MC	0.18	0.18
Chikamin Creek[1]									
1	0.00-0.94	0.013	Glacial Drift over Chumstick Formation	Glacial Valley	UCV Alluvial	NC	G	0.02	0.02
						NC	P	0.21	0.05
						NC	R	0.32	0.03
						MC	MC	0.09	0.09
Rock Creek									
1	0.00-0.73	0.020	Glacial Drift over Swakane Gneiss	Glacial Valley	UCV Alluvial	NC	P	0.18	0.04
						NC	R	0.36	0.05
						MC	MC	0.07	0.07
Unnamed Creek									
1	0.00-0.05		Pre-upper Jurassic Gneiss	Glacial Valley	MCV Alluvial	NC	P	0.00	0.00
						NC	R	0.00	0.00
Big Meadow Creek									
1	0.00-0.35	0.025	Glacial Drift over Chumstick Formation	Glacial Valley	MCV Alluvial	NC	G	0.01	0.01
						NC	P	0.17	0.08
						NC	R	0.13	0.05
						NC	MC	0.00	0.00
Alder Creek									
1	0.00-0.01		Glacial Drift over Chumstick Formation	Glacial Valley	MCV Alluvial	NC	P	0.003	0.003
						NC	R	0.007	0.007
Brush Creek									
1	0.00-0.01		Glacial Drift over Chumstick Formation	Glacial Valley	UCV Alluvial	NC	P	0.002	0.002
						NC	R	0.006	0.006
Clear Creek									
1	0.00-0.05		Glacial Drift over Chumstick Formation	Glacial Valley	UCV Alluvial	NC	P	0.002	0.002
						NC	R	0.004	0.004
Y Creek									
1	0.00-0.05		Glacial Drift over Swakane Gneiss	Glacial Valley	UCV Alluvial	NC	P	0.000	0.000
						NC	R	0.000	0.000

[1] Includes the lower 0.2 miles of Minnow Creek.

Table 2. Estimated mean densities (fish/hectare and fish/m^3), total numbers, 95% confidence bounds on total numbers, and error of the estimated total number of age-0 Chinook salmon in reaches in the Chiwawa River basin, Washington, August 2016.

Reach	Mean density		Surface area (ha)			Volume (m^3)		
	Fish/ha	Fish/m^3	Total No.	95% C.B.	± Error	Total No.	95% C.B.	± Error
Chiwawa River								
1	197.7	0.061	3,621	±480	0.13	3,975	±311	0.08
2	349.8	0.079	2,886	±597	0.21	3,004	±601	0.20
3	167.7	0.041	1,739	±97	0.06	1,726	±97	0.06
4	365.3	0.080	1,348	±153	0.11	1,365	±128	0.09
5	86.6	0.020	1,002	±57	0.06	897	±69	0.08
6	188.3	0.051	802	±107	0.13	753	±116	0.15
7	1,301.4	0.186	36,608	±7,797	0.21	35,873	±8,470	0.24
8	1,078.2	0.177	25,272	±7,382	0.29	22,786	±10,263	0.45
9	2,420.1	0.410	24,685	±7,779	0.32	23,332	±7,993	0.34
10	4,942.0	1.393	37,856	±5,774	0.15	39,575	±9,230	0.23
Phelps Creek								
1	594.4	0.301	107	±0	0.00	107	±0	0.00
Chikamin Creek[1]								
1	2,568.8	1.178	1,644	±519	0.32	1,576	±654	0.41
Rock Creek								
1	1,624.6	0.641	991	±302	0.30	1,018	±388	0.38
Unnamed Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Big Meadow Creek								
1	4,928.1	2.265	1,508	±408	0.27	1,435	±801	0.56
Alder Creek								
1	2000.0	2.326	20	±0	0.00	20	±0	0.00
Brush Creek								
1	7,250.0	9.508	58	±0	0.00	58	±0	0.00
Clear Creek								
1	5,000.0	4.808	25	±0	0.00	25	±0	0.00
Y Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Grand Total	**1,098.1**	**0.217**	**140,172**	**±14,502**	**0.10**	**137,525**	**±18,108**	**0.13**

[1] Includes lower 0.2 miles of Minnow Creek.

Table 3. Estimated mean densities (fish/hectare and fish/m^3), total numbers, 95% confidence bounds on total numbers, and error of the estimated total number of age-1+ Chinook salmon in reaches in the Chiwawa River basin, Washington, August 2016.

Reach	Mean density		Surface area (ha)			Volume (m^3)		
	Fish/ha	Fish/m^3	Total No.	95% C.B.	± Error	Total No.	95% C.B.	± Error
Chiwawa River								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
2	1.0	0.000	8	±10	1.25	8	±12	1.50
3	0.0	0.000	0	±0	0.00	0	±0	0.00
4	1.9	0.000	7	±0	0.00	7	±0	0.00
5	0.0	0.000	0	±0	0.00	0	±0	0.00
6	0.5	0.000	2	±0	0.00	1	±0	0.00
7	0.4	0.000	11	±12	1.09	19	±13	0.68
8	2.8	0.000	65	±56	0.86	52	±72	1.38
9	14.6	0.003	149	±96	0.64	142	±119	0.84
10	1.7	0.001	12	±12	1.00	13	±16	1.23
Phelps Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Chikamin Creek[1]								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Rock Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Unnamed Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Big Meadow Creek								
1	91.5	0.041	28	±47	1.68	26	±30	1.15
Alder Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Brush Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Clear Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Y Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Grand Total	**2.2**	**0.000**	**282**	**±122**	**0.43**	**268**	**±144**	**0.54**

[1] Includes lower 0.2 miles of Minnow Creek.

Table 4. Summary of the five productivity models of juvenile (age-0) Chinook salmon in the Chiwawa River basin. Models are shown, including the number of parameters (K), AIC_c values, AIC_c difference scores (Δ_i), the likelihood of the model given the data ($\pounds(g_i|x)$), Akaike weights (w_i), and adjusted R^2 values. The sample size (n) for all models was 24. Models describe the relationship between juvenile Chinook numbers (dependent variable) and redd numbers (independent variable).

Model	K[a]	AIC_c	Δ_i	$\pounds(g_i\|x)$	w_i	Adj R^2
Beverton-Holt	3	-130.391	0.000	1.000	0.661	0.841
Smooth Hockey Stick	3	-128.692	1.698	0.428	0.283	0.829
Gamma[b]	4	-123.826	6.565	0.038	0.025	0.805
Ricker	3	-123.279	7.112	0.029	0.019	0.786
Cushing	3	-122.355	8.036	0.018	0.012	0.777

[a] $\boldsymbol{K}$ is the number of structural parameters in the model plus 1 for σ^2.
[b] The γ parameter in the Gamma model was greater than 0, which means that this model is nearly identical to the Ricker model.

Table 5. Estimated mean densities (fish/hectare and fish/m^3), total numbers, 95% confidence bounds on total numbers, and error of the estimated total number of age-0 (<4 in) steelhead/rainbow in reaches in the Chiwawa River basin, Washington, August 2016.

Reach	Mean density		Surface area (ha)			Volume (m^3)		
	Fish/ha	Fish/m^3	Total No.	95% C.B.	± Error	Total No.	95% C.B.	± Error
Chiwawa River								
1	139.0	0.044	2,546	±280	0.11	2,861	±221	0.08
2	234.8	0.053	1,937	±336	0.17	2,035	±342	0.17
3	264.7	0.064	2,745	±179	0.07	2,679	±162	0.06
4	191.9	0.043	708	±174	0.25	743	±163	0.22
5	97.7	0.022	1,130	±20	0.02	997	±33	0.03
6	70.7	0.018	301	±44	0.15	265	±55	0.21
7	57.0	0.008	1,604	±598	0.37	1,546	±703	0.45
8	0.0	0.000	0	±0	0.00	0	±0	0.00
9	0.0	0.000	0	±0	0.00	0	±0	0.00
10	0.0	0.000	0	±0	0.00	0	±0	0.00
Phelps Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Chikamin Creek[1]								
1	2,217.2	1.053	1,419	±467	0.33	1,409	±501	0.36
Rock Creek								
1	1,632.8	0.607	996	±261	0.26	963	±311	0.32
Unnamed Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Big Meadow Creek								
1	8,892.2	4.131	2,721	±2,003	0.74	2,618	±2,887	1.10
Alder Creek								
1	5,000.0	5.581	50	±0	0.00	48	±0	0.00
Brush Creek								
1	7,750.0	10.164	62	±0	0.00	62	±0	0.00
Clear Creek								
1	5,000.0	4.808	25	±0	0.00	25	±0	0.00
Y Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Grand Total	**127.3**	**0.026**	**16,244**	**±2,217**	**0.14**	**16,251**	**±3,066**	**0.19**

[1] Includes lower 0.2 miles of Minnow Creek.

Table 6. Estimated mean densities (fish/hectare and fish/m^3), total numbers, 95% confidence bounds on total numbers, and error of the estimated total number of age-1+ (4-8 in) steelhead/rainbow in reaches in the Chiwawa River basin, Washington, August 2016.

Reach	Mean density		Surface area (ha)			Volume (m^3)		
	Fish/ha	Fish/m^3	Total No.	95% C.B.	± Error	Total No.	95% C.B.	± Error
Chiwawa River								
1	54.9	0.017	1,005	±145	0.14	1,126	±141	0.13
2	41.9	0.010	346	±162	0.47	363	±164	0.45
3	93.9	0.024	974	±49	0.05	986	±45	0.05
4	60.4	0.014	223	±117	0.52	233	±112	0.48
5	44.3	0.010	513	±34	0.07	453	±45	0.10
6	32.2	0.008	137	±31	0.23	121	±36	0.30
7	7.8	0.001	220	±185	0.84	213	±171	0.80
8	0.0	0.000	0	±0	0.00	0	±0	0.00
9	0.0	0.000	0	±0	0.00	0	±0	0.00
10	0.0	0.000	0	±0	0.00	0	±0	0.00
Phelps Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Chikamin Creek[1]								
1	400.0	0.180	256	±392	1.53	241	±320	1.33
Rock Creek								
1	65.6	0.025	40	±0	0.00	40	±0	0.00
Unnamed Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Big Meadow Creek								
1	1,009.8	0.466	309	±307	0.99	295	±396	1.34
Alder Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Brush Creek								
1	1,000.0	1.312	8	±0	0.00	8	±0	0.00
Clear Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Y Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Grand Total	**31.6**	**0.006**	**4,031**	**±590**	**0.15**	**4,079**	**±594**	**0.15**

[1] Includes lower 0.2 miles of Minnow Creek.

Table 7. Estimated mean densities (fish/hectare and fish/m^3), total numbers, 95% confidence bounds on total numbers, and error of the estimated total number of steelhead/rainbow larger than 8 inches in reaches in the Chiwawa River basin, Washington, August 2016.

Reach	Mean density		Surface area (ha)			Volume (m^3)		
	Fish/ha	Fish/m^3	Total No.	95% C.B.	± Error	Total No.	95% C.B.	± Error
Chiwawa River								
1	0.3	0.000	5	±6	1.20	7	±10	0.42
2	0.4	0.000	3	±2	0.67	4	±4	1.00
3	0.0	0.000	0	±0	0.00	0	±0	0.00
4	0.0	0.000	0	±0	0.00	0	±0	0.00
5	0.0	0.000	0	±0	0.00	0	±0	0.00
6	0.0	0.000	0	±0	0.00	0	±0	0.00
7	0.0	0.000	0	±0	0.00	0	±0	0.00
8	0.3	0.000	6	±8	1.33	6	±10	1.67
9	0.0	0.000	0	±0	0.00	0	±0	0.00
10	0.0	0.000	0	±0	0.00	0	±0	0.00
Phelps Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Chikamin Creek[1]								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Rock Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Unnamed Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Big Meadow Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Alder Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Brush Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Clear Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Y Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Grand Total	**0.1**	**0.000**	**14**	**±10**	**0.71**	**17**	**±15**	**0.88**

[1] Includes lower 0.2 miles of Minnow Creek.

Table 8. Estimated mean densities (fish/hectare and fish/m^3), total numbers, 95% confidence bounds on total numbers, and error of the estimated total number of juvenile bull trout (2-8 in) in reaches in the Chiwawa River basin, Washington, August 2016.

Reach	Mean density		Surface area (ha)			Volume (m^3)		
	Fish/ha	Fish/m^3	Total No.	95% C.B.	± Error	Total No.	95% C.B.	± Error
Chiwawa River								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
2	1.6	0.000	13	±17	1.31	15	±20	1.33
3	0.0	0.000	0	±0	0.00	0	±0	0.00
4	0.0	0.000	0	±0	0.00	0	±0	0.00
5	0.0	0.000	0	±0	0.00	0	±0	0.00
6	0.0	0.000	0	±0	0.00	0	±0	0.00
7	0.0	0.000	0	±0	0.00	0	±0	0.00
8	0.0	0.000	0	±0	0.00	0	±0	0.00
9	7.6	0.001	78	±38	0.49	74	±44	0.59
10	21.9	0.011	168	±40	0.24	310	±43	0.14
Phelps Creek								
1	144.4	0.073	26	±0	0.00	26	±0	0.00
Chikamin Creek[1]								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Rock Creek								
1	9.8	0.004	6	±0	0.00	6	±0	0.00
Unnamed Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Big Meadow Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Alder Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Brush Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Clear Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Y Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Grand Total	**2.3**	**0.001**	**291**	**±58**	**0.20**	**431**	**±65**	**0.15**

[1] Includes lower 0.2 miles of Minnow Creek.

Table 9. Estimated mean densities (fish/hectare and fish/m^3), total numbers, 95% confidence bounds on total numbers, and error of the estimated total number of adult bull trout (>8 in) in reaches in the Chiwawa River basin, Washington, August 2016.

Reach	Mean density		Surface area (ha)			Volume (m^3)		
	Fish/ha	Fish/m^3	Total No.	95% C.B.	± Error	Total No.	95% C.B.	± Error
Chiwawa River								
1	1.1	0.000	20	±15	0.75	20	±15	0.75
2	2.3	0.001	19	±15	0.79	19	±28	1.47
3	2.0	0.001	21	±3	0.14	21	±4	0.19
4	3.3	0.001	12	±4	0.33	12	±5	0.42
5	0.3	0.000	4	±0	0.00	5	±0	0.00
6	1.4	0.000	6	±0	0.00	6	±0	0.00
7	8.6	0.001	242	±74	0.31	232	±133	0.57
8	7.3	0.001	171	±46	0.27	155	±117	0.75
9	22.8	0.004	233	±39	0.17	222	±96	0.43
10	74.5	0.020	519	±117	0.23	540	±92	0.17
Phelps Creek								
1	38.9	0.020	7	±0	0.00	7	±0	0.00
Chikamin Creek[1]								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Rock Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Unnamed Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Big Meadow Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Alder Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Brush Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Clear Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Y Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Grand Total	**9.8**	**0.002**	**1,254**	**±152**	**0.12**	**1,239**	**±224**	**0.18**

[1] Includes lower 0.2 miles of Minnow Creek.

APPENDIX A. Numbers of redds, eggs, age-0 Chinook salmon, parr per redd, and percent egg-to-parr survival in the Chiwawa River basin, brood years 1991-2016; NS = not sampled. Numbers of eggs were calculated as the number of redds times the mean fecundity of females collected for broodstock.

Brood Year	Chinook Salmon			Parr/Redd	Egg-to-parr survival (%)
	Redds	Eggs	Age-0 (parr)		
1991	104	478,400	45,483	437	9.5
1992	302	1,570,098	79,113	262	5.0
1993	106	556,394	55,056	519	9.9
1994	82	485,686	55,240	674	11.4
1995	13	66,248	5,815	447	8.8
1996	23	106,835	16,066	699	15.0
1997	82	374,740	68,415	834	18.3
1998	41	218,325	41,629	1,015	19.1
1999	34	166,090	NS	NS	NS
2000	128	642,944	114,617	895	17.8
2001	1,078	4,984,672	134,874	125	2.7
2002	345	1,605,630	91,278	265	5.7
2003	111	648,684	45,177	407	7.0
2004	241	1,156,559	49,631	206	4.3
2005	332	1,436,564	79,902	241	5.6
2006	297	1,284,228	60,752	205	4.7
2007	283	1,256,803	82,351	291	6.6
2008	689	3,163,888	106,705	155	3.4
2009	421	1,925,233	128,220	305	6.7
2010	502	2,165,628	141,510	282	6.5
2011	492	2,157,420	103,940	211	4.8
2012	880	3,716,240	149,563	185	4.4
2013	714	3,367,224	121,240	170	3.6
2014	485	1,961,825	111,224	229	5.7
2015	543	2,631,921	140,172	258	5.3
Average	**333**	**1,525,131**	**84,499**	**388**	**8.0**

APPENDIX B. Estimated numbers of salmonids (based on fish/ha) in the Chiwawa River basin, Washington, 1992-2016; NS = not sampled.

Survey year	Chinook salmon		Steelhead/Rainbow			Bull trout		Cutthroat trout
	Age-0	Age-1+	Age-0	Age-1+	>8 in[1]	2-8 in	>8 in	
1992[2]	45,483	563	4,927	2,533	1,869	299	208	NS
1993	79,113	174	4,004	2,860	768	158	156	NS
1994	55,056	18	1,410	5,856	67	90	76	NS
1995	55,241	13	7,357	9,517	140	97	664	NS
1996	5,815	22	4,245	11,849	78	79	343	NS
1997	16,066	5	8,823	6,905	48	220	472	56
1998	68,415	63	3,921	10,585	78	300	900	93
1999	41,629	41	5,838	22,130	33	130	423	80
2000	NS	NS	NS	NS	NS	NS	NS	NS
2001	114,617	69	45,727	10,623	420	505	542	108
2002	134,874	32	20,521	9,090	181	217	521	111
2003	91,278	134	18,020	6,179	49	196	282	52
2004	45,177	21	10,380	8,190	8	140	157	22
2005	49,631	79	11,463	6,188	48	125	346	23
2006	79,902	388	16,245	10,533	50	238	686	68
2007	60,752	41	14,073	8,448	77	95	520	47
2008	82,351	189	15,230	10,576	144	124	510	109
2009	106,705	54	17,179	5,629	85	82	618	128
2010	128,220	291	25,018	9,616	63	79	547	252
2011	141,510	967	39,446	14,903	65	86	621	240
2012	103,940	767	27,134	8,576	65	159	768	188
2013	149,563	852	21,682	7,253	76	299	820	358
2014	121,240	939	16,083	5,084	87	259	875	761
2015	111,224	620	10,208	754	18	239	2,286	292
2016	140,172	282	16,244	4,031	14	291	1,254	544

[1]During 1992-1993, numbers of steelhead/rainbow greater than 8 inches included both hatchery and wild rainbow trout. Thereafter, only wild trout were observed.
[2]Only the Chiwawa River was sampled in 1992. No tributaries were sampled in that year.

APPENDIX C. Proportion of total habitat available, fraction of all age-0 Chinook within each habitat type, and densities (fish/ha) and numbers of age-0 Chinook within each habitat type in the Chiwawa River basin, survey years 1992-2016; NS = not sampled.

Habitat	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002
Proportion of total habitat available											
Glide	0.10	0.09	0.10	0.10	0.10	0.09	0.09	0.09	NS	0.07	0.08
Pool	0.19	0.19	0.21	0.18	0.18	0.17	0.16	0.17	NS	0.15	0.16
Riffle	0.61	0.61	0.57	0.59	0.57	0.57	0.58	0.55	NS	0.49	0.48
M. Chan	0.10	0.11	0.12	0.14	0.14	0.17	0.17	0.19	NS	0.29	0.28
Fraction of all age-0 Chinook within habitat types											
Glide	0.07	0.03	0.02	0.01	0.02	0.01	0.01	0.01	NS	0.03	0.01
Pool	0.30	0.28	0.22	0.21	0.30	0.16	0.17	0.14	NS	0.23	0.24
Riffle	0.19	0.16	0.12	0.11	0.43	0.23	0.08	0.11	NS	0.18	0.15
M. Chan	0.45	0.53	0.64	0.67	0.24	0.60	0.74	0.74	NS	0.57	0.60
Densities of age-0 Chinook within habitat types (fish/ha)											
Glide	254	251	93	55	11	12	78	13	NS	351	187
Pool	584	1,049	619	541	82	122	607	257	NS	1,392	1,468
Riffle	116	188	124	91	38	52	79	62	NS	336	300
M. Chan	1,710	3,408	2,985	2,328	84	449	2,620	1,201	NS	1,820	2,069
Number of age-0 Chinook within habitat types											
Glide	2,967	2,458	857	623	137	130	837	157	NS	3,231	1,931
Pool	13,468	21,814	12,131	11,294	1,755	2,553	11,454	5,933	NS	25,890	32,612
Riffle	8,531	12,616	6,698	6,197	2,525	3,699	5,392	4,626	NS	20,629	19,754
M. Chan	20,517	42,225	35,370	36,965	1,396	9,682	50,728	30,912	NS	64,866	80,576

APPENDIX C. Continued.

Habitat	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Proportion of total habitat available											
Glide	0.07	0.07	0.08	0.08	0.07	0.09	0.08	0.08	0.08	0.07	0.07
Pool	0.17	0.16	0.16	0.16	0.17	0.23	0.22	0.23	0.18	0.23	0.23
Riffle	0.49	0.50	0.47	0.47	0.47	0.51	0.54	0.53	0.57	0.53	0.53
M. Chan	0.26	0.27	0.29	0.30	0.29	0.17	0.15	0.16	0.17	0.17	0.17
Fraction of all age-0 Chinook within habitat types											
Glide	0.02	0.01	0.01	0.03	0.02	0.03	0.02	0.02	0.04	0.01	0.02
Pool	0.23	0.07	0.19	0.31	0.46	0.40	0.36	0.34	0.34	0.41	0.37
Riffle	0.15	0.14	0.07	0.12	0.12	0.11	0.11	0.11	0.19	0.15	0.13
M. Chan	0.60	0.77	0.73	0.54	0.40	0.45	0.51	0.53	0.43	0.43	0.48
Densities of age-0 Chinook within habitat types (fish/ha)											
Glide	200	58	49	237	113	238	230	286	526	173	321
Pool	951	155	492	1,240	1,211	1,210	1,453	1,436	1,805	1,360	1,890
Riffle	216	101	60	166	118	156	175	200	330	221	281
M. Chan	1,626	1,008	1,057	1,147	603	1,872	2,993	3,293	2,515	2,061	3,190
Number of age-0 Chinook within habitat types											
Glide	1,884	540	442	2,498	1,120	2,668	2,371	3,164	6,122	1,535	2,822
Pool	21,091	3,183	9,626	26,754	28,851	34,314	39,382	44,765	48,846	42,209	55,651
Riffle	13,783	6,501	3,367	10,753	7,809	9,773	11,558	14,446	27,883	15,418	19,619
M. Chan	54,519	34,952	36,196	46,580	25,409	38,275	55,607	69,609	61,944	44,779	73,057

APPENDIX C. Concluded.

Habitat	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	Mean
Proportion of total habitat available											
Glide	0.07	0.07	0.06								**0.08**
Pool	0.22	0.24	0.24								**0.19**
Riffle	0.54	0.53	0.54								**0.53**
M. Chan	0.17	0.16	0.16								**0.20**
Fraction of all age-0 Chinook within habitat types											
Glide	0.01	0.01	0.01								**0.02**
Pool	0.37	0.31	0.35								**0.30**
Riffle	0.11	0.05	0.08								**0.13**
M. Chan	0.51	0.63	0.56								**0.55**
Densities of age-0 Chinook within habitat types (fish/ha)											
Glide	133	66	114								**169**
Pool	1,569	1,300	1,628								**1,079**
Riffle	190	98	168								**163**
M. Chan	2,957	3,768	3,789								**1,923**
Number of age-0 Chinook within habitat types											
Glide	1,120	518	931								**1,711**
Pool	44,321	34,993	49,103								**25,916**
Riffle	13,085	6,017	11,550								**10,926**
M. Chan	62,713	69,969	78,589								**46,893**

Appendix B

Fish Trapping at the Chiwawa and Wenatchee Rotary Smolt Traps during 2016

Monitoring Juvenile Salmonids in the Wenatchee River basin:

Activities in the Chiwawa River and Lower Wenatchee River during 2016

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INTRODUCTION

Background

Monitoring and Evaluation

Productivity indicators in the freshwater environment provide data essential to inform evolving salmon and steelhead hatchery programs. In the Wenatchee River subbasin, the Juvenile Monitoring Component of the Monitoring and Evaluation Plan for PUD Hatchery Programs gather data directed at informing these productivity indicators (see Hillman et al. 2013). More specifically, this data directly addresses Objective 2 of the monitoring and evaluation framework:

"Determine if the proportion of hatchery fish on the spawning grounds affects the freshwater productivity of supplemented stocks."

Objectives

The Washington Department of Fish and Wildlife monitors juvenile salmonids in the Wenatchee River basin with the primary objective of estimating: natural productivity, migration timing, and age with size at migration. This has occurred at the tributary level (Chiwawa River since 1991) and population level (Wenatchee River since 1997). Target species include spring Chinook Salmon *Oncorhynchus tshawytscha* and summer steelhead *O. mykiss* in the Chiwawa River, and is expanded to include sockeye Salmon *O. nerka* and summer Chinook Salmon *O. tshawytscha* in the mainstem Wenatchee River.

Monitoring has primarily been conducted with rotary smolt traps that capture emigrating salmonids from spring through fall. In an effort to reduce biases in emigrant estimates, and to improve understanding of survival and movement during non-trapping periods (December through February), WDFW began remote sampling spring Chinook Salmon in the Chiwawa Basin in 2012.

Study Area

Chiwawa River

The Chiwawa River is a fourth-order river draining a 474-km^2 basin and has a mean annual discharge of 14.4 cubic meters per second (m^3/s); contributing about 15% of the mean annual discharge of the Wenatchee River. The Chiwawa basin is dominated by the snow melt cycle with peak discharge occurring May through July with occasional fall freshets (Figure 1). The Chiwawa River originates in the North Cascades and flows southeast for 60 km before joining the Wenatchee River. This confluence with the Wenatchee River is approximately 9km downstream of Lake Wenatchee and 76 km upstream of the Columbia River (Figure 2). The Chiwawa River basin is relatively natural, with 96% managed as part of the Wenatchee National Forest and the upper 32% designated wilderness.

Precipitation in the basin varies between 76 cm near the confluence and 356 cm at the peaks, while elevations range from 573 to 2,768 m. The river is dynamic with generally shallow pool

riffle segments as it meanders through a U-shaped valley formed by ancient glaciers in the region. Gradients remain well under 1% for the majority of the river.

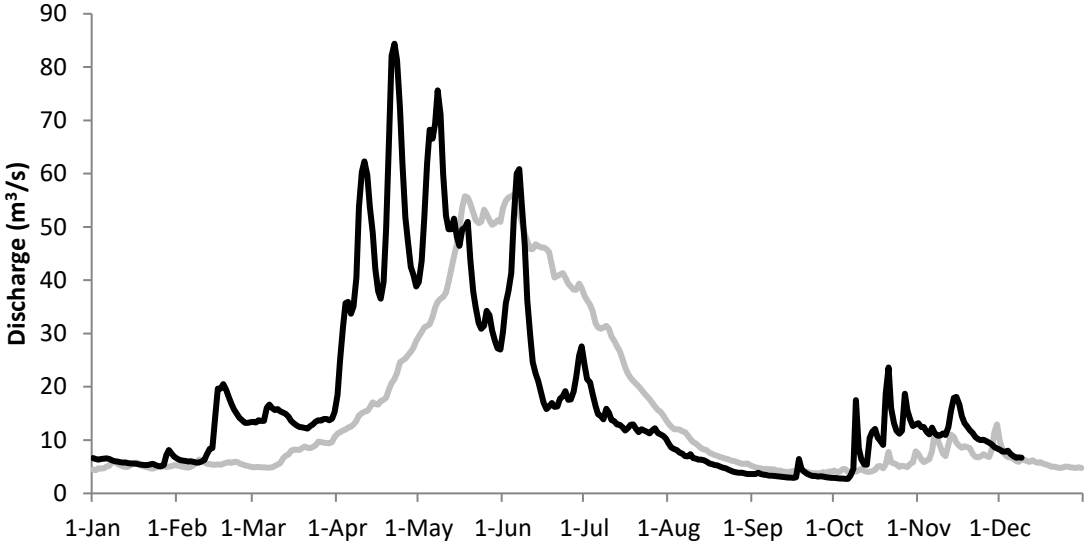

Figure 1. Discharge of the Chiwawa River at Plain, USGS gauge # 12456500. Black line represents 2016 discharge and grey line represents mean discharge from 1990-2015.

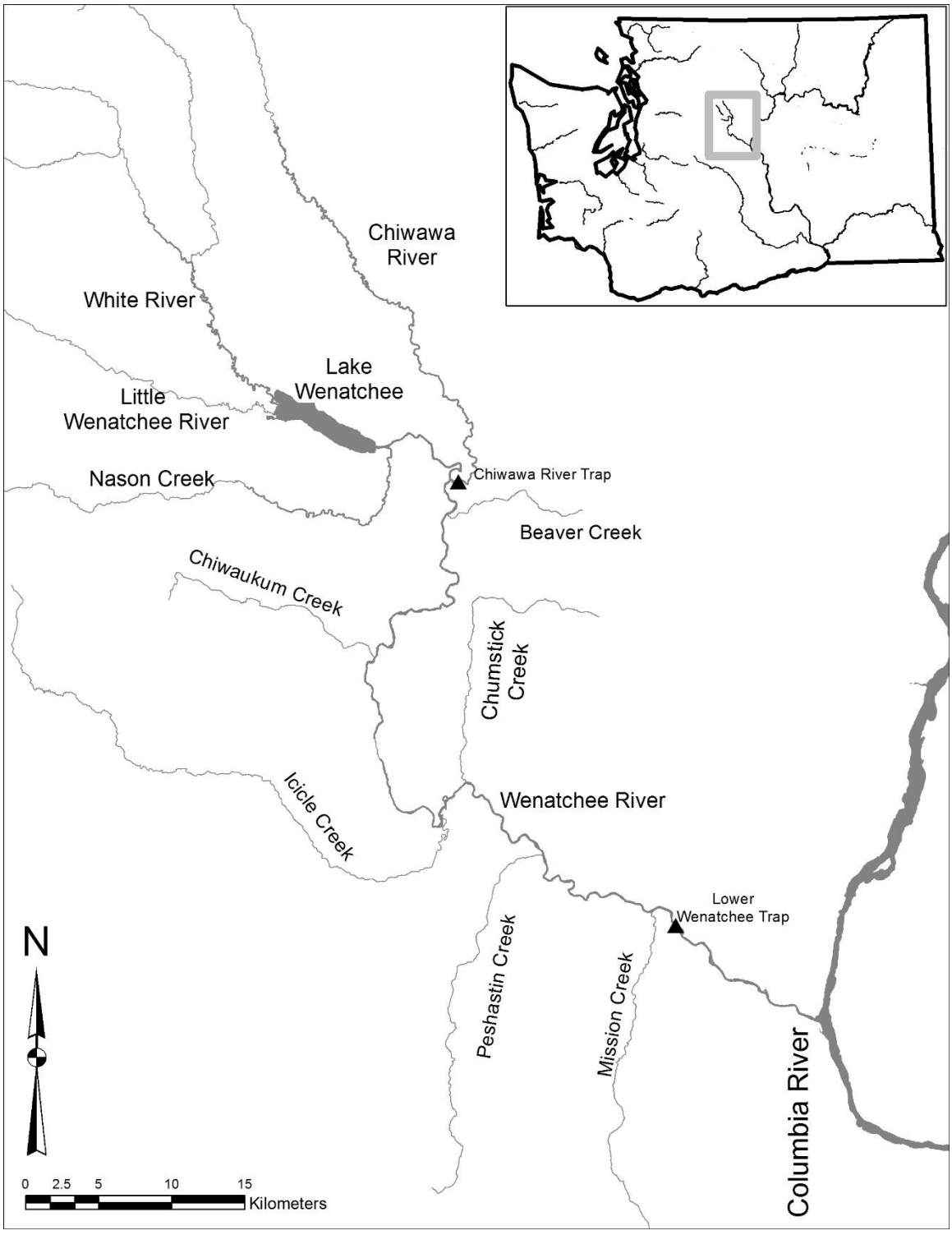

Figure 2. Wenatchee River basin (with rotary smolt trap locations).

Wenatchee River

The Wenatchee River is a fourth-order river draining a 3,437-km^2 basin and has a mean annual discharge of 91.4 m^3/s. The hydrograph is dominated by the snow melt cycle with peak discharge occurring May through July with occasional fall freshets (Figure 3). The mainstem originates at the outlet of Lake Wenatchee and flows southeast 84.5 km before joining the Columbia River, 753 km upstream of the Pacific Ocean (Figure 2). While most of the lowlands (17%) are private, the majority (83%) of basin is public land.

Precipitation in the basin varies from 22 cm near the Columbia River confluence to 381 cm at the crest of the Cascade Mountains with elevations ranging from 237 to 2,768 m. The Wenatchee River has a relatively low gradient except from rkm 40 – 64 where the river flows through a bedrock canyon (Tumwater Canyon) and has a gradient of approximately 9.8 meters per kilometer.

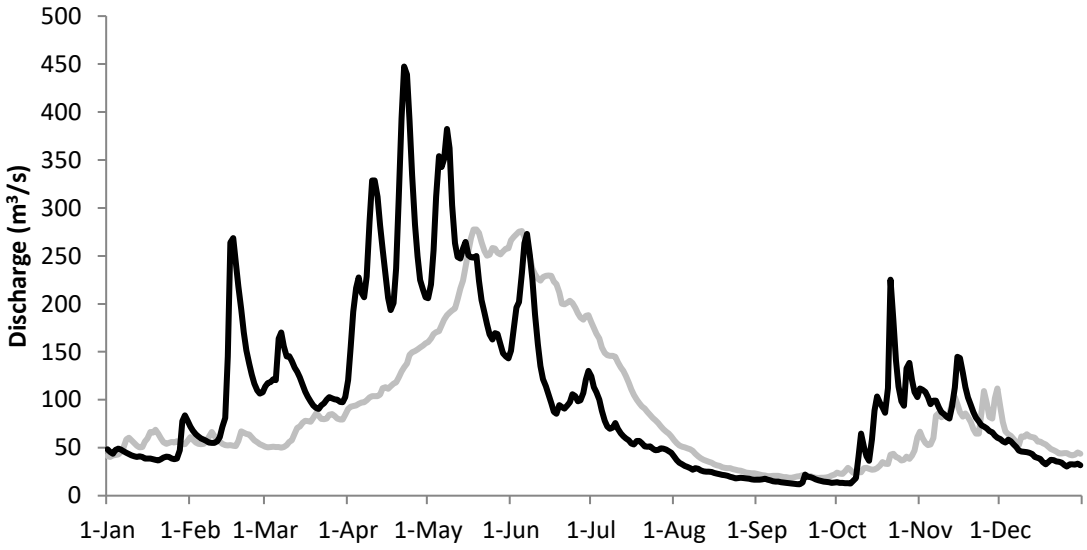

Figure 3. Discharge of the Wenatchee River at Monitor, USGS gauge # 12462500. Black line represents 2016 discharge and grey line represents mean discharge from 1990-2015.

METHODS

Rotary Smolt Traps

Trap Operations

The Chiwawa River trap consists of a single 2.4m cone and has been operating since 1991 at its current location, 0.6 km upstream from the confluence with the Wenatchee River. Trap operations usually begin in late February and continue until ice suspends operations in late fall. The Lower Wenatchee trap consists of two 2.4m cones and has been operating in its current location (rkm 12.5) since 2013. Trap operations usually begin in late January and continue until fall, when river conditions force its removal.

Operational procedures and techniques follow the standardized basin-wide monitoring plan developed by the Upper Columbia Regional Technical Team for the Upper Columbia Salmon Recovery Board (UCSRB; Hillman 2004), which was adapted from Murdoch and Petersen (2000). The traps remain in operation 24 hours a day unless environmental condition (high/low flow, extreme temperature, and high debris), hatchery releases, mechanical failure or human recreational activities halt operations. During periods of high recreational activities in the spring and summer the Lower Wenatchee trap is pulled during daylight hours to minimize human danger.

Fish Sampling

At a minimum of once a day, all fish collected at the traps were identified to genus or species, enumerated, weighed, and fork length (FL) measured. All salmonids were classified as hatchery, wild, or unknown and visually classified as fry, parr, transitional, or smolt. All hatchery salmonids in the basin are marked (adipose fin-clip, coded-wire tags, or Passive Integrated Transponder (PIT) with the exception of coho. Based on length subsamples of known hatchery coho at Leavenworth Fish Hatchery, all coho collected at the Lower Wenatchee rotary smolt trap were considered wild if < 80mm FL or unknown origin if ≥ 80mm FL. All coho collected in the Chiwawa River were considered wild. Target species (≥ 65 mm FL) were tagged using 12.5 mm FDX PIT tags and all PIT tagging information was uploaded to a reginal PIT tag database (PTAGIS) maintained by the Pacific States Marine Fisheries Commission.

A combination of length, time of year, and trap location was used to determine race (spring or summer) of captured juvenile Chinook Salmon. All Chinook Salmon captured in the Chiwawa River trap were considered spring Chinook, regardless of size since summer Chinook Salmon spawning has not been documented upstream of the trap. All yearling (age-1) Chinook captured at the Lower Wenatchee River trap during the spring migration period were considered spring Chinook Salmon because spring Chinook Salmon are yearling migrants and summer Chinook Salmon are typically subyearling migrants. All subyearling fry and parr (age-0) Chinook captured at the Lower Wenatchee River trap during spring were considered summer Chinook Salmon.

Mark–Recapture Trials

Groups of marked juveniles were released during a range of stream discharges in order to determine trapping efficiencies under the varied flow regime. Natural origin fish were marked with a PIT tag if ≥65mm FL or stained with Bismarck Brown dye if <65 mm FL and hatchery origin fish were marked using a caudal fin clip. All marked fish were released evenly upstream on both sides of the river between 1800 hours and 2000 hours. Marked fish from the Lower Wenatchee River trap were transported and released 14.5 km upstream of the trap site while fish from the Chiwawa River trap were released 2.6 km upstream. Each trial was conducted over a four-day (96 hour) period to allow time for passage or capture. Target mark group sizes were based on historical data, location and species, ranging from 100 to over 500 individual fish. See appendix D for mark-recapture trails.

Emigrant Estimates

All emigration estimates were calculated using estimated daily trap efficiency derived from the regression formula using trap efficiency (dependent variable) and discharge (independent variable). Trap efficiency models used a modified Bailey estimator (recaptures + 1) in the calculation of efficiency as a method of bias correction. If a significant relationship ($R^2 > 0.5$ and $P < 0.05$) could not be found a pooled trap efficiency estimate was used. Estimates of emigrating spring Chinook were calculated with and without fry (<50mm FL) due to the uncertainty that these fish were actively migrating to the ocean (UCRTT, 2001). See appendices A and B for detailed equations and information on how the point estimate, variance, and standard error were calculated.

During minor breaks in operation (less than seven days), the number of individual fish collected was estimated. This estimate was calculated using the mean number of fish captured two days prior and two days after the break in operation. For major breaks in operations (greater than seven days), an estimate based on historical run timing was developed. This estimate of daily capture was incorporated into the overall emigration estimate.

Egg-to-emigrant Survival

The estimated total egg deposition (d) was calculated by multiplying the mean fecundity (f) of the brood spawners by the total number of redds (r) found during surveys (Hillman et al. 2015). Egg-to-emigrant survival (s) was calculated by dividing total emigrants (e) by estimated egg deposition (d).

Backpack Electrofishing

Sampling Procedure

From 2012 to present, WDFW has had a goal of PIT tagging 3,000 juvenile spring Chinook Salmon each year. In order to representatively tag the population throughout all reaches, the number of fish tagged in each reach was based on the reach specific abundance encountered during snorkeling surveys in late summer. See Appendix C for further explanation.

Detections and Calculations

Detections occur at PIT tag interrogation sites in and out of the basin as well as rotary smolt traps downstream of the sampling reaches. Calculations of non-trapping emigrant estimates are based on a flow-detection efficiency regression developed using mark-groups previously released to test smolt trap efficiencies. The total number of tagged fish (t) divided by the estimated total parr abundance (p), as based off of standard snorkeling techniques (Hillman et al. 2013), resulted in an overall tag rate (t_i). See Appendix C for further explanation.

RESULTS

Rotary Smolt Traps – Chiwawa

Trap Operation

The Chiwawa trap operated between 2 March and 21 November 2016. During that time the trap was inoperable for 72 days as a result of low or high discharge, debris, hatchery fish releases, and mechanical issues. Forty seven of those days came during the fall when there was not enough discharge to operate the trap. Throughout the year the trap was operated in a single upper position.

Fish Sampling

A total of 27,172 individual fish were collected, with wild spring Chinook Salmon and steelhead comprising 71% and 6% of the total catch, respectively. Additionally, 2,525 hatchery spring Chinook, 1,518 hatchery steelhead, and 3 wild coho were collected. Throughout the sampling period 11,396 PIT tag were deployed into wild spring Chinook and steelhead (10,083 and 1,313 respectively). Spring Chinook mortality for the season totaled 4 yearling, 74 subyearling parr, and 15 fry (0.1%, 0.6%, and 0.4%, respectively). Mortality of steelhead throughout the season totaled 10 (0.6%). The mean fork length (SD) of captured yearling and subyearling spring Chinook Salmon (fry excluded) was 91 (8.5) mm and 71 (12.78) mm, respectively (Table 1).

Table 1. Mean fork length (mm) and weight (g) of spring Chinook Salmon captured in the Chiwawa rotary smolt trap during 2016.

	Yearling transitional/smolts			Subyearling parr		
	Mean	SD	*N*	Mean	SD	*N*
Fork length	91.3	8.5	2,789	71.1	12.8	12,198
Weight	8.3	3.1	2,784	4.7	2.2	10,947

Yearling Spring Chinook (Brood Year 2014)

Wild yearling spring Chinook Salmon were primarily captured between 2 March and 31 May (Figure. 4). A total of 2,807 yearling Chinook Salmon were captured and an estimated 3,414 would have been captured if the trap had operated without interruption. Six mark/recapture efficiency trials using PIT tags were conducted producing a mean trap efficiency of 9.4%. In 2016, mark/recapture trials were conducted at all desired discharge levels and a statistically

significant flow-efficiency regression model was obtained (R^2 = 0.84, P < 0.028). The estimated number (95% C.I.) of yearling spring Chinook Salmon that emigrated from the Chiwawa River in 2015 was 37,170 (±6,524). Smolt survival (SE) to McNary of those tagged fish was 43% (5%) using the Cormack-Jolly-Seber estimator.

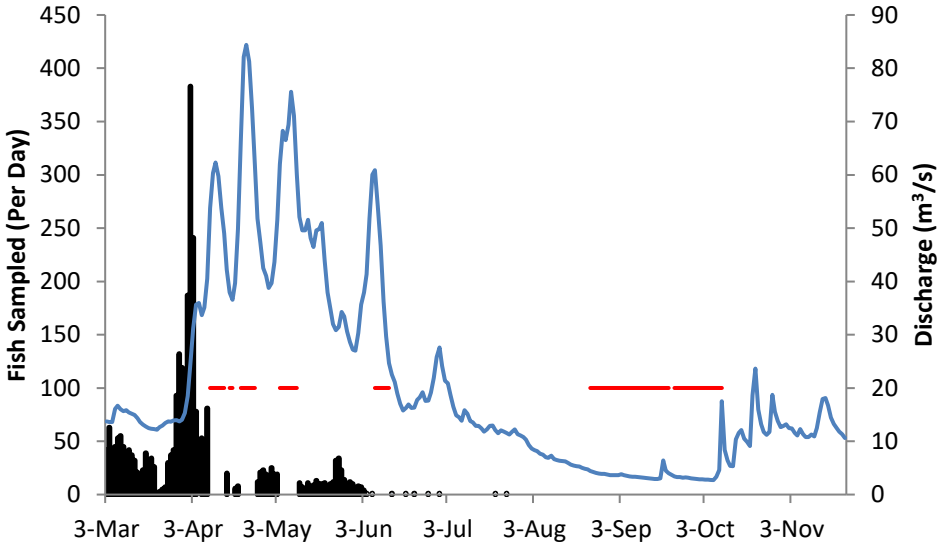

Figure 4. Daily catch of yearling spring Chinook Salmon at the Chiwawa rotary smolt trap. Blue line indicates river discharge and red horizontal line indicates non-trapping period.

Subyearling Spring Chinook (Brood Year 2015)

Wild subyearling spring Chinook Salmon were captured throughout the sampling period, with peak catches of parr in August, October, and November and fry occurring in March and April (Figures 5 and 6, respectively). A total of 12,429 subyearling parr and 3,835 fry were captured with an estimated 13,319 subyearling parr and 4,063 fry had the trap operated without interruption. Twelve mark/recapture efficiency trials were conducted (eight PIT tagged and four Bismarck Brown groups) with a mean trap efficiency of 19.1%. These 12 trials were used to develop a significant regression model for the trap (R^2 = 0.64, P < 0.002). In 2016, the estimated number of subyearling spring Chinook Salmon emigrating from the Chiwawa River during the sampling period was 80,543 (± 27,967) if you do not include fry or 145,971 (±48,393) if fry are included.

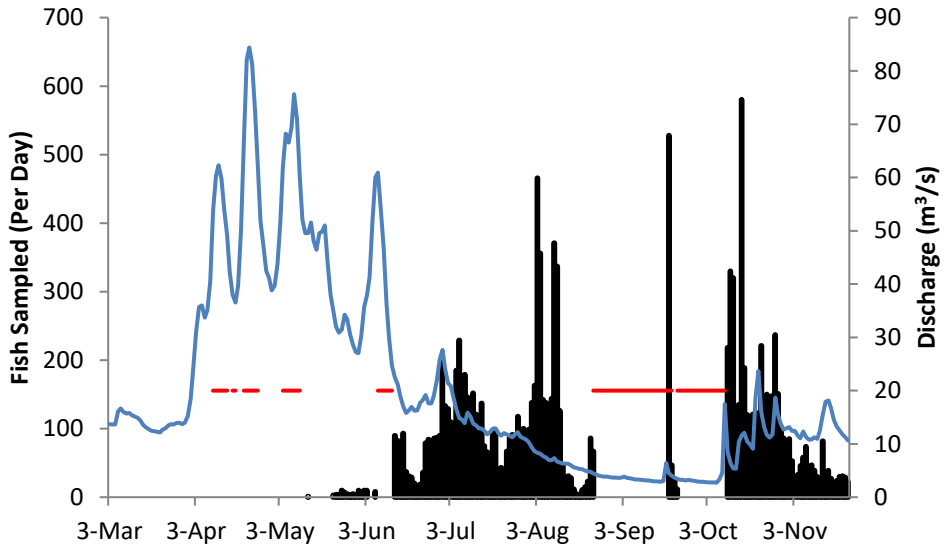

Figure 5. Daily catch of wild spring Chinook subyearling parr at the Chiwawa rotary smolt trap. Blue line indicates river discharge and red horizontal line indicates non-trapping period.

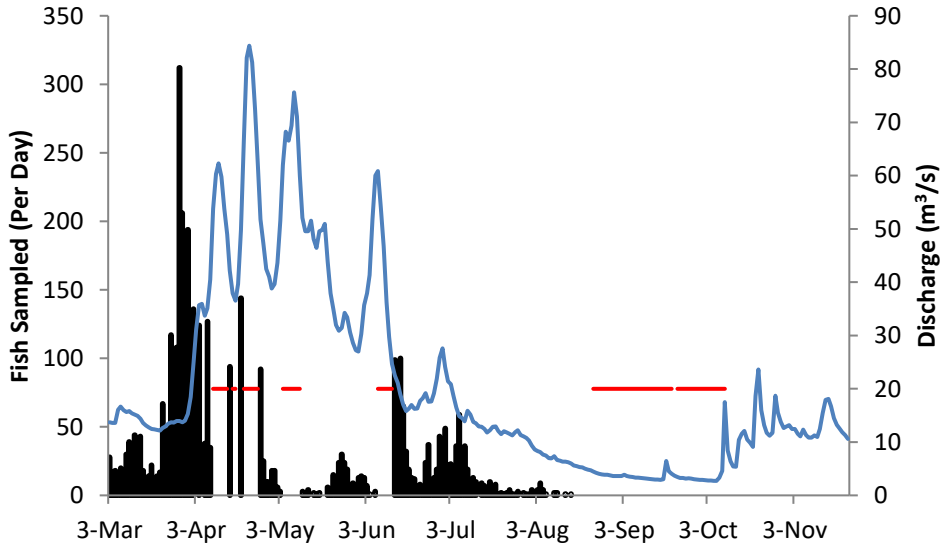

Figure 6. Daily catch of wild spring Chinook fry at the Chiwawa rotary smolt trap. Blue line indicates river discharge and red horizontal line indicates non-trapping period.

Summer Steelhead

During the trapping period, 195 steelhead transitional/smolts and 1,522 steelhead/rainbow parr and fry were captured. While collections occurred in moderate numbers throughout the year, peak collections occurred during September and October (Figure 7). The mean fork length (SD) of steelhead parr and transitional/smolts captured was 83.6 (23.1) and 146.7 (33.4) mm, respectively (Table 2).

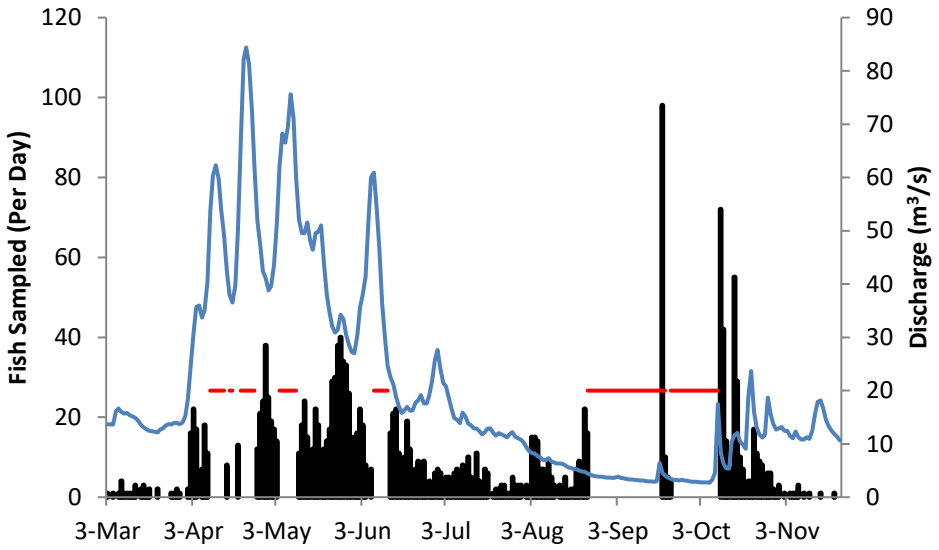

Figure 7. Daily catch of all wild steelhead at the Chiwawa rotary smolt trap. Blue line indicates river discharge and red horizontal line indicates non-trapping period.

Table 2. Mean fork length (mm) and weight (g) and of steelhead/rainbow captured in the Chiwawa rotary smolt trap during 2016.

	Transitional/smolts			Parr		
	Mean	SD	*N*	Mean	SD	*N*
Fork length	146.7	33.4	195	83.6	23.1	1,406
Weight	37.3	23.7	194	7.8	9.4	1,393

Egg-to-emigrant Survival

For BY 2014, 485 redds were counted in the Chiwawa River Basin with an estimated 1,961,825 eggs being deposited. A total of 114,680 emigrants were estimated resulting in an egg-to-emigrant survival of 5.8% (Table 3). This is up from a five year moving average of 3.8%.

Table 3. Estimated egg deposition and egg-to-emigrant survival rates for Chiwawa River spring Chinook Salmon.

Brood Year	Number of redds	Estimated egg deposition	Estimated number				Egg-to-emigrant survival (%)
			Sub-yearling	Non trapping	Yearling	Total emigrants	
1992	302	1,570,098	25,818		39,723	65,541	4.2
1993	106	556,394	14,036		8,662	22,698	4.1
1994	82	485,686	8,595		16,472	25,067	5.2
1995	13	66,248	2,121		3,830	5,951	9.0
1996	23	106,835	3,708		15,475	19,183	18.0
1997	82	374,740	16,228		28,334	44,562	11.9

Brood Year	Number of redds	Estimated egg deposition	Estimated number				Egg-to-emigrant survival (%)
			Sub-yearling	Non trapping	Yearling	Total emigrants	
1998	41	207,675	2,855		23,068	25,923	11.9
1999	34	166,090	4,988		10,661	15,649	9.4
2000	128	642,944	14,854		40,831	55,685	8.7
2001	1,078	4,836,704	459,784		86,482	546,266	11.0
2002	345	1,605,630	93,331		90,948	184,279	11.5
2003	111	648,684	16,881		16,755	33,637	5.2
2004	241	1,156,559	44,079		72,080	116,158	10.0
2005	333	1,436,564	108,595		69,064	177,659	12.3
2006	297	1,284,228	62,922		45,050	107,972	8.4
2007	283	1,241,521	60,196		25,809	86,006	6.9
2008	689	3,163,199	85,161		35,023	120,184	3.8
2009	421	1,925,233	30,996		30,959	61,955	3.2
2010[a]	502	2,165,628	53,619		47,511	101,130	4.7
2011[a]	492	2,157,420	67,982	3,665	37,185	108,832	5.0
2012[a]	880	3,716,240	49,774	25,305	34,334	109,413	2.9
2013[a]	714	3,367,224	73,695	NA	39,396	113,091	3.4
2014[a]	485	1,961,825	77,510	NA	37,170	114,680	5.8
2015[a]	312	1,372,800	80,543	--	--	--	--

[a]calculated with Bailey model

Non-target Taxa

Bull trout (*Salvelinus confluentus*) also comprised a large proportion of incidental species captured. During the trapping period 118 bull trout (15 ≥ 300 mm FL and 103 <300 mm FL) were captured. Additionally, 43 westslope cutthroat trout (*O. clarki lewisi*), and three Eastern brook trout (*S. fontinalis*) were collected. In all, 109 bull trout and 41 westslope cutthroat trout were released with PIT tags. Monthly and annual totals of all fish captured are presented in Appendix E and Appendix F, respectively.

Rotary Smolt Traps – Lower Wenatchee

Trap Operation

The Lower Wenatchee trap operated from 29 January through 26 July 2016. During this time the trap was inoperable for a total of 23 days due to high/low flows, high temperatures, heavy debris, major hatchery releases, and mechanical issues. Extreme river temperatures and low flows resulted in trapping operations being suspended for the season on 26 July. Throughout the season, the trap cones were operated in a single lower position.

Fish Sampling

A total of 43,685 individual fish were collected, with wild summer Chinook Salmon comprising 89% of the total catch. Additionally, 610 wild yearling spring Chinook Salmon, 7,701 hatchery yearling Chinook Salmon, 1,346 wild sockeye, 417 wild steelhead, and 259 hatchery steelhead were captured. Throughout the sampling period 567, 1,065, and 131 PIT tag were deployed into wild yearling spring Chinook, sockeye, and steelhead, respectively. Mortality for the season totaled 2 yearling spring Chinook, 184 subyearling summer Chinook, 63 sockeye, and 6 steelhead (0.3%, 0.7%, 4.7%, and 1.4%, respectively).

Wild Yearling Spring Chinook (Brood Year 2014)

Wild yearling spring Chinook Salmon were primarily captured in February and March (Figure 8). Throughout the trapping period 610 spring Chinook were collected and an estimated 708 would have been collected had the trap operated without interruption. A combination of 2013, 2014, and 2015 trials were used to develop a significant relationship between discharge and trap efficiency (R^2 = 0.62, P = 0.02). This model was used to calculate an emigrant estimate of 36,752 (±5,330). The mean fork length (SD) of captured yearling Chinook was 94 (9.4) mm (Table 4).

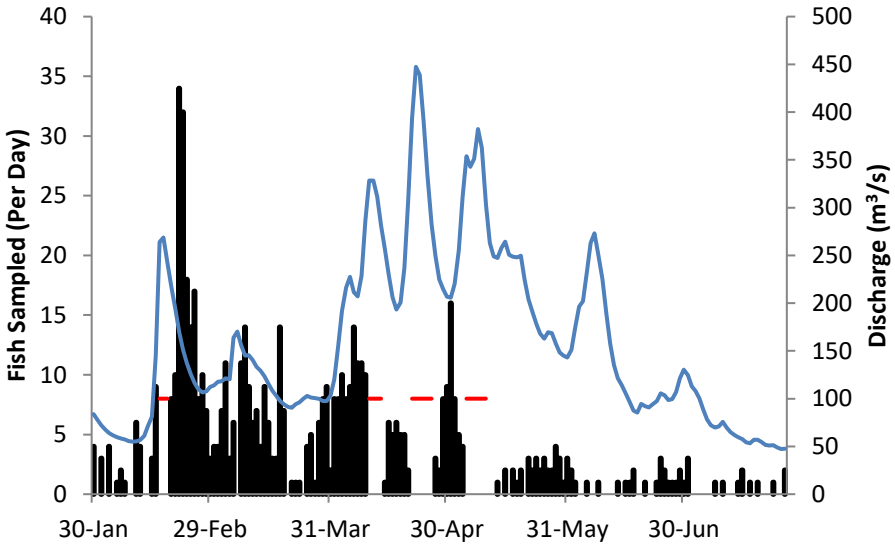

Figure 8. Daily capture of wild yearling Chinook Salmon at the Lower Wenatchee rotary smolt trap. Blue line indicates river discharge and red horizontal line indicates non-trapping period.

Table 4. Mean fork length (mm) and weight (g) for wild yearling spring Chinook Salmon sampled at the Lower Wenatchee rotary trap during 2016.

	Mean	SD	N
Fork length	94	9.4	600
Weight	9.0	2.9	598

Wild Subyearling Summer Chinook (Brood Year 2015)

Wild subyearling summer Chinook dominated the catch (63%) with 27,407 fish being processed, most being collected in April and May (Figure 9). An estimated 35,815 would have been captured had the trap operated without interruption. Over the season, four mark/recapture efficiency trials were carried out using Bismarck Brown dye. When combined with trials from 2014 and 2015 a significant discharge efficiency relationship was developed ($R^2 = 0.56$, $P < 0.001$) and an emigrant estimate (95% C.I.) of 4,023,310 (±676,633) was calculated. The mean fork length (SD) for captured subyearling parr and fry summer Chinook was 64 (10.1) and 40 (3.7), respectively (Table 5). Over the sampling period 18 PIT tags were deployed in summer Chinook.

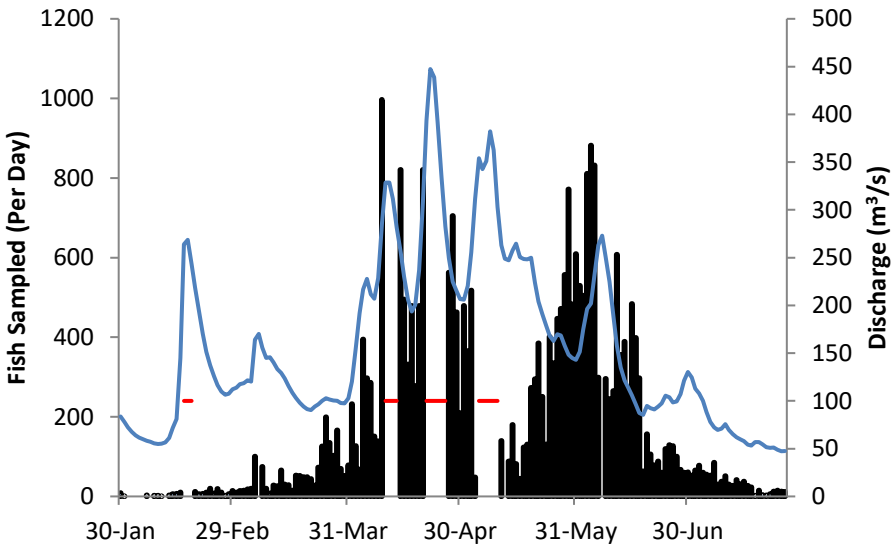

Figure 9. Daily capture of wild summer Chinook Salmon at the Lower Wenatchee rotary smolt trap. Blue line indicates river discharge and red horizontal line indicates non-trapping period.

Table 5. Mean fork length (mm) and weight (g) of subyearling summer Chinook Salmon sampled at the Lower Wenatchee rotary smolt trap.

	Transition / Smolt			Parr			Fry		
	Mean	SD	N	Mean	SD	N	Mean	SD	N
Fork length	82.8	7.3	216	64.1	10.1	2,799	40.9	3.7	3,143
Weight	6.4	1.8	216	3.1	1.6	2,778	0.6	0.3	3,005

Wild Sockeye

A total of 1,346 juvenile sockeye were collected in the 2016 season and an estimated 1,916 had the trap operated without interruption. Almost all of these fish (84%) were collected in April (Figure 10). No mark/recapture efficiency trials were carried out due to mechanical issues during the peak of the run. Mark/recapture efficiency trials from the 2013, 2014, and 2015 seasons created a significant discharge efficiency model ($R^2 = 0.52$, $P < 0.043$). This model

produced a 2016 emigrant population estimate (95% C.I.) for juvenile sockeye at 208,250 (±29,447). Smolt survival (SE) to McNary of those tagged fish was 26% (5%) using the Cormack-Jolly-Seber estimator. In 2016, while most were Age 1+ (78%), we saw a large jump in Age 2+ (22%) when compared to 2014 and 2013 (Table 6). Mean fork length (SD) for captured sockeye was 81 (12.1) mm (Table 7).

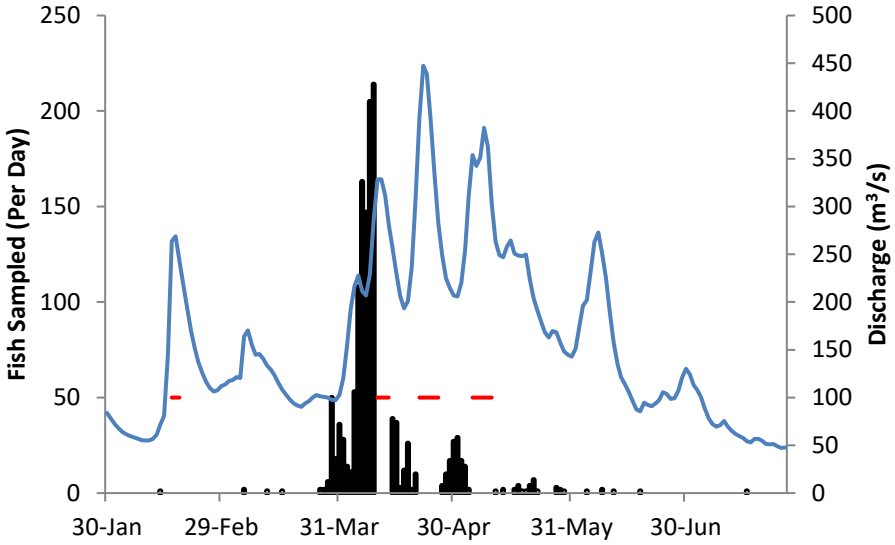

Figure 10. Daily capture of wild sockeye Salmon at the Lower Wenatchee rotary smolt trap. Blue line indicates river discharge and red horizontal line indicates non-trapping period.

Table 6. Age structure and estimated number of wild sockeye smolts that emigrated from Lake Wenatchee in 2013-2015.

Run year	Proportion of Wild Smolts			Total Wild Smolts
	Age 1+	Age 2+	Age 3+	
2013	0.932	0.068	0.00	873,096
2014	0.924	0.076	0.00	1,275,027
2015	0.780	0.220	0.00	1,065,614
2016	NA	NA	NA	208,250

Table 7. Mean fork length (mm) and weight (g) of wild sockeye Salmon smolts sampled at the Lower Wenatchee rotary smolt trap.

	Mean	SD	N
Fork length	81.0	12.1	1,164
Weight	4.7	2.9	1,147

Wild Summer Steelhead

Capture of wild steelhead at the Lower Wenatchee site for all life stages was low, totaling 417 fry, parr, and smolts combined and an estimated 505 collected had the trap operated without

interruption. Peak catches of steelhead occurred in July (Figure 11). One mark/recapture trial was conducted using hatchery steelhead transitional/smolts in 2016. When combined with two trials using hatchery steelhead transitional/smolts 2014 a pooled efficiency of 0.028 was used to estimate (95% C.I.) the emigrant population (no fry) at 10,135 (±102,145) parr and smolt emigrant steelhead. If you include fry, the emigrant population was estimated at 18,400 (± 185,447). However, due to the low number of trials, small sample sizes, use of hatchery transitional/smolts surrogates and the relationship not being significant, caution should be used in the interpretation and use of the estimate. Mean length (SE) of transitional/smolts and parr was 159 (29.6) and 83 (24.0) mm, respectively (Table 8).

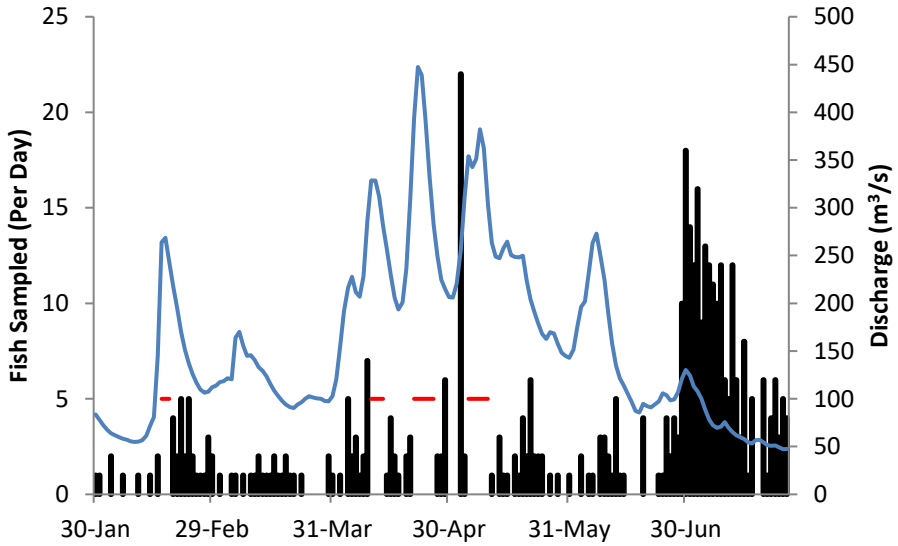

Figure 11. Daily capture of wild steelhead at the Lower Wenatchee rotary smolt trap. Blue line indicates river discharge and red horizontal line indicates non-trapping period.

Table 8. Mean fork length (mm) and weight (g) of wild steelhead sampled at the Lower Wenatchee rotary smolt trap.

	Transitional/Smolt			Parr		
	Mean	SD	N	Mean	SD	N
Fork length	159.4	29.6	66	83.1	24.0	102
Weight	45.7	27.4	66	7.7	6.6	99

Survival

For BY 2014, 885 spring Chinook Salmon redds were surveyed in the Wenatchee Basin producing an estimated 3,894,000 eggs. An estimate of 36,752 emigrants results in an estimated egg-to-emigrant survival of 0.94%. This is down from the last three year average of 1.45% (Table 9).

Table 9. Estimated egg deposition and egg-to-smolt survival rates for Wenatchee Basin spring

Chinook Salmon.

Brood Year	Number of redds	Estimated egg deposition	Estimated number	
			Total emigrants	Egg-to-emigrant survival (%)
2000	350	1,758,050	76,643	4.36
2001	1,876	8,674,624	243,516	2.81
2002	1,139	5,300,906	165,116	3.11
2003	323	1,887,612	70,738	3.75
2004	555	2,663,445	55,619	2.09
2005	829	3,587,083	302,116	8.42
2006	588	2,542,512	85,558	3.37
2007	466	2,069,506	60,219	2.91
2008	1,411	6,479,312	82,137	1.27
2009	--	--	--	--
2010	--	--	--	--
2011	872	3,823,720	89,917	2.35
2012	1,704	7,195,992	67,973	0.94
2013	1,159	5,512,204	58,595	1.06
2014	885	3,894,000	36,752	0.94

For BY 2015, 2,725 summer Chinook Salmon redds were surveyed in the Wenatchee Basin, 95.8% being upstream of the Lower Wenatchee smolt trap. After extrapolating by the proportion of redds above the trap a total emigrant population of 4,023,310 was estimated resulting in an egg-to-emigrant survival of 36.55%. This is down from the last three year average of 83.54% (Table 10).

Table 10. Estimated egg deposition and egg-to-emigrant survival rates for Wenatchee Basin summer Chinook Salmon.

Brood year	Peak total redd expansion	Estimated egg deposition	Redds above trap / total redds	Estimated number		
				Trap estimate	Total emigrants	Egg-to-emigrant survival (%)
1999	2,738	13,654,406	0.988	9,572,392	9,685,591	70.93
2000	2,540	13,820,140	0.983	1,299,476	1,322,383	9.57
2001	3,550	18,094,350	0.987	8,229,920	8,340,342	46.09
2002	6,836	37,488,624	0.977	13,167,855	13,475,368	35.95
2003	5,268	28,241,748	0.996	20,336,968	20,426,149	72.33
2004	4,874	26,207,498	0.989	14,764,141	14,935,745	56.99
2005	3,538	17,877,514	0.993	11,612,939	11,695,581	65.42
2006	8,896	45,663,168	0.979	9,397,044	9,595,512	21.01

Brood year	Peak total redd expansion	Estimated egg deposition	Redds above trap / total redds	Estimated number		
				Trap estimate	Total emigrants	Egg-to-emigrant survival (%)
2007	1,970	10,076,550	0.983	4,470,672	4,546,838	45.12
2008	2,800	14,302,400	0.978	4,309,496	4,405,473	30.8
2009	3,441	18,206,331	0.983	6,695,977	6,814,805	37.43
2010	3,261	16,184,343	0.957	--	--	--
2011	3,078	15,122,214	0.958	--	--	--
2012	2,504	12,021,704	0.93	9,333,214	10,034,508	83.47
2013	3,241	16,162,867	0.947	11,936,928	12,605,925	77.99
2014	3,458	16,556,904	0.959	14,157,778	14,763,064	89.17
2015	2,725	11,491,325	0.958	4,023,310	4,199,697	36.55

Non-target Taxa

No westslope cutthroat trout or bull trout where sampled at the Lower Wenatchee Trap. No PIT tags were applied to non-target taxa. Monthly and annual totals of all fish captured are presented in Appendix G and Appendix H, respectively.

Backpack Electrofishing

Fish Sampling

Between 19 October and 12 November 2015, WDFW personnel sampled the Chiwawa River for a total of 36,782 seconds. During this sampling, a total of 1,103 subyearling Chinook were collected of which 1,054 received a PIT tag. The greatest concentration of juvenile Chinook occurred between rkm 31 and 45 which had a mean sample rate of one Chinook collected for every 24 seconds of sampling. Over the sample period 20 Chinook died resulting in a mortality rate of 1.8%. Additionally, 63 juvenile bull trout were collected and 43 received a PIT tag. Highest catch rates for bull trout were around rkm 47. No mortality was observed for bull trout.

Detections and Calculations

Between the non-trapping season of 25 November 2015 through 1 March 2016, a total of three detections of remotely tagged Chinook were recorded at the lower Chiwawa antenna array. During the 2015 fall (19 October through 24 November) and 2016 spring trapping season (2 March and 30 June), the Chiwawa rotary smolt trap collected 29 and 26 remotely tagged Chinook, respectively. Due to relatively low sample size and poor detection rates at the Chiwawa antenna no emigrant estimate for the non-trapping period was calculated for the BY 2014.

DISCUSSION

Chiwawa River Rotary Smolt Trap

Over the last five years the Chiwawa River smolt trap has had an average installation date of 1 March. With a relatively normal winter the smolt trap was installed on 2 March. However the spring proved to be one of the warmest leading to a record high discharge for much of the spring and very low flows in the fall. In the spring the trap was pulled due to high flow/debris for 22 days and in the fall it was pulled for 47 days due to low flow.

Floods in the fall of 2015 – spring 2016 also caused the substrate to sift and altered the range of flows the Chiwawa River rotary smolt trap is considered operable. New discharge limits are estimated to be between 4.5 and 55.2 m^3/s. For the 2017 field season we will adjust our methodology to allow for sampling during low discharge levels by replacing our 2.4 m smolt trap with a 1.5 m smolt trap as needed.

Due to the assumed change in trap efficiencies associated with a single cone positions and altered substrate new trap efficiency models were developed for subyearling and yearling Chinook. However, a continued reliance upon historic mark/recapture trials for steelhead had to be used. This model will continue to be improved and updated as conditions allow. Historically, emigrant estimates were calculated using the Peterson estimator of abundance (Seber 1982), however more accurate estimates currently utilize a modified Bailey estimator (Murdoch et al. 2012).

The total production estimate for brood year 2014 was 114,680 and comprises estimates of subyearling emigrants in 2015 and yearling emigrants in 2016. Unfortunately, high flows, low antenna detections, and concerns related to spawning bull trout resulted in an abbreviated sampling window and prevented the completion of 2015 remote tagging efforts. This resulted in no estimate being calculated for the 2015 non-trapping season and a known underestimate of the total brood year production. Protocols and field sampling will be continually adapted to fit within environmental and permit constraints and estimates will be improved upon when possible.

Due to the large fall break in trapping historic run timing was used to extrapolate what the catch would have been had the trap been able to operate without interruption. It was estimated 6.5% of subyearling Chinook emigrated during this fall break in trapping so our subyearling Chinook emigrant estimate was adjusted accordingly.

The 2016 field season represented the first year the smolt trap operated with a single cone position. This allowed for a single model to be developed for each life stage and species regardless of when it emigrated, thus removing bias and improving our estimates for subyearling and yearling Chinook. In 2017 we will continue to develop and modify our mark/recapture models paying particular attention to improving our steelhead model.

Lower Wenatchee River Rotary Smolt Trap

Historically, the smolt trap on the mainstem Wenatchee River has moved location numerous

times due to poor trap efficiencies of target species and environmental factors causing abbreviated trapping seasons. At the lower Wenatchee site, the smolt trap has been able to operate into September in 2013 and October in 2014. This marks a relatively large increase in operational length over the old site (located 2.5 km downstream) which had an average trap removal date of 14 August. However, since 2014 river discharge and water temperatures have hampered the trapping season for the Lower Wenatchee trap. At this site, the trap is considered operable between discharges of 36.8 and 283.2 m^3/s. In 2016, record high spring discharge resulted in the trap being pulled for 19 days, mostly in April and May. Complicating things further, river temperatures exceeded starting 20°C starting 27 July and trapping operations were again suspended. River temperatures remained elevated and low flow persisted through summer and on 19 August the decision was made to remove the smolt trap. Additionally, mechanical issues hindered catch totals and subsequent emigrant estimates. This was particularly evident when mechanical issues led to only one cone being operable for five days during the peak sockeye emigration. This caused a known underestimate of total catch and emigrant estimate. Overall however, river discharge and temperature continue to be the main issues that impact our trapping season. Adaptive management will be use to ensure maximum efficiency and number of days trapping.

Significant discharge efficiency models were obtained for three of the four target species at the Lower Wenatchee trap during the 2016 trapping season (wild spring and summer Chinook Salmon and sockeye Salmon). Collections of wild steelhead continue to be inadequate for conducting mark–recapture trials. In 2017, hatchery steelhead from the Chiwawa acclimation site will be used in mark/recapture trials in an effort to improve emigrant estimates of this target species. This approach requires the assumption that hatchery fish behave in a similar manner to wild fish, an assumption we will test over time as possible. While the new trap location has allowed for greater operational flexibility, it does require the development of new flow-efficiency models. While this can be accomplished relatively quickly with species that are relatively abundant (e.g., summer Chinook and sockeye), it may take several years for those in low abundance (e.g., steelhead). Fortunately, given similar operation parameters across time, we will be able to reexamine past abundance estimates when those models are fully developed.

Backpack Electrofishing

Remote sampling in the Chiwawa Basin started in 2012. Some success occurred early with PIT tag targets being met, however, there have been substantial obstacles since 2013. Permit restrictions limit field operations until bull trout spawning has concluded; which typically occurs early October. At this time, weather becomes increasingly unfavorable and elevated discharge along with cold air and water temperatures hinder sampling efforts. Since 2014, early high water events hindered sampling efforts and limited not only the area that was sampled, but also the number of fish that were processed. Future investigations will look into alternative sampling techniques and the allocation of personnel to maximize sampling efforts in the basin.

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APPENDICES

Appendix A. Peterson Population and Variance Equations.

Trap efficiency was calculated using the following formula:

$$\text{Trap efficiency} = E_i = R / Mi,$$

Where E_i is the trap efficiency during time period *i*; M_i is the number of marked fish released during time period *i*; and R_i is the number of marked fish recaptured during time period *i*. The number of fish captured was expanded by the estimated daily trap efficiency (*e*) to estimate the daily number of fish migrating past the trap using the following formula:

$$\text{Estimated daily migration} = \hat{N}_i = C_i / \hat{e}_i$$

where N_i is the estimated number of fish passing the trap during time period *i*; C_i is the number of unmarked fish captured during time period *i;* and e_i is the estimated trap efficiency for time period *i* based on the regression equation.

The variance for the total daily number of fish migrating past the trap was calculated using the following formulas:

$$\text{Variance of daily migration estimate} = \text{var}\left[\hat{N}_i\right] = \hat{N}_i^2 \frac{\text{MSE}\left(1 + \frac{1}{n} + \frac{(X_i - \overline{X})^2}{(n-1)s_X^2}\right)}{\hat{e}_i^2}$$

where X_i is the discharge for time period *i*, and *n* is the sample size. If a relationship between discharge and trap efficiency was not present (i.e., $P < 0.05$; r^2 ?0.5), a pooled trap efficiency was used to estimate daily emigration:

$$\text{Pooled trap efficiency} = e_p = \sum R / \sum M$$

The daily emigration estimate was calculated using the formula:

$$\text{Daily emigration estimate} = \hat{N}_i = C_i / e_p$$

The variance for daily emigration estimates using the pooled trap efficiency was calculated using the formula:

Variance for daily emigration estimate = $$\mathrm{var}\left[\hat{N}_i\right] = \hat{N}_i^2 \frac{e_p(1-e_p)/\sum M}{e_p^2}$$

The total emigration estimate and confidence interval was calculated using the following formulas:

Total emigration estimate = $$\sum \hat{N}_i$$

95% confidence interval = $$1.96 \times \sqrt{\sum \mathrm{var}\left[\hat{N}_i\right]}$$

Appendix B. Bailey Population and Variance Equations.

Trap efficiency was calculated using the following formula:

$$\text{Trap efficiency} = E_i = R+1 \;/\; Mi,$$

$$\text{Estimated daily emigration} = \hat{N}_i = \frac{C_i + 1}{\hat{e}_i}$$

The variance of the total population abundance was calculated as follows:

$$Var\left(\sum_{i=1}^{n} \hat{N}_i\right) = \underbrace{\sum_i Var\left(\frac{(C_i+1)}{\hat{e}_i}\right)}_{Part\ A} + \underbrace{\sum_i \sum_j Cov\left(\frac{(C_i+1)}{\hat{e}_i}, \frac{(C_j+1)}{\hat{e}_j}\right)}_{Part\ B}$$

Part A is the variance of the daily estimates where C_i is the number of fish caught in period *i*, e_i is the estimated trap efficiency for period *i*, and *Cov* is the between day covariance for days that the same linear model is used (part B). For a more details and derivation of Peterson and Bailey estimation methods see Murdoch et al. (2012).

Appendix C. Emigration during non-trapping periods.

A flow-efficiency regression model was developed for the lower Chiwawa River PIT tag interrogation site (CHL) using the same mark/recapture trials used for estimating efficiency at the smolt trap. This CHL model was used to calculate emigration outside of the trapping period by incorporating the tag rate into the Bailey estimator.

$$\text{Estimated daily emigration} = \left(\hat{N}_i = \frac{C_i + 1}{\hat{e}_i} \right) \Big/ t_i$$

Where t_i is equal to the tag rate = $t_i = \frac{t}{p}$

Appendix D: Mark–recapture groups used to developing emigrant estimates. YCW = Yearling spring Chinook wild, YCH = Yearling spring Chinook hatchery, SKW = Sockeye wild, SUCH = summer Chinook wild, SBC = subyearling Chinook wild.

Species	Date	Position	Released	Recaptured	Efficiency (%)	Discharge (m^3/s)
			Lower Wenatchee River rotary smolt trap			
YCW	20-Mar-13	Low	223	5	2.24	88.2
YCW	05-Apr-13	Low	216	4	1.85	211.6
YCW	09-Apr-13	Low	186	3	1.61	187.2
YCW	13-Mar-14	Low	156	2	1.28	121.8
YCW	21-Mar-14	Low	243	4	1.65	102.8
YCW	31-Mar-14	Low	306	9	2.94	82.9
YCW	14-Apr-14	Low	165	4	2.42	127.6
YCH	17-Apr-15	Low	2,045	82	4.01	63.1
SKW	27-Apr-13	Low	565	6	1.06	141.6
SKW	31-Mar-14	Low	322	1	0.31	83.1
SKW	04-Apr-14	Low	599	2	0.33	81.7
SKW	07-Apr-14	Low	633	2	0.32	99.6
SKW	16-Apr-14	Low	591	3	0.51	126.2
SKW	19-Apr-14	Low	385	4	1.04	130.4
SKW	23-Apr-14	Low	504	2	0.40	125.5
SKW	12-Apr-15	Low	540	2	0.37	73.9
SUCH	14-May-14	Low	521	3	0.58	236.4
SUCH	20-May-14	Low	999	5	0.50	289.5
SUCH	27-May-14	Low	1,039	4	0.38	263.3
SUCH	31-May-14	Low	1,129	17	1.51	223.4
SUCH	05-Jun-14	Low	993	3	0.30	287.9
SUCH	08-Jun-14	Low	1,023	5	0.49	259.8
SUCH	16-Jun-14	Low	911	6	0.66	182.2
SUCH	19-Jun-14	Low	960	13	1.35	175.4
SUCH	07-Jul-14	Low	931	13	1.40	153.8
SUCH	11-Jul-14	Low	511	6	1.17	125.0
SUCH	17-Jul-14	Low	407	7	1.72	105.8
SUCH	20-Jul-14	Low	448	4	0.89	91.1
SUCH	24-Jul-14	Low	364	4	1.10	74.4
SUCH	03-Apr-15	Low	540	5	0.93	114.7
SUCH	07-Apr-15	Low	1,170	44	3.76	88.1
SUCH	10-Apr-15	Low	755	13	1.72	76.5

Species	Date	Position	Released	Recaptured	Efficiency (%)	Discharge (m^3/s)
SUCH	23-Apr-15	Low	1,035	17	1.64	99.4
SUCH	22-May-15	Low	974	12	1.23	159.5
SUCH	28-May-15	Low	1,109	3	0.27	164.6
SUCH	25-May-16	Low	1,051	10	0.95	171.5
SUCH	02-Jun-16	Low	1,071	22	2.05	167.6
SUCH	11-Jun-16	Low	685	11	1.61	85.1
		Chiwawa River rotary smolt trap				
YCW	06-Mar-16	Upper	132	15	11.36	14.7
YCW	09-Mar-16	Upper	106	12	11.32	15.8
YCW	12-Mar-16	Upper	126	14	11.11	15.1
YCW	02-Apr-16	Upper	178	11	6.18	22.7
YCW	04-Apr-16	Upper	240	13	5.42	34.4
SBC	16-Jun-16	Upper	265	21	7.92	17.6
SBC	26-Jun-16	Upper	241	32	13.28	17.7
SBC	01-Jul-16	Upper	326	34	10.43	24.9
SBC	07-Jul-16	Upper	246	34	13.82	14.5
SBC	11-Jul-16	Upper	80	13	16.25	14.0
SBC	27-Jul-16	Upper	101	22	21.78	12.1
SBC	04-Aug-16	Upper	209	96	45.93	8.2
SBC	10-Aug-16	Upper	162	51	31.48	6.5
SBC	12-Oct-16	Upper	199	73	36.68	5.7
SBC	17-Oct-16	Upper	185	37	20.00	10.9
SBC	28-Oct-16	Upper	200	22	11.00	16.8
SBC	04-Nov-16	Upper	156	17	10.90	11.8

Appendix E. Monthly collection information for the Chiwawa River rotary smolt trap.

	2016											
Species/Origin	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Total**
Chinook												
Wild												
Yearling	--	--	1,252	1,202	324	27	2	0	0	0	0	2,807
Subyearling	--	--	1,662	985	256	1,863	3,557	2,856	611	3,725	878	16,393
Hatchery	--	--	0	2,523	2	0	0	0	0	0	0	2,525
Steelhead												
Wild												
Smolt	--	--	8	56	46	44	8	16	16	1	0	195
Parr and fry	--	--	21	178	439	201	115	140	101	316	11	1,522
Hatchery	--	--	0	2	1,505	10	0	1	0	0	0	1,518
Coho												
Wild												
Smolt	--	--	0	0	0	0	0	0	0	0	0	0
Parr and fry	--	--	0	0	3	0	0	0	0	0	0	3
Hatchery	--	--	0	0	0	0	0	0	0	0	0	0
Bull trout												
Juvenile	--	--	0	3	2	1	0	4	9	71	13	103
Adult	--	--	1	0	0	2	1	0	7	4	0	15
Westslope cutthroat trout	--	--	0	0	5	13	6	14	4	1	0	43
Eastern brook trout	--	--	0	0	1	1	0	0	0	0	1	3
Rainbow trout	--	--	0	0	0	0	0	0	0	0	0	0
Mountain whitefish	--	--	14	1	6	6	211	570	6	25	44	883
Longnose dace	--	--	5	19	51	213	57	122	388	111	13	979
Northern pikeminnow	--	--	0	0	0	1	26	42	0	0	0	69
Sculpin spp.	--	--	7	5	12	16	21	15	4	9	5	94
Sucker spp.	--	--	0	0	0	0	1	1	0	1	0	3
Dace spp.	--	--	0	5	3	0	1	6	0	0	1	16
Yellow Perch	--	--	0	0	0	0	0	1	0	0	0	1
Redside shiner	--	--	0	0	0	0	0	0	0	0	0	0

Appendix F. Annual collection information from the Chiwawa River rotary smolt trap.

Species origin	2016	2015	2014	2013	2012	2011
Chinook						
Wild						
Yearling	2,807	6,350	5,419	3,199	7,626	4,848
Subyearling	16,393	31,152	23,755	27,621	14,831	20,561
Hatchery	2,525	7,162	5,293	15,909	30,751	25,620
Steelhead						
Wild						
Smolt	195	259	49	85	183	195
Parr and Fry	1,522	3,004	1,889	1,949	1,738	981
Hatchery	1,518	3,151	290	1,539	1,664	8,250
Coho						
Wild						
Smolt	0	0	0	1	1	3
Parr and fry	3	38	12	0	0	4
Hatchery	0	0	1	10	3	0
Bull trout						
Juvenile	103	266	260	310	488	351
Adult	15	32	75	51	31	7
Westslope cutthroat trout	43	72	59	86	60	38
Eastern brook trout	3	8	12	13	66	3
Mountain whitefish	883	5,544	2,970	2,108	3,291	990
Longnose dace	979	2,663	2,633	2,257	1,762	1,526
Northern pikeminnow	69	331	5	71	34	20
Sculpin spp.	94	225	131	91	157	129
Sucker spp.	3	30	4	6	0	0
Dace spp.	16	NA	NA	NA	NA	NA
Redside shiner	0	13	0	0	0	0
Yellow perch	1	0	0	0	0	0

Appendix G. Monthly collection information for the Lower Wenatchee River rotary smolt trap.

	2016											
Species/Origin	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Total**
Chinook												
Wild												
Yearling	4	194	166	141	69	23	13	--	--	--	--	610
Subyearling	10	148	1,752	8,338	7,612	8,677	870	--	--	--	--	27,407
Hatchery	1,858	3,197	37	2,538	69	2	0	--	--	--	--	7,701
Steelhead												
Wild												
Smolt	0	7	3	29	43	5	1	--	--	--	--	88
Parr and fry	2	28	20	15	11	62	191	--	--	--	--	329
Hatchery	0	0	0	101	146	12	0	--	--	--	--	259
Sockeye												
Wild	0	1	118	1,130	91	5	1	--	--	--	--	1,346
Coho												
Wild												
Smolt	0	7	2	0	0	1	0	--	--	--	--	10
Fry and parr	0	45	13	11	18	36	12	--	--	--	--	135
Hatchery	0	0	0	0	212	7	0	--	--	--	--	219
Unknown	0	0	5	1,776	829	17	3	--	--	--	--	2,630
Bull trout												
Juvenile	0	0	0	0	0	0	0	--	--	--	--	0
Adult	0	0	0	0	0	0	0	--	--	--	--	0
Westslope cutthroat trout	0	0	0	0	0	0	0	--	--	--	--	0
Mountain whitefish	0	0	2	7	3	3	0	--	--	--	--	15
Lamprey spp.	35	162	343	89	286	397	185	--	--	--	--	1,497
Longnose dace	1	23	11	28	17	39	44	--	--	--	--	163
Sculpin spp.	1	5	6	7	8	10	19	--	--	--	--	56
Sucker spp.	2	23	14	49	79	86	16	--	--	--	--	269
Dace spp.	1	3	20	25	32	37	15	--	--	--	--	133
Fathead minnow	0	0	0	3	5	0	1	--	--	--	--	9
Redside shiner	0	1	2	1	69	90	26	--	--	--	--	189
Stickleback (3-spined)	0	0	0	0	2	0	0	--	--	--	--	2
Northern pikeminnow	0	11	7	54	181	274	25	--	--	--	--	552
Chiselmouth	0	0	0	1	2	57	6	--	--	--	--	66
Peamouth	0	0	0	0	0	0	0	--	--	--	--	0

Appendix H. Annual collection information from the Lower Wenatchee River rotary smolt trap.

Species/Origin	2016	2015	2014	2013
Chinook				
Wild				
Yearling	610	1,559	1,700	1,854
Subyearling	27,407	252,293	81,445	52,652
Hatchery	7,701	9,920	31,290	13,979
Steelhead				
Wild				
Smolt	88	231	80	173
Parr	329	100	102	537
Hatchery	259	2,288	494	819
Sockeye				
Wild	1,346	4,178	7,678	4,520
Hatchery	0	0	0	72
Coho				
Wild				
Smolt	10	22	220	597
Fry and parr	135	4,972	393	923
Hatchery	219	6,566	16,908	12,960
Unknown	2,630	143	NA	NA
Bull trout				
Juvenile	0	0	3	6
Adult	0	0	0	0
Westslope cutthroat trout	0	1	3	0
Mountain whitefish	15	9	27	110
Lamprey spp.	1,497	283	292	762
Longnose dace	163	242	541	1,382
Sculpin spp.	56	52	128	242
Sucker spp.	269	51	134	240
Redside shiner	189	19	94	423
Stickleback (3-spined)	2	13	66	196
Dace spp.	133	NA	NA	NA
Fathead minnow	9	NA	NA	NA
Northern pikeminnow	552	12	37	39
Chiselmouth	66	6	69	10
Peamouth	0	3	9	10

Appendix C

Summary of PIT-Tagging Activities in the Wenatchee Basin, 2016

Appendix C. Numbers of fish captured, recaptured, PIT tagged, trap and hand mortality, shed tags, and total tags released in the Wenatchee River basin during January through November, 2016.

Sampling Location	Species and Life Stage	Number collected	Number of recaptures	Number tagged	Number died	Shed tags	Total tags released	Percent mortality
Chiwawa Trap	Wild Subyearling Chinook	16,393	89	7,355	82	1	7354	0.50
	Wild Yearling Chinook	2,807	79	2,729	4	3	2,729	0.14
	Wild Steelhead/Rainbow	1,717	18	1,323	10	10	1,313	0.58
	Hatchery Steelhead/Rainbow	1,518	0	1	0	0	1	0.00
	Wild Coho	3	0	0	0	0	0	0.00
	Total	22,438	186	11,408	96	14	11,397	0.43
Chiwawa Remote (Electrofishing)	Wild Subyearling Chinook	1,829	24	1,776	5	0	1,776	0.27
	Wild Yearling Chinook	0	0	0	0	0	0	0.00
	Wild Steelhead/Rainbow	0	0	0	0	0	0	0.00
	Hatchery Steelhead/Rainbow	0	0	0	0	0	0	0.00
	Wild Coho	0	0	0	0	0	0	0.00
	Total	1,829	24	1,776	5	0	1,776	0.27
Nason Creek Trap	Wild Subyearling Chinook	791	48	434	6	0	434	0.76
	Wild Yearling Chinook	61	4	61	0	0	61	0.00
	Wild Steelhead/Rainbow	1,007	6	531	1	1	530	0.10
	Hatchery Steelhead/Rainbow	98	7	0	0	0	0	0.00
	Wild Coho	6	0	6	0	0	6	0.00
	Total	1,963	65	1,032	7	1	1,031	0.36
Nason Creek Remote (Electrofishing)	Wild Subyearling Chinook	828	10	802	14	0	802	1.69
	Wild Yearling Chinook	0	0	0	0	0	0	0.00
	Wild Steelhead/Rainbow	0	0	0	0	0	0	0.00
	Hatchery Steelhead/Rainbow	0	0	0	0	0	0	0.00
	Wild Coho	0	0	0	0	0	0	0.00
	Total	828	10	802	14	0	802	1.69
White River Trap	Wild Subyearling Chinook	197	3	137	2	1	136	1.02
	Wild Yearling Chinook	3	0	3	0	0	3	0.00
	Wild Steelhead/Rainbow	5	0	5	0	0	5	0.00
	Hatchery Steelhead/Rainbow	0	0	0	0	0	0	0.00
	Wild Coho	0	0	0	0	0	0	0.00
	Total	205	0	145	2	1	144	0.98
Lower Wenatchee Trap	Wild Subyearling Chinook	27,407	38	18	184	0	18	0.67
	Wild Yearling Chinook	610	4	538	2	0	538	0.33
	Wild Steelhead/Rainbow	417	0	131	6	0	131	1.44
	Hatchery Steelhead/Rainbow	259	0	0	1	0	0	0.39
	Wild Coho	145	0	0	0	0	0	0.00
	Unknown Coho	2,630	0	2	3	0	2	0.11
	Wild Sockeye	1,346	1	1,065	64	0	1,065	4.75

Sampling Location	Species and Life Stage	Number collected	Number of recaptures	Number tagged	Number died	Shed tags	Total tags released	Percent mortality
	Total	32,814	43	1,754	260	0	1,754	0.79
Total:	**Wild Subyearling Chinook**	47,445	212	10,522	293	2	10,520	0.62
	Wild Yearling Chinook	3,481	87	3,331	6	3	3,331	0.17
	Wild Steelhead/Rainbow	3,146	24	1,990	17	11	1,979	0.51
	Hatchery Steelhead/Rainbow	1,875	7	1	1	0	1	0.05
	Wild Coho	154	0	6	0	0	6	0.00
	Unknown Coho	2,630	0	2	3	0	2	0.11
	Wild Sockeye	1,346	1	1,065	64	0	1,065	4.75
Grand Total:		60,077	331	16,917	384	16	16,904	0.64

Appendix D

Wenatchee Steelhead Spawning Escapement Estimates, 2016

Estimates of Wenatchee Steelhead Spawners in 2016

Kevin See

January 06, 2017

Introduction

Redd counts are an established method to provide an index of adult spawners (Gallagher et al. 2007). In the Wenatchee and Methow subbasins, index reaches are surveyed weekly during the steelhead spawning season (Mar 07, 2016 - May 26, 2016) and non-index reaches are surveyed once during the peak spawning period. The goal of this work is to:

- Predict observer net error, based on a model developed with data from steelhead redd surveys in the Methow, similar to that described in Murdoch et al. (2014).
- Use estimates of observer net error rates and the mean survey interval to estimate the number of redds in each index reach, using a Gaussian area under the curve (GAUC) technique described in Millar et al. (2012).
- Estimate the total number of redds in the non-index reaches by adjusting the observed counts with the estimated net error.
- Convert these estimates of redds in the mainstem areas (surveyed for redds) into estimates of spawners.
- Use PIT-tag based estimates of escapement for all tributaries in the Wenatchee, and combine those estimates with the redd-based estimates of spawners in the mainstem areas to estimate the total number of spawners in the Wenatchee.

Methods

Mainstem areas

The model for observer net error (observed redd counts / true number of redds) is a model averaging of the 2 best models that were fit to 43 data points in the Methow. Both models contained covariates of observed redd density (redds / m) and mean thalweg CV as a proxy for channel complexity. One model also contained discharge while the other also contained total redd survey experience as an additional covariate. Predictions were made using model averaged coefficients (based on AICc model weights) and the 2016 steelhead data. From these survey specific estimates of net error, a mean and standard error of net error was calculated for each reach. The standard deviation was calculated by taking the square root of the sum of the squared standard errors for all predictions within a reach.

Estimates of total redds were made for each index reach using the GAUC model described in Millar et al. (2012). The GAUC model was developed with spawner counts in mind. As it is usually infeasible to mark every individual spawner, only total spawner counts can be

used, and an estimate of average stream life must be utilized to translate total spawner days to total unique spawners. However, in adapting this for redd surveys, two modification could be used. The first would fit GAUC models to data showing all visible redds at each survey, and use an estimate of redd life as the equivalent of spawner stream life. However, because conditions can lead to many redds not disappearing before the end of the survey season, the estimates of redd life can be biased low. The second method relies on the fact that individual redds can be marked, and therefore the GAUC model can be fit to new redds only. The equivalent of stream life thus became the mean and standard deviation of the survey interval. We utilized the second method for this analysis.

For non-index reaches, which were surveyed only once during peak spawning, the estimate of total redds was calculated by dividing the observed redds by the estimate of net error associated with that survey. This assumes that no redds were washed out before the non-index survey, and that no new redds appeared after that survey. As the number of redds observed in the non-index reaches ranged from 0 to 3, any violoation of this assumption should not affect the overall estimates very much. Based on the peak spawning time for the associated index reaches, the surveys in the non-index reaches were conducted either at peak spawning, or within 10 days after peak spawning (Figure 2}).

To convert estimates of total redds into estimates of natural and hatchery spawners, total redds were multiplied by a fish per redd (FpR) estimate and then by the proportion of hatchery or wild fish. The fish per redd estimate was based on PIT tags from the branching patch-occupany model (see below) observed to move into the lower or upper Wenatchee (below or above Tumwater dam). FpR was calculated as the ratio of male to female fish, plus 1. This was 1.65 above Tumwater dam, and 1.61 below Tumwater. Reaches W1 - W7 are below Tumwater, while reaches W8 - W10 are above Tumwater. Similarly, the proportion of hatchery and natural origin fish was calculated from the same group of PIT tags for areas above and below Tumwater. The proportion of hatchery origin fish was 0.45 above Tumwater dam, and 0.35 below Tumwater (Table 2).

Tributary areas

Esimates of escapement to various tributaries in the Wenatchee were made using a branching patch-occupancy model (Waterhouse, L. et al., *in prep*) based on PIT tag observations of fish tagged at Priest Rapids dam. All fish that escaped to the various tributaries were assumed to be spawners (i.e. pre-spawn mortality only occurs in the mainstem).

Total spawners

When summing spawner estimates from index reaches to obtain estimates of total spawners in the Wenatchee, an attempt was made to incorporate the fact that the reaches within a stream are not independent. Estimates of correlation between the reaches within a stream were made based on weekly observed redds. Because correlations are often quite high between reaches, this is a better alternative than to naively assume the standard errors between reaches are independent of one another. These estimates of correlation were combined with estimates of standard error for each index reach to calculate a

covariance matrix for the Wenatchee index reaches (W6, W8, W9, W10), which was used when summing estimates of spawners to estimate the total standard error. Failure to incorporate the correlations between reaches would result in an underestimate of standard error at the population scale. Non-index reaches were only surveyed once, so it is impossible to estimate a correlation coefficient between non-index reaches and index reaches. Therefore, they were assumed to be independent from the index reachs when summing the estimates of spawners. Because the estimates of tributary spawners were made separately (see above), they were also treated as independent when summing spawner estimates. The uncertainty in each step was carried through the entire analysis via the delta method (Casella and Berger 2002).

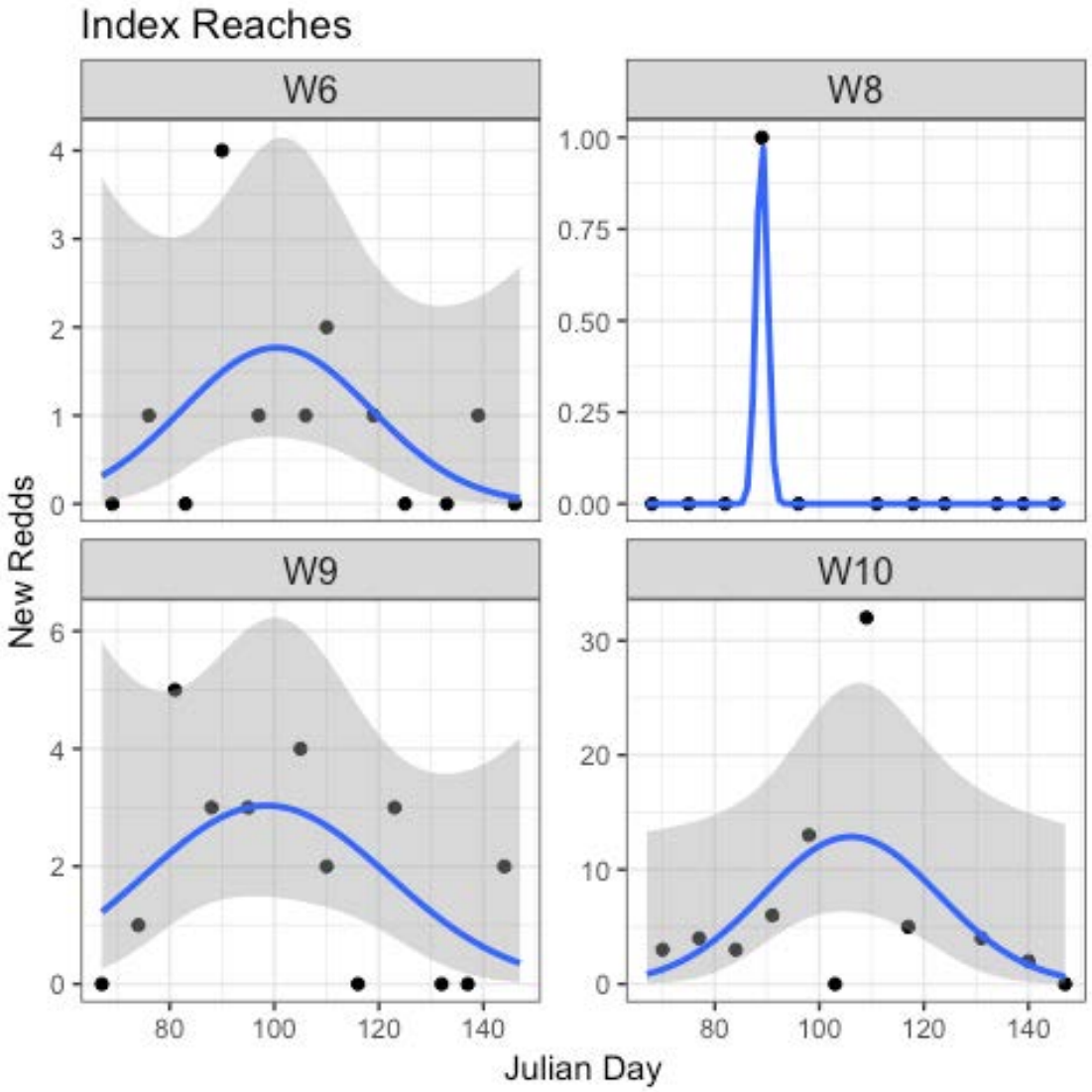
Results

Redd estimates

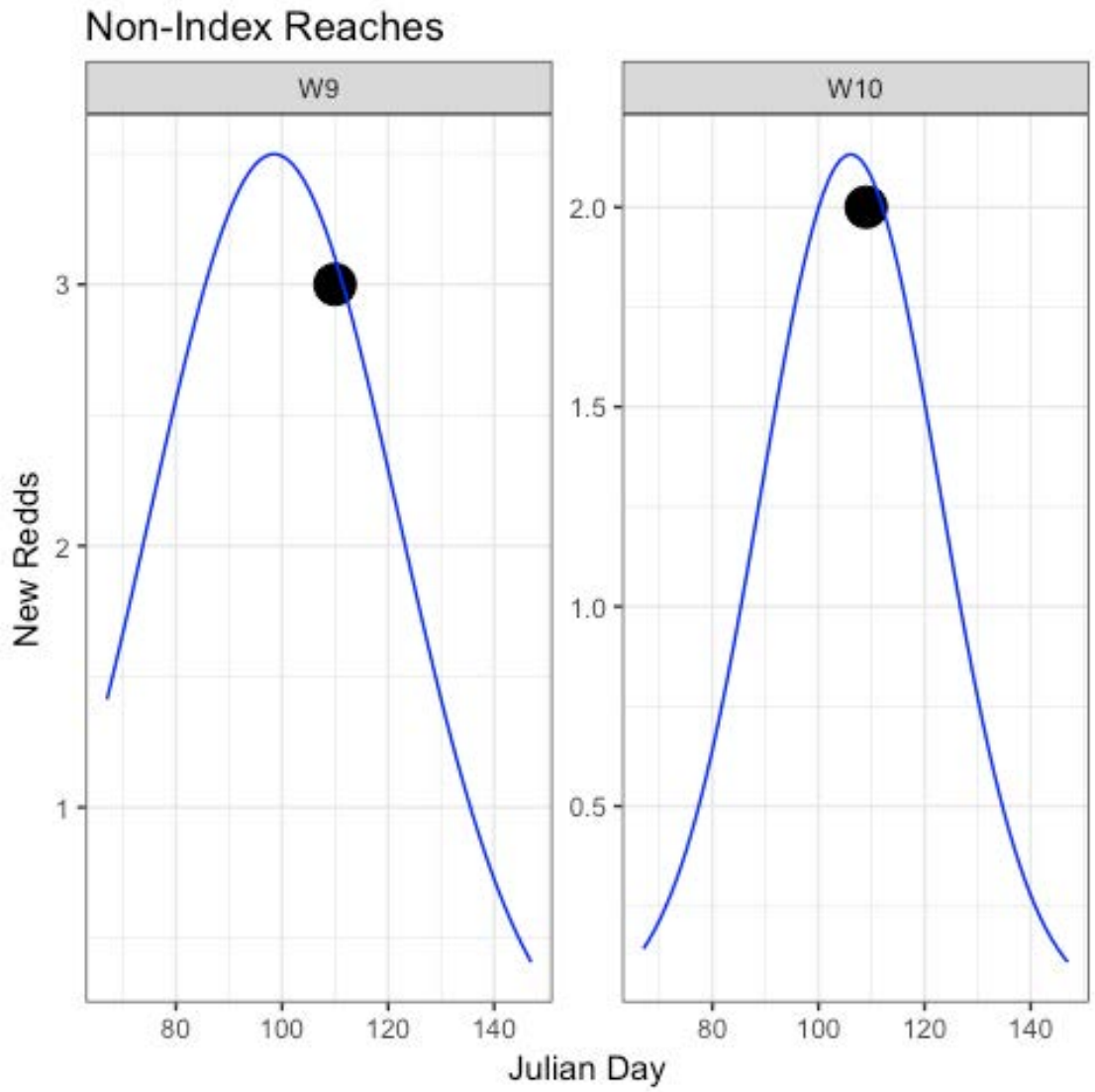
It should be noted that the GAUC parameters from index reaches were not used to estimate total redds in the associated non-index reaches. Figure 4 does illustrate that the non-index reach surveys were conducted close to the period of peak spawning (as determined by the associated index reaches), thus helping to validate the assumptions that go into estimating total redds in non-index reaches.

Table 1: Estimates of mean net error and total redds for each reach.

Reach	Type	Index.Reach	Net.Error	Net.Error.CV	Redds.Counted	Redds.Est	Redds.CV
C1	Index	-	NA	NA	0	0	NA
N1	Index	-	NA	NA	0	0	NA
P1	Index	-	NA	NA	0	0	NA
P1	Non-Index	NA	NA	NA	0	0	NA
W1	Non-Index	W2	NA	NA	0	0	NA
W2	Index	-	0.91	1.98	0	0	NA
W3	Non-Index	W2	NA	NA	0	0	NA
W4	Non-Index	W6	NA	NA	0	0	NA
W5	Non-Index	W6	NA	NA	0	0	NA
W6	Index	-	1.01	1.36	11	11	1.42
W6	Non-Index	W6	1.28	0.52	0	0	NA
W8	Index	-	0.85	1.47	1	1	0.59
W9	Index	-	0.93	1.46	23	26	1.48
W9	Non-Index	W9	0.99	0.42	3	3	0.42
W10	Index	-	0.84	1.31	72	82	1.39
W10	Non-Index	W10	0.66	0.34	2	3	0.34
Total	NA	NA	NA	NA	112	126	1.04



Plots of observed redd counts (black dots) through time for each index reach, and the fitted curve from the GAUC model (blue line) with associated uncertainty (gray).



Observed redd counts for non-index reaches with non-zero peak redd counts. The blue curve shows the GAUC estimated spawning curve, demonstrating how close to peak spawning the non-index surveys were conducted.

Spawner estimates

Table 2: Fish per redd and hatchery / natural origin proportion estimates.

Area	Fish/redd	FpR Std. Error	Prop. Hatchery	Prop Std. Error
Above TUF	1.652	0.070	0.447	0.036
Below TUF	1.613	0.084	0.347	0.043

Table 3: Estimates (CV) of spawners by area and origin.

Area	Type	Hatchery	Natural
Little Wenatchee	Trib	0 (--)	0 (--)
White River	Trib	0 (--)	8 (0.8)
C1	Index	0 (--)	0 (--)
Chiwaukum	Trib	11 (1)	64 (0.36)
Chiwawa	Trib	134 (0.35)	45 (0.44)
Chumstick	Trib	39 (0.37)	74 (0.27)
Icicle	Trib	18 (0.53)	72 (0.25)
Mission	Trib	13 (0.69)	33 (0.38)
N1	Index	0 (--)	0 (--)
Nason	Trib	94 (0.32)	57 (0.39)
P1	Index	0 (--)	0 (--)
P1	Non-Index	0 (--)	0 (--)
Peshastin	Trib	0 (--)	151 (0.19)
W1	Non-Index	0 (--)	0 (--)
W10	Index	61 (1.39)	75 (1.39)
W10	Non-Index	2 (0.35)	3 (0.35)
W2	Index	0 (--)	0 (--)
W3	Non-Index	0 (--)	0 (--)
W4	Non-Index	0 (--)	0 (--)
W5	Non-Index	0 (--)	0 (--)
W6	Index	6 (1.43)	12 (1.42)
W6	Non-Index	0 (--)	0 (--)
W8	Index	1 (0.6)	1 (0.6)
W9	Index	19 (1.48)	24 (1.48)
W9	Non-Index	2 (0.43)	3 (0.42)
Total		400 (0.31)	621 (0.25)

Discussion

We have estimated the number of steelhead redds based on redd surveys, while incorporating potential observation error. After translating these to estimates of spawners by origin, we can then compare the spawner estimates to escapement estimates made using PIT tags, and estimate a pre-spawn mortality rate (Table 4). Taking the total PIT-tag based escapement estimate to the Wenatchee (after subtracting the 327 hatchery and 66 wild fish removed at Tumwater, as well as the 27 hatchery fish removed at Dryden, and the 56 and 8 deaths to hatchery and wild fish due to harvest), and subtracting the total estimate of spawners, including the tributaries, then dividing by the total escapement

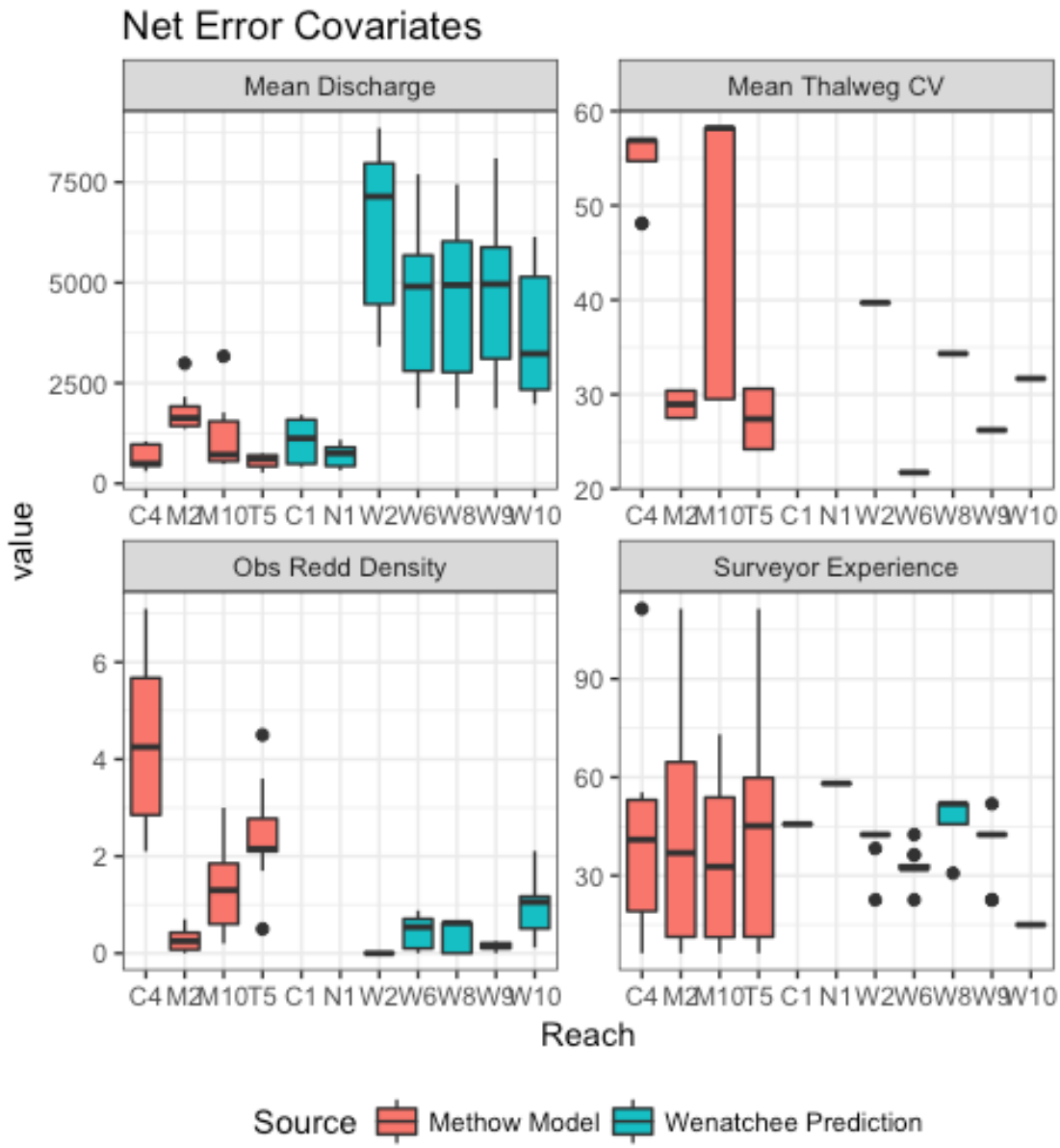
estimate provides an estimate of pre-spawn mortality across the entire Wenatchee population. We did this for natural and hatchery origin fish, and found that natural fish had a higher pre-spawn mortality rate this year.

Table 4: Wenatchee pre-spawn mortality rates.

Origin	Pre-spawn_Mort	CV
Natural	0.26	0.0009
Hatchery	0.09	0.0077

Caveats

The predictions of surveyor net error were made using a model that had been fit to data in the Methow. Most covariates in the Wenatchee were within the range of values in the Methow study, but mean discharge was higher in all reaches in the Wenatchee than in the modeled reaches in the Methow (Figure 3). The mean discharge in the Methow study was 1069.2, while it was 3837.5 in the Wenatchee reaches in 2016. That difference alone would change net error predictions by 0.5, not an insignificant amount. However, the observed covariate values in the Wenatchee did not lead to unrealistic estimates of net error. The ranges of net error estimates for the Methow study and the Wenatchee in 2016 were very similar.



Net error covariate values from the study in the Methow and the predicted reaches in the Wenatchee.

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Appendix E

Genetic Diversity of Wenatchee Summer Steelhead

Examining the Genetic Structure of Wenatchee Basin Steelhead and Evaluating the Effects of the Supplementation Program

Developed for

Chelan County PUD

and the

Rock Island Habitat Conservation Plan Hatchery Committee

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17 January 2012

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Executive Summary

In 1997, Wenatchee River summer steelhead, as part of the upper Columbia River evolutionarily significant unit (ESU), were listed as threatened under the Endangered Species Act (ESA). To address concerns about effects of hatchery supplementation, the hatchery program for hatchery produced (HOR) summer steelhead to be planted in the Wenatchee River changed from using mixed ancestry broodstock collected in the Columbia River to using Wenatchee River broodstock collected in the Wenatchee River. Three monitoring and evaluation (M&E) indicators were developed to measure the genetic effects of hatchery production on wild fish populations. To address these indicators, temporal collections of tissue samples from Wenatchee River hatchery-produced (HOR) and natural origin (NOR) adults captured and sampled at Dryden and Tumwater dams and from NOR juveniles from three Wenatchee River tributaries and the Entiat River were surveyed for genetic variation with 132 genetic (SNPs) markers. Peshastin Creek (a Wenatchee River tributary) and the Entiat River served as no-hatchery-outplant controls, meaning they have stopped receiving HOR juvenile outplants. As per the M&E plan, we interrogated these data for the presence or absence of spatial and temporal trends in allele frequencies, genetic distances, and effective population size.

Allele frequencies – Changes to the summer steelhead hatchery supplementation program had no detectable effect on genetic diversity of wild populations. On average, HOR adults had higher minor allele frequencies (MAF) than NOR adults, which may simply reflect the mixed ancestry of HOR adults. Both HOR and NOR adults had MAF similar to juveniles collected in spawning tributaries and in the Entiat River. There was no temporal trend in allele frequencies or observed heterozygosity in adult or juvenile collections and allele frequencies in control populations were no different than those still receiving hatchery outplants. This suggests that the hatchery program has had little effect on allele frequencies since broodstock sources changed in 1998.

Genetic distances – As intended, interbreeding of Wenatchee River HOR and NOR adults reduced the genetic differences between Wells Hatchery HOR adults and Wenatchee River NOR adults observed in the first few years after changing the broodstock collection protocol. Though there were detectable genetic differences between HOR and HOR adults, the magnitude of that

difference declined over time. HOR adults were genetically quite different from NOR adults and juveniles based on pair-wise F_{ST} and principal components analysis (PCA), most likely because of the much smaller effective population size (N_b) in the hatchery population (see below). Pair-wise F_{ST} estimates and genetic distances between HOR and NOR adults collected the same year declined over time suggesting that the interbreeding of HOR and NOR adults in the hatchery (and presumably in the wild) is slowly homogenizing Wenatchee River summer steelhead. Analyses using brood year (the year fish were hatched, determined using scale-based age estimates) were inconclusive because of limitations of the data.

Effective population size (N_b) – Although the effective population size of the Wenatchee River hatchery summer steelhead program was consistently small, it does not appear to have caused a reduction in the effective population size of wild populations. On average, estimates of N_b were much lower and varied less for HOR adults than for NOR adults and juveniles. Estimates of N_b for HOR adults declined from the earliest brood years to a stable new low value after broodstock practices were changed in 1997. There was no indication that this had any effect on N_b in NOR adults and juveniles; N_b estimates for NOR adults and juveniles were, on average, higher and varied considerably over the time period covered by our dataset (1998 – 2010) and showed no temporal trend.

Introduction

The National Marine Fisheries Service (NMFS) recognizes 15 Evolutionary Significant Units (ESU) for west coast steelhead (*Oncorhynchus mykiss*). The Upper Columbia ESU, which contains steelhead in the Wenatchee Basin, was listed as endangered under the Endangered Species Act (ESA) in 1997. Included in this listing were the Wells hatchery steelhead (program initiated in the late 1960s) that originated from a mixed group of native steelhead and are considered to be genetically similar to natural spawning populations above Wells Dam. Juvenile steelhead from Wells Fish Hatchery was the primary stock released into the Wenatchee River (Murdoch et al. 2003). The 1998 steelhead status review identified several areas of concern for this ESU including the risk of genetic homogenization due to hatchery practices and the high proportion (65% for the Wenatchee River) of hatchery fish present on the spawning grounds (Good et al. 2005). The Biological Review Team (BRT) further identified the relationship between the resident and anadromous forms of *O. mykiss* and possible changes in the population structure ('genetic heritage of the naturally spawning fish') in the basin as two areas requiring additional study. Furthermore, the West Coast Steelhead BRT (2003) recommended that stocks in the Wenatchee, Entiat, and Methow rivers, within the Upper Columbia ESU, be managed as separate populations.

A review of the presence of resident *O. mykiss* in the Upper Columbia ESU (Good et al. 2005) shows that rainbow trout are relatively abundant in upper Columbia River tributaries currently accessible to steelhead as well as in upriver tributaries unavailable to anadromous access by Chief Joseph and Grand Coulee dams (Kostow 2003). U.S. Fish and Wildlife Service (USFWS) biologists surveyed the abundance of trout and steelhead juveniles in the Wenatchee, Entiat, and Methow river drainages in the mid-1980s and found adult trout (defined as those with fork length > 20 cm) in all basins (Mullan et al. 1992). The results also supported the hypothesis that resident *O. mykiss* are more abundant in tributary or mainstem areas upstream of the areas used by steelhead for rearing. No samples of rainbow trout from the Wenatchee were available for this study.

In addition to the mixed ancestry Wells Hatchery steelhead, Skamania Hatchery (Washougal River steelhead ancestry) steelhead were also released into the Wenatchee River basin for several years in the late 1980s (L. Brown, Washington Dept. of Fish and Wildlife [WDFW], personal communication). In 1996, broodstock for the Wenatchee River steelhead program were collected from Priest Rapids Dam and Dryden (rkm 24.9) and Tumwater (rkm 52.6) dams on the Wenatchee River. Because of the ESA listing, broodstock collection after 1996 was restricted to the Wenatchee River in an effort to develop a localized broodstock (Murdoch et al. 2003). Thus, starting in 1998, all juvenile steelhead released into the Wenatchee River and Wenatchee River tributaries were offspring of only Wenatchee River captured broodstock.

In response to the need for evaluation of the supplementation program, both a monitoring and evaluation plan (Murdoch and Peven 2005) and the associated analytical framework (Hays et al. 2006) were developed for the Habitat Conservation Plans Hatchery Committee through the joint effort of the fishery co-managers (Confederated Tribes of the Colville Reservation [CCT], NMFS, USFWS, WDFW, and Yakama Nation [YN]) and Chelan County, Douglas County, and Grant County Public Utility Districts (PUD). These reports outline 10 objectives to be applied to various species assessing the impacts of hatchery operations mitigating the operation of Rock Island and Rocky Reach Dams. This report pertains to Wenatchee River basin steelhead (*O. mykiss*) and the steelhead supplementation program as addressed by objective 3, specifically the first three evaluation indicators.

> **Objective 3:** Determine if genetic diversity, population structure, and effective population size have changed in natural spawning populations as a result of the hatchery program. Additionally, determine if hatchery programs have caused changes in phenotypic characteristics of natural populations.
>
> **3.1 Allele Frequency**
>
> **3.2 Genetic Distances Between Populations**
>
> **3.3 Effective Spawning Population**

To address these evaluation indicators the WDFW Molecular Genetics Lab (MGL) obtained pertinent tissue collections and samples, surveyed genetic variation with SNP markers using our standard laboratory protocols, and calculated the relevant genetic metrics and statistics. We used collections from both the Entiat River and Wenatchee River basins. Both have received hatchery plants from non-local stocks [i.e. Entiat was stocked with both Wenatchee and Wells program juveniles averaging 12K and 18K respectively during 1995-2001, and Wenatchee received on average 177K juveniles from the Wells program during 1995-2001; (Good et al. 2005)], and both have all or some part of the basin designated as natural production "reference" drainage – no hatchery outplanting (i.e., the entire Entiat Basin, and Peshastin Creek in the Wenatchee River basin) (Good et al. 2005).

Materials and methods

Sample collections

To address objectives 3.1 through 3.3, we obtained samples from hatchery (HOR, adipose fin clipped) and natural origin (NOR, adipose fin intact) adult summer steelhead captured at Dryden or Tumwater diversion dams in the summer and fall of 1997 through 2009 (excepting 2004 and 2005; Table 1). All or some fraction of these fish was later used as hatchery broodstock the calendar year following the sampling year. In order to keep things simple we have reported years as the spawning year, i.e., the calendar year the fish were spawned, not the calendar year they were captured.

To address objective 3.2, it was necessary to have samples from natural origin fish from each of the spawning populations in the basin. It is difficult to obtain adult samples from known spawning populations due to the life history and behavior of steelhead, without tributary weirs or some other blocking method of collection. The NOR adult samples used as broodstock collected from Dryden and Tumwater Dams were a mixed collection representing all of the spawning populations located upstream. Therefore to determine population substructure within the basin we obtained collections of juvenile fish from smolt traps located within tributaries representing three major populations in the basin and from the Entiat River (Chiwawa River, Nason Creek, and Peshastin Creek; Table 2). We also obtained two collections of juvenile fish caught in a

smolt trap in the lower Wenatchee River. These, like the NOR adult collections, were a mixed collection presumably representing all populations located upstream. Fin tissue was taken from each fish and preserved in 95% ethanol.

Sample processing

Fin tissue samples were processed for 1468 HOR and NOR adult steelhead broodstock (Table 1) and for 1542 juvenile *O. mykiss* from the Wenatchee and Entiat Rivers (Table 2). Samples were genotyped at 152 single nucleotide polymorphism loci (SNPs, Tables 3, 4). We originally proposed to use microsatellites, but WDFW MGL and other regional genetic laboratories (Columbia River Inter-Tribal Fish Commission [CRITFC], Idaho Fish and Game [IDFG], USFWS) are moving toward using SNPs and they provide the same kinds of information with faster processing. Twenty SNP loci were developed to discriminate among trout species; 14 distinguish *O. mykiss* from coastal cutthroat trout (*O. clarkii clarkii*) and westslope cutthroat (*O. clarkii lewisi*), and 6 distinguish steelhead and coastal cutthroat from westslope cutthroat (Table 4). The remaining 132 SNP loci were developed to be used for population structure, parentage assignment, or other population genetic studies of *O. mykiss* (Table 3). These markers comprised the current standard set of SNP markers used for genetic studies of *O. mykiss* at WDFW MGL.

We used Qiagen DNEasy ® kits (Qiagen Inc., Valencia, CA), following the recommended protocol for animal tissues, to extract and isolate DNA from fin tissue. SNP genotypes were obtained through PCR and visualization on Fluidigm EP1 integrated fluidic circuits (chips). Protocols followed Fluidigm's recommendations for TaqMan SNP assays as follows: Samples were pre-amplified by Specific Target Amplification (STA) following Fluidigm's recommended protocol with one modification. The 152 assays were pooled to a concentration of 0.2X and mixed with 2X Qiagen Multiplexing Kit (Qiagen, Inc., Valencia CA), instead of TaqMan PreAmp Master Mix (Applied Biosystems), to a volume of 3.75µl, to which 1.25µl of unquantified sample DNA was added for a total reaction volume of 5µl. Pre-amp PCR was conducted on a MJ Research or Applied Biosystems thermal cycler using the following profile: 95°C for 15 min followed by 14 cycles of 95°C for 15 sec and 60°C for 4 minutes. Post-PCR reactions were diluted with 20µl dH_2O to a final volume of 25µl.

Specific SNP locus PCRs were conducted on the Fluidigm chips. Assay loading mixture contained 1X Assay Loading Reagent (Fluidigm), 2.5X ROX Reference Dye (Invetrogen) and 10X custom TaqMan Assay (Applied Biosystems); sample loading mixture contains 1X TaqMan Universal PCR Master Mix (Applied Biosystems), 0.05X AmpliTaq Gold DNA polymerase (Applied Biosystems), 1X GT sampling loading reagent (Fluidigm) and 2.1 µL template DNA. Four µL assay loading mix and 5 µL sample loading mix were pipetted onto the chip and loaded by the IFC loader (Fluidigm). PCR was conducted on a Fluidigm thermal cycler using a two step profile. Initial mix thermal profile was 70°C for 30min, 25°C for 5 min, 52.3° for 10 sec, 50.1°C for 1 min 50sec, 98°C for 5 sec, 96°C for 9 min 55 sec, 96°C for 15 sec, 58.6°C for 8 sec, and 60.1°C for 43 sec. Amplification thermal profile was 40 cycles of 58.6°C for 10 sec, 96°C for 5 sec, 58.6°C for 8 sec and 60.1°C for 43 sec with a final hold at 20°C.

The SNP assays were visualized on the Fluidigm EP1 machine using the BioMark data collection software and analyzed using Fluidigm SNP genotyping analysis software. To ensure all SNP markers were being scored accurately and consistently, all data were scored by two researchers and scores of each researcher were compared. Disputed scores were called missing data (i.e., no genotype).

Evaluation of loci

A two-tailed exact test of Hardy–Weinberg equilibrium (HWE) was performed for each locus in each collection or population using the Markov Chain method implemented in GENEPOP v4.1 (dememorization number 1000, 100 batches, 1000 iterations per batch; Raymond and Rousset 1995; Rousset 2008). Significance of probability values was adjusted for multiple tests using false discovery rate (Verhoeven et al. 2005). F_{IS}, a measure of the fractional reduction in heterozygosity due to inbreeding in individuals within a subpopulation and an additional indicator of scoring issues, was calculated according to Weir and Cockerham (1984) using GENEPOP v4.1. Allele frequencies were calculated using CONVERT v1.0 (Glaubitz 2004). Expected and observed heterozygosities were calculated using GDA v1.1 (Lewis and Zaykin 2001).

Allele frequencies, genetic distances and population differentiation

To evaluate Q1 of Objective 3.1 and 3.2, we evaluated trends and patterns in allele frequencies, genetic distances and population differentiation. To test for temporal patterns in allele frequencies, we compared sample or spawn year to two diversity metrics, allele frequency and observed heterozygosity, from each adult and juvenile collection. Each SNP locus had only one or two alleles, so we used the minor allele frequency (MAF) of each SNP locus for each adult collection and averaged across loci. We also calculated the average observed heterozygosity (Ho) for each SNP locus within each adult and juvenile collection. We examined the presence or absence of a temporal trend in average allele frequency and observed heterozygosity with logistic regression analysis in R (R Development Core Team 2009).

To partition genetic variance into temporal, spatial (juvenile) and origin (adult) fractions, we performed hierarchical analysis of molecular variance (AMOVA) using ARLEQUIN v3.0 (Excoffier et al. 2005) with 1,000 permutations. We performed this analysis separately for juvenile and adult collections. Juveniles were grouped by sampling location (tributary) and adults were grouped by origin (HOR or NOR). To estimate the magnitude of genetic differences among temporal and spatial collections we calculated pairwise F_{ST} estimates among collections using FSTAT (Goudet 1995) with 1000 permutations. Statistical significance was adjusted using false discovery rate (Verhoeven et al. 2005).

To evaluate the temporal changes in genetic relationships, we compared spawn year to within spawn year pairwise F_{ST} estimates between NOR and NOR adults using beta regression (Simas and Rocha 2010). We used beta regression because the dependent variable was bound by zero and one but not binomial. Analysis was performed in R (package "betareg", Cribari-Neto and Zeileis 2010), with a loglog link.

We used principal component analyses (PCA) to explore the relationship between the covariation among the SNP loci within each collection and genetic differentiation between HOR and NOR collections, and to determine if the degree of differentiation has changed with time. Since each SNP is represented by only two alleles, only one allele per SNP is necessary to fully describe the covariation among all SNPs. We used MATLAB® scripts (2007a, The Mathworks, Natlick, MA)

to calculate the principal components from SNP allele frequencies using only the major allele (1-MAF) for each SNP. We defined the major allele as the allele with the higher mean frequency across all collections, regardless of its status within any individual collection. We conducted three PCA analyses using: (1) all adult samples, aggregated based on origin (HOR versus NOR) and spawn year (i.e., the year the adult fish were used as broodstock) (N = 1437, 22 collections), (2) same as #1, but with the addition of all juvenile samples (N = 2938, 37 collections), and (3) only those adults samples with available age information (Mike Hughes, WDFW, personal communication) aggregated based on origin, and spawn year or brood year (i.e., the year the fish were hatched) (N = 1313, 20 spawn-year or 25 brood-year collections).

Molecular differentiation between HOR and NOR adults within a year was calculated based on principal component scores using Euclidian distances. We calculated pair-wise Euclidian distances between HOR and NOR fish within a spawn year or brood year using the first three principal components, and standardized each distance by subtracting from it the mean Euclidian distance calculated across all pair-wise distances. We used Mahalanobis distances to calculate the variation among HOR and NOR collections (calculated separately), again using the first three principal components. Here, we calculated Mahalanobis distances as the Euclidian distances between each collection and the centroid of all collections (HOR and NOR combined), but the Euclidian distances are scaled based on the dispersion of collections around the centroid (i.e., the variance). Euclidian and Mahalanobis distances were calculated using MATLAB scripts.

Effective spawning population

To evaluate Q1 of Objective 3.3, we estimated N_e using the single-sample linkage disequilibrium methods implemented in the program LDNE (Waples and Do 2008). This method requires that you input the P_{crit} value, the minimum frequency at which alleles were included in the analysis, since results can be biased depending on this setting (Waples and Do 2010). SNP markers typically have only one or two alleles; if one of two alleles is excluded based on its frequency in the collection it essentially excludes the locus, reducing the overall dataset. Therefore, we used P_{crit} values ranging from 0.1 to 0.001 to evaluate whether trends in N_e changed given which loci were used. Confidence intervals were calculated using a jackknife procedure.

We calculated an estimate of N_e for all adult and juvenile collections individually. However, the intention of an integrated hatchery program such as the Wenatchee River steelhead hatchery program is that HOR and NOR fish are integrated and progress as a single population through intentional interbreeding in the hatchery and presumed natural interbreeding in the wild. Thus, we also combined annual HOR and NOR collections to calculate an overall N_e estimate as has been done in other genetic monitoring and evaluation analyses (e.g., Small et al. 2007, [Chinook salmon, *O. tshawytscha*]).

Estimates of N_e from linkage refer to the generations that produced the sample. To calculate the ratio of effective population size to census size (N_e/N), we obtained the number of fish spawned in the hatchery (1993 through 2006, i.e., those that produced the adipose fin clipped adults that returned to spawn in the Wenatchee River 1998 through 2010) and the estimated escapement of fish spawning naturally (HOR and NOR separately) for the same time period. Estimates of census population size in spawning tributaries was obtained by multiplying the fraction of redds counted within tributaries (Chad Herring ,WDFW, unpublished data) by the total Wenatchee River census population estimate (Andrew Murdoch, WDFW, unpublished data). To calculate N_e/N, we performed two analyses. First, for adults, we assumed a five year generation time for natural origin adults and a four year generation time for hatchery origin adults and divided the N_e estimate by the census population estimate from four or five years earlier. For juveniles, we assumed an age at outmigration of two years and divided the N_e estimates by the estimate of census population size for the appropriate tributary. Second, we used available adult age data to parse individuals into cohorts originating in brood years (rather than spawn years) and then used LDNE to estimate N_e from cohort collections. We performed both analyses to make full use of all available data; age data were not available for many adults, and because of variable survival and sampling not all cohorts had sufficient numbers of HOR and NOR adults. According to Luikart et al. (2010), estimates produced using linkage disequilibrium should be interpreted as something between effective population size (N_e) and the effective number of breeders (N_b). Using cohorts, the estimate produced by LDNE is clearly an estimate of N_b rather than N_e. In order to keep things simple, we have referred to all estimates as N_b.

Results and Discussion

Collections and samples received

From 1468 samples from HOR and NOR adult steelhead broodstock, 1437 produced sufficient genetic data for further analysis (Table 1). From 1542 samples from NOR juvenile steelhead from Wenatchee River tributaries and the Entiat River, 1501 produced sufficient genetic data for further analysis and were genetically identified as *O. mykiss* (Table 2). Samples genetically identified as *O. clarki* (2 samples from the Chiwawa River, 1 from the Entiat River) or *O. clarki*/*O. mykiss* hybrids (4 – lower Wenatchee River, 4 – Nason Creek, 4 – Chiwawa River, and 1 – Entiat River) were omitted from further analysis.

Evaluation of loci

Three loci showed deviations from HWE in 10 or more of 37 Wenatchee steelhead collections before correcting for multiple tests (AOmy016, AOmy051, AOmy252, Table A1) indicating possible scoring issues. These loci were omitted from further analysis. Nine of the remaining loci were monomorphic or nearly monomorphic in all collections (average MAF < 0.1, AOmy023, AOmy028, AOmy123, AOmy129, AOmy132, AOmy209, AOmy229, AOmy270, AOmy271, Table A1) contributing little or nothing to analytical power. These loci were also omitted from further analysis. No genetic data was available for collection 10FD due to poor PCR amplification at locus AOmy213 for the entire collection. AOmy213 had a relatively low MAF in most collections so rather than re-processing this collection at this locus or running different sets of loci for different tests, we omitted this locus from further analysis. Only six tests of deviation from HWE were significant after correcting for 4348 tests using false discovery rate. Two of these tests were in loci already omitted. The remaining four tests were spread among the remaining loci, indicating no more loci needed to be omitted from further analysis.

Objective 3.1, 3.2 – Allele frequencies and Genetic distances

Allele frequencies

Average MAF of SNP loci ranged from 0.00 to 0.60 in HOR adult collections and from 0.00 to 0.61 in NOR adult collections (Table A1). Observed heterozygosity ranged from 0.00 to 0.75 in HOR adult collections and from 0.01 to 0.67 in NOR adult collections. Juvenile collections produced similar ranges of MAF and Ho (Table A1). Average MAF and Ho of HOR adult collections appeared to be greater than those of natural origin collections. However, logistic regression analysis indicated there was no significant temporal trend in either diversity statistic (Figure 1). Similarly, there was no consistent temporal trend in MAF or Ho of juvenile collections (Figure 2). Both the Chiwawa River and Nason Creek, the two tributaries that currently still receive hatchery juvenile outplants, both appeared to have declining allele frequencies, but neither was statistically significant ($P > 0.90$). However, the power to detect significant trends was limited by the small sample sizes (n = 3 sample years).

Analysis of Molecular Variance

Analysis of molecular variance (AMOVA) of adult collections (i.e., temporal and origin structure) indicated most of the genetic variance was among individuals or among individuals within populations (99.04%). Most of the remaining variance was temporal variation within hatchery and natural origin groups (0.61%) with the remaining variation from origin (0.35%). AMOVA of juvenile collections (i.e., spatial structure) indicated most of the genetic variance was among individuals (98.44%) or among individuals within populations (0.94%). Most of the remaining variance existed among temporal collections within tributary collections (0.37%) with the smallest fraction as among tributary variance (0.24%). Thus, overall, there was more variability among years than among tributaries or origins, but no trend in the temporal variability.

Pair-wise F_{ST} *estimates*

HOR adults were genetically different that NOR adults as estimated by F_{ST} (full pair-wise table in Table A2, all pair-wise F_{ST} estimates with P-values ≤ 0.05 before correcting for multiple tests

were significantly different from zero after correcting for multiple tests using false discovery rate). On average, HOR adult collections were as different from one another (mean F_{ST} = 0.011) as they were from NOR adult collections among years (mean F_{ST} = 0.009) or from NOR adult collections within years (mean F_{ST} = 0.010). Among year comparisons of NOR adult collections were, on average, nearly an order of magnitude lower (mean = 0.002). These patterns held whether spawn year or brood year (data not shown) was used to group individuals. Over time, within spawn year pair-wise F_{ST} estimates between HOR and NOR adults declined over time (β = -0.014, P = 0.0185; Figure 3), suggesting that the integration of hatchery and wild fish is slowly genetically homogenizing the groups. That relationship disappeared when adults were grouped by brood year (i.e., comparing fish produced the same year) and all brood years were used (β = -0.009, P = 0.615, data not shown). However, when the dataset was restricted to just those brood years when all typical (age at maturation frequency among all years > 0.10) age classes were present in the dataset (HOR = age 3, 4; NOR = age 4, 5, 6; brood years 1996-1998, 2004-2005) a non-significant (P = 0.278) negative relationship (β = -0.12) of F_{ST} and brood year was apparent. When the data were further restricted to just the years after the hatchery program changed to only collecting broodstock in the Wenatchee River (brood years 1998, 2004-2005), the slope was also negative (β = -0.09), but the relationship was not statistically significant (P = 0.962).

Within tributary among sample year pair-wise comparisons of juvenile collections were, on average, only very slightly smaller than comparisons among tributaries (0.005 vs. 0.006, respectively, Table 5, all pair-wise F_{ST} estimates with P-values $\leq$ 0.05 before correcting for multiple tests were significantly different from zero after correcting for multiple tests using false discovery rate). Nason Creek and Peshastin Creek on average showed higher among sample year F_{ST} estimates (0.010 and 0.007, respectively) than the Chiwawa or Entiat Rivers (0.004 and 0.002, respectively). The pair-wise comparison of the two collections of lower Wenatchee River smolts, presumably a mix of Chiwawa, Nason, Peshastin smolts and smolts from other spawning tributaries, was an order of magnitude smaller (F_{ST} = 0.0002), and not significantly different than zero (Table 5). There was no temporal trend in pair-wise comparisons of juvenile collections. However with, at most, four annual collections, detecting any temporal trend was unlikely. We also had no collections from years prior to 1998 (the first year of new hatchery program

broodstock collecting protocols) with which to compare contemporary data, nor could we find any reports or papers containing pre-hatchery-program-change genetic comparisons among Wenatchee River tributary populations, making it impossible to determine whether or not changing the hatchery program has had any effect at all on population structure. However, these data will be useful for future studies.

Principal Components

Each principal component analysis (Figures 4, 5) indicated that the genetic structure among HOR collections differed from that among NOR collections, and that this difference has decreased with time. When adult fish were aggregated based on origin and spawn-year, there was a clear differentiation between HOR and NOR adult collections along PC 1, and a separation among HOR collections, differentiating the early spawn-years (1998 – 2003) from the later spawn-years (2004 – 2010) along PC 2 and PC 3, respectively (Figure 4). The pair-wise genetic distances between HOR and NOR collections from the same spawn year (i.e., the HOR and NOR fish used as broodstock within the same year) decreased from the largest distance in 1998 to small distances in 2009 and 2010, although the smallest distance occurred in 2004 (Figure 4, top right). That is, within hatchery broodstock, the genetic difference between HOR and NOR fish decreased, on average, from 1998 to 2010, and the decrease appeared to be a mutual convergence of NOR fish shifting right along PC 1 and HOR fish shifting downward along PC 2 and PC 3. This increasing similarity in adult fish mirrored that seen in within year pair-wise F_{ST} estimates between HOR and NOR adults which also declined over time (Figure 3).

Overall, there was considerably more genetic variation among the HOR collections than there was among the NOR collections with average Mahalanobis distances (distance between each collection and the overall centroid [0,0,0]) among the HOR and NOR collections being 4.2 and 1.5, respectively. Since each NOR collection was generally composed of 3-4 brood-years, while HOR collections rarely were composed of more than two brood-years, we attributed the lower year-to-year genetic variability of the NOR broodstock to the greater homogenizing effect of including four or more brood-years compared with only two brood years for the HOR broodstock.

Including the 15 juvenile collections, along with the 22 adult collections, did not materially alter the principal component structure (Figure 6), although the total genetic variation accounted for by the three principal components decreased from 44% using only the adults to 33% when juveniles were included. For the most-part, the juvenile fish appeared intermediate between HOR and NOR fish, but there was greater overlap in principal component scores (and therefore greater genetic similarity) of the juvenile and NOR collections, than of the juvenile and HOR collections. The average Euclidian distance between the juvenile and HOR collections was 0.49, compared to 0.23 between the juvenile and NOR collections, which was no different than 0.23 and 0.22 for the within juvenile and NOR collections, respectively.

By using the available adult age data, we were able to compare the genetic differentiation among the same set of fish when they are aggregated by origin (hatchery versus natural) and brood-year (year fish were hatched) with aggregates based on origin and spawn-year (year adult fish were spawned). A brood-year analysis compares within a year the genetic diversity generated from hatchery broodstock with that naturally produced in the spawning grounds. A spawn-year analysis compares the HOR and NOR genetic diversity that was mixed among cohorts of the parental generations. The same basic pattern of genetic structure that we have seen in spawn-year analyses (Figure 4, Figure 6, and the right side of Figure 5) also occurred in the brood-year analysis (left side of Figure 5). That is, from Figure 5 we saw (1) that HOR and NOR fish were differentiated from each other; (2) there was considerably more genetic variation (temporal variation) among the hatchery-origin collections than there was among the natural-origin collections (for brood-year, Mahalanobis distances = 5.18 and 0.75, respectively; for spawn-year, Mahalanobis distances = 4.25 and 1.25, respectively), and (3) that the genetic distances between HOR and NOR collections were lower in the more recent brood- and spawn-years, than in the earlier brood- and spawn-years (Figure 7; $R^2 = 0.41$ or 41%, $P < 0.05$). This indicated that the HOR and NOR fish used as broodstock in 2010 were more similar to each other than they were at the inception of the new hatchery program.

The relationship between genetic distance and brood-year was not the same as the relationship between genetic distance and spawn-year. For brood-year, although the slope was negative (i.e.,

trending downward or decreased differentiation with time) and the two most-recent brood years (2005-2006) showed relatively small HOR and NOR adult differentiation, the negative slope was not significantly different from zero and the regression accounted for only 7% of the variation. This was likely the result of insufficient sampling of certain age classes from many brood years (especially from NOR adults) due to two un-processed sample years (2005 and 2006).

Objective 3.3 – Effective spawning population

There was no difference in the temporal trends in estimates of N_b with P_{crit} set from 0.1 to 0.001 (Figure 8, data not shown for all collections), so we have reported only results with $P_{crit} = 0.001$, i.e., the full genetic dataset. Using either spawn-year or brood year, estimates of NOR adult N_b were higher and varied more than those of HOR adults (Figures 9, 10), concordant with the PCA analysis. Estimates for HOR adults ranged from 17 to 174 (by spawn year, mean = 65) or from 6 to 130 (by brood year, mean = 39). Estimates for NOR adults ranged from 36 to 982 (by spawn year, mean = 405) or from 59 to 2966 (by brood year, mean = 645). Many N_b estimates for NOR adults had confidence intervals extending to infinity on the upper bound. This reflected the difficulty in obtaining precise estimates of N_b for large populations (Waples and Do 2010).

Estimates of N_b for HOR steelhead dropped by approximately half from 1994, when broodstock were still collected at Wells Hatchery, to 1998, when the program used Wenatchee River trapped adults only, suggesting an effect of changing broodstock collection practices, which began in 1997 (Figures 8, 9). Since 1997, the hatchery population N_b remained at a relatively stable lower level (Figures 8, 9, and 10). There was no obvious change in N_b for NOR steelhead since 1993; the N_b estimate for 1993 was the largest, however the confidence interval overlapped estimates from many other years. The temporal trend in N_b estimates from combined collections mirrored those of the HOR collections alone, though estimates using combined collections were slightly larger (Figure 11).

As with N_b estimates, estimates of the ratio of N_b/N for NOR adults varied more than those of HOR adults (Figures 12, 13). However, using spawn year, i.e., mixtures of cohorts, the average N_b/N ratio for HOR adults was equal to that of NOR adults (mean $N_b/N = 0.26$), whereas when using brood year, the average N_b/N ratio for NOR adults was double that of HOR adults (NOR

average =0.40, HOR average = 0.20). This is likely a consequence of the homogenizing effect of mixed cohorts. Estimates of N_b for HOR adults using spawn year were close to those estimated using brood year because of the lower diversity in age at maturation, whereas for NOR, grouping by brood year produces different estimates than when grouping by spawn year because of higher diversity in age at maturation. Regardless of which estimate was used, there was no temporal trend in N_b/N for either NOR or HOR adults.

Summary

On average, HOR adults had higher minor allele frequencies (MAF) than NOR adults, and both had similar MAF as juveniles collected in spawning tributaries and in the Entiat River. There was no temporal trend in allele frequencies or observed heterozygosity in adult or juvenile collections and allele frequencies in control populations were no different than those still receiving hatchery outplants suggesting that the hatchery program has had little effect on allele frequencies since 1998.

HOR adults were genetically quite different from NOR adults and juveniles based on pair-wise F_{ST} and principal components analysis (PCA), most likely because of the much smaller effective population size (N_b) in the hatchery population. Pair-wise F_{ST} estimates and genetic distances between HOR and NOR adults collected the same year declined over time suggesting that the interbreeding of HOR and NOR adults in the hatchery (and presumably in the wild) is slowly homogenizing Wenatchee River summer steelhead. Analyses using brood year (the year fish were hatched, determined using scale-based age estimates) were inconclusive because of limitations of the data.

On average, estimates of N_b were much lower and varied less for HOR adults than for NOR adults and juveniles. Estimates of N_b for HOR adults declined from the earliest brood years to a stable new low value after broodstock practices were changed in 1997. There was no indication that this had any effect on N_b in NOR adults and juveniles; N_b estimates for NOR adults and juveniles were, on average, higher and varied considerably over the time period covered by our dataset (1998 – 2010) and showed no temporal trend. Small N_b sizes increase the risk of loss of

genetic diversity due to inbreeding and random effects (genetic drift). The N_b of the hatchery component of the population may be increased by spawning more families, using specific mating designs, and minimizing variance in reproductive success. However, given the apparent lack of effects overall, changes to the hatchery protocol may not be necessary.

Overall, hatchery practices appear to have had little effect on natural origin Wenatchee summer steelhead neutral genetic diversity or N_b. We cannot accurately assess their effects on population structure at this time. However, it is interesting to note that when juvenile collections are analyzed separately from adult collections, Peshastin Creek, which has received fewer hatchery outplants in the past and is currently a refuge from hatchery outplants, is genetically different than other tributaries and the Entiat River (data not shown). On the other hand, the Entiat River, which is also a refuge from hatchery outplants and is not a tributary of the Wenatchee River, is genetically very similar to Nason Creek and the Chiwawa River, both Wenatchee River tributaries. This suggests, though it does not conclude, that within basin population structure may have existed before summer steelhead hatchery production began in the upper Columbia River and that the population structure was eliminated by hatchery influence long before 1998.

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Figures

Figure 1. Observed average minor allele frequencies (MAF) and observed heterozygosities (Ho) of 119 SNP loci from 11 annual collections of hatchery-produced (HOR) and natural origin (NOR) adult steelhead from the Wenatchee River. Trend lines are from a logistic regression. Note the X axis does not cross the Y axis at the origin. Neither the slopes nor the intercepts were statistically significant.

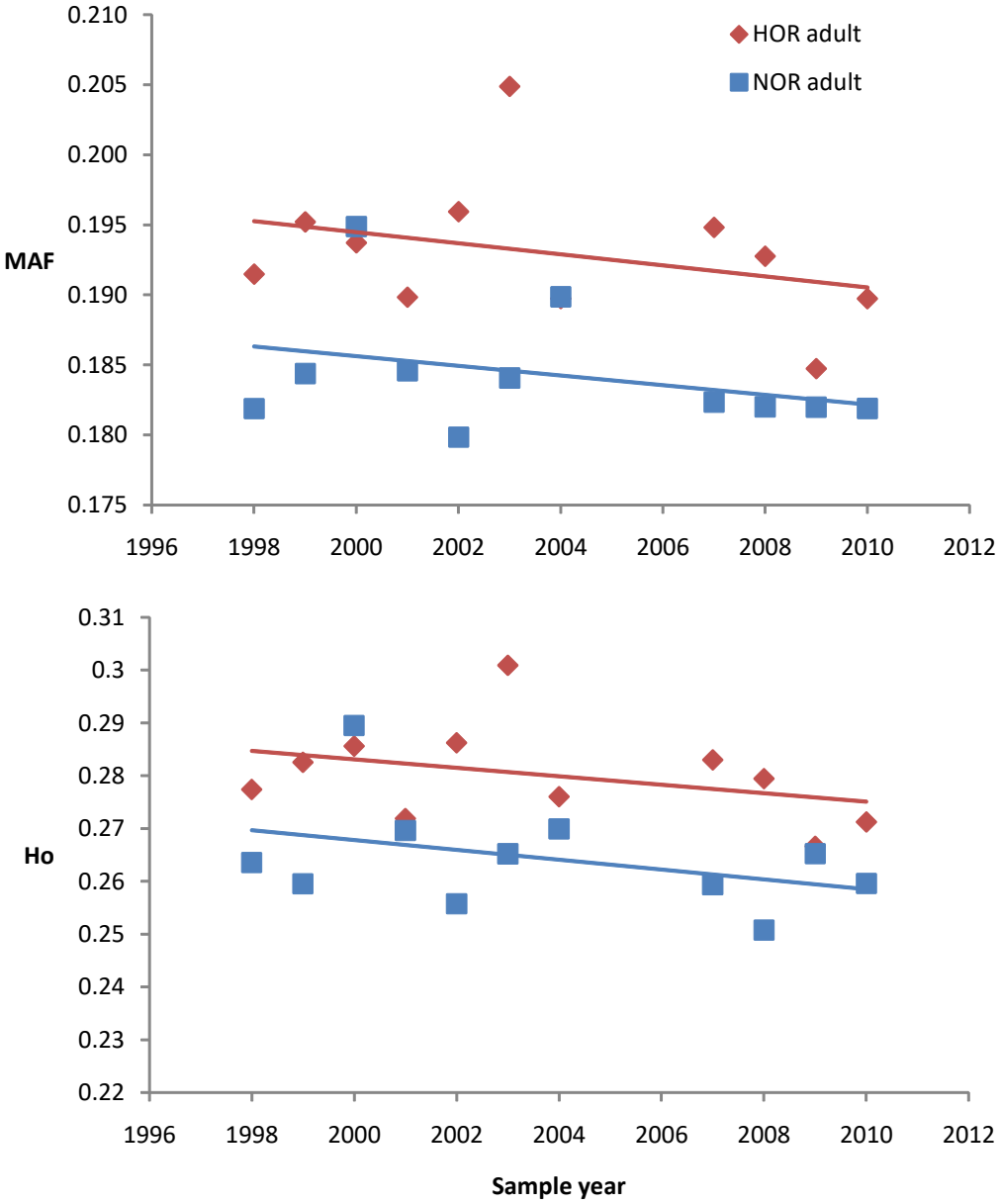


Figure 2. Observed average minor allele frequencies (MAF) and observed heterozygosities (Ho) of 119 SNP loci from 15 collections of natural origin juvenile steelhead from Wenatchee River tributaries, the lower Wenatchee River and the Entiat River. There were no consistent temporal trends in MAF or Ho in these collections.

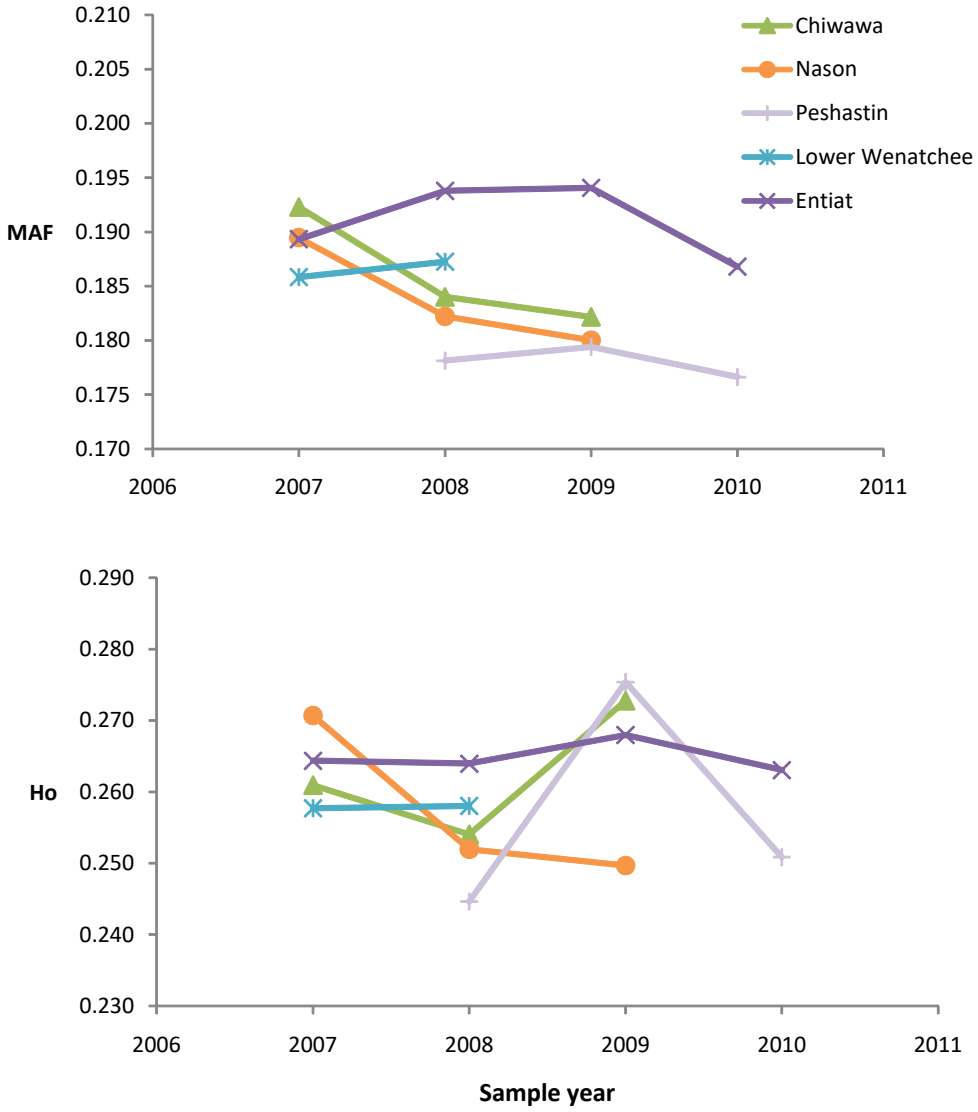


Figure 3. The relationship of time with pairwise F_{ST} estimates between hatchery-produced (adipose fin clipped) and natural origin (unclipped) adults of the same sample year. The line is the prediction based on beta regression.

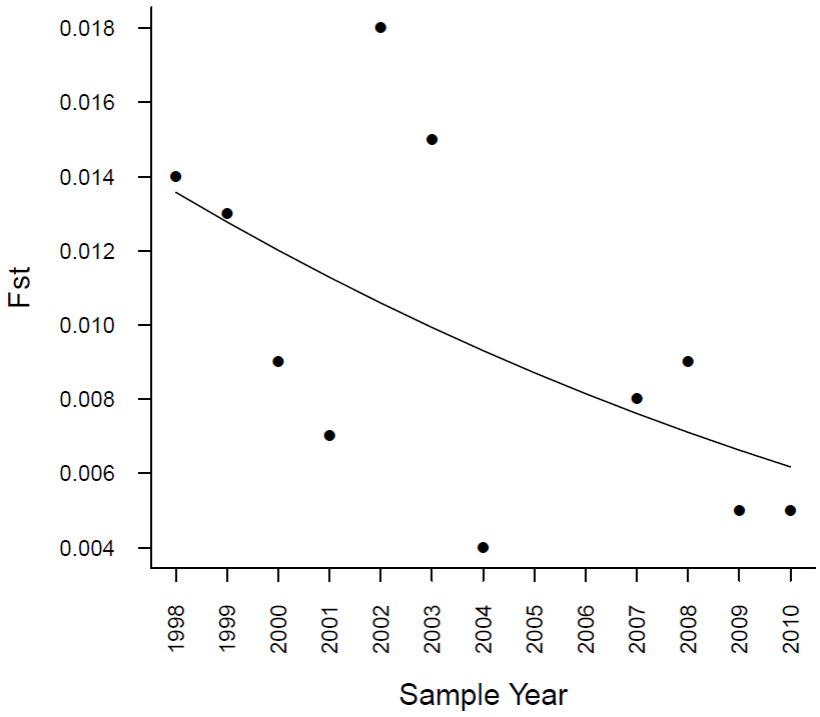


Figure 4. Principal component (PC) 1 versus 2 (top left), PC 1 versus 3 (bottom left), and PC 2 versus 3 (bottom right) based on an analysis using all adults aggregated into origin and spawn-year collections. Natural-origin spawn-years are shown in italicized typeface. The percentage within the label of each axis convey the percent of total genetic variance that is accounted for by that axis. Taken together, the three principal components account for 44% of the total SNP variation. Top right shows pairwise Euclidian distances versus spawn-year, with zero distance equal to average distance across all pairwise distances. Blue line is least-squares fit with $R^2 = 0.45$.

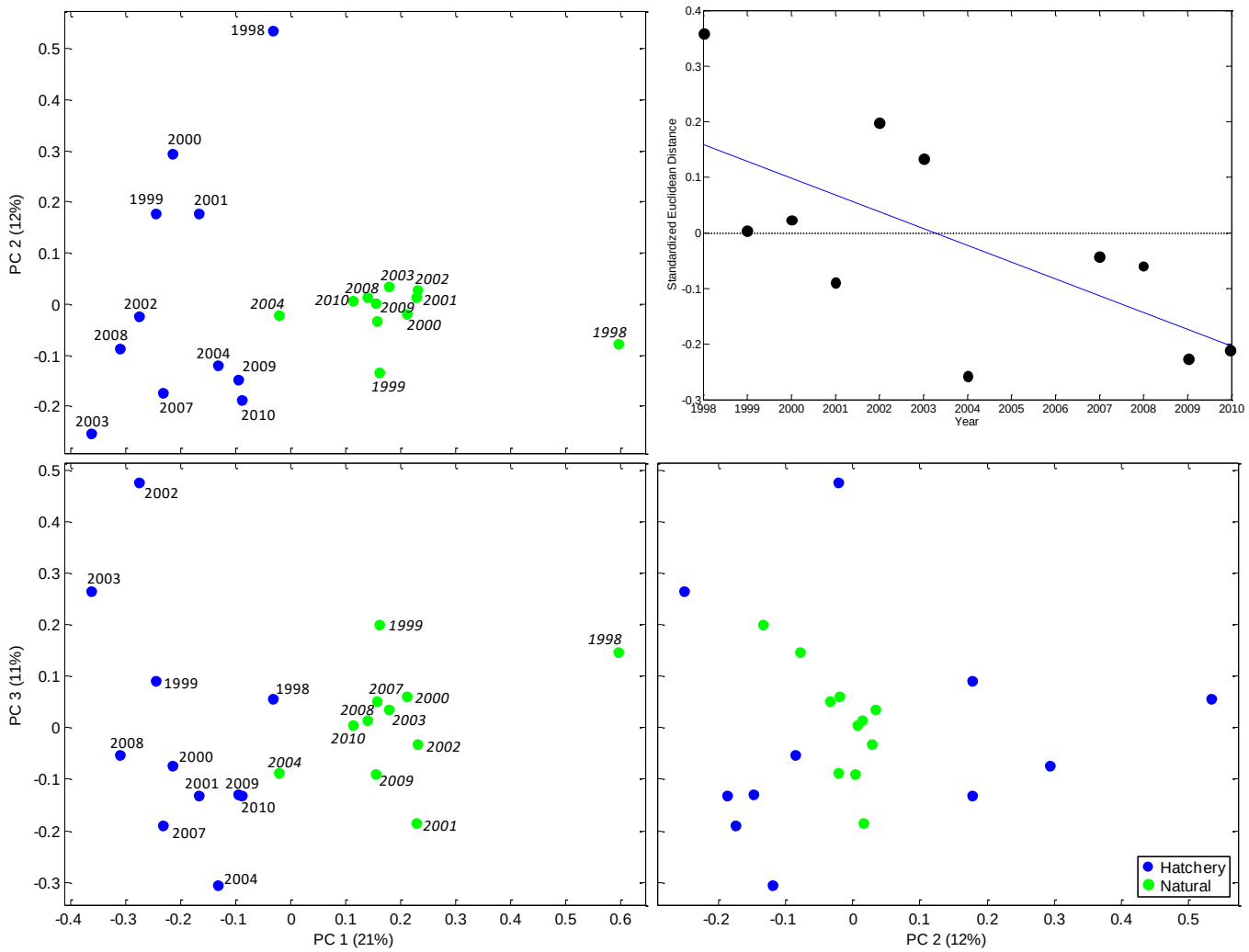


Figure 5. Principal components (PC) 1 versus 2 (top) and 3 (bottom) for adults aggregated into brood-year (BY; left) and spawn-year (SY; right). Spawn-year analysis is the same as in Figure x1, except fewer individuals per collection were included (see methods). Note that for the SY analysis here PC 2 and 3 are similar to PC 3 and 2, respectively, in Figure x1. Only BY1995 (earliest year with paired hatchery-natural data), BY2000 (extreme PC 1 score), and BY2006 (latest year with paired hatchery-natural data) are labeled. Hatchery- and natural-origin individuals from BY1995, BY2000, and BY2006, returned to spawn (spawn-year) in 1999 (hatchery)/1999-2001 (natural), 2003-2004 (hatchery)/2004 and 2007 (natural), and 2009-2010 (hatchery)/2010 (natural), respectively. These years are labeled in the upper right figure. Only 4 year-old BY 2006 natural-origin fish are represented in the SY 2010 collection.

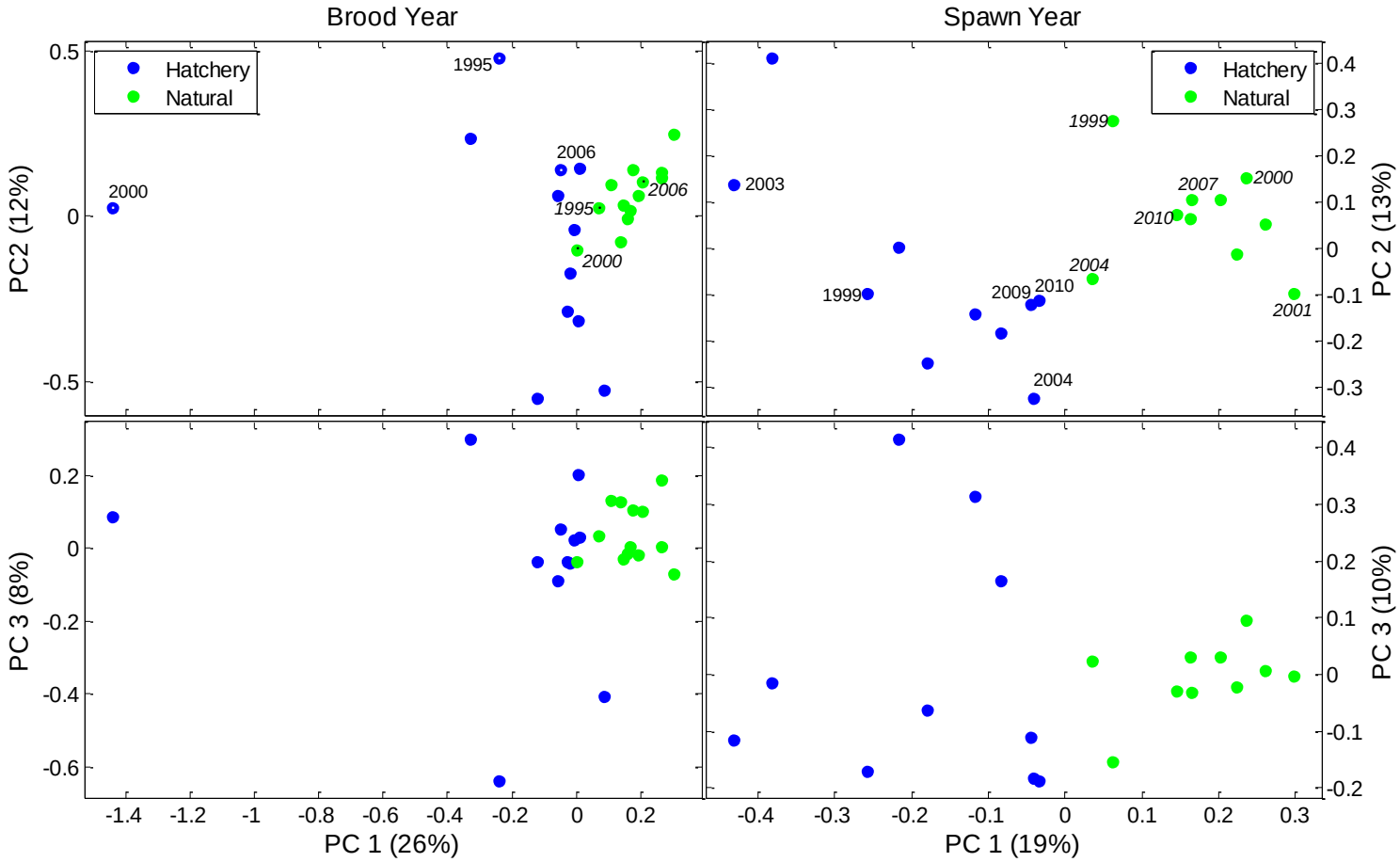


Figure 6. Principal component (PC) 1 versus 2 (top) and PC 1 versus 3 (bottom) based on an analysis using all adult and juvenile fish aggregated into age (juvenile versus adult), origin (hatchery versus adult) and spawn-year collections.

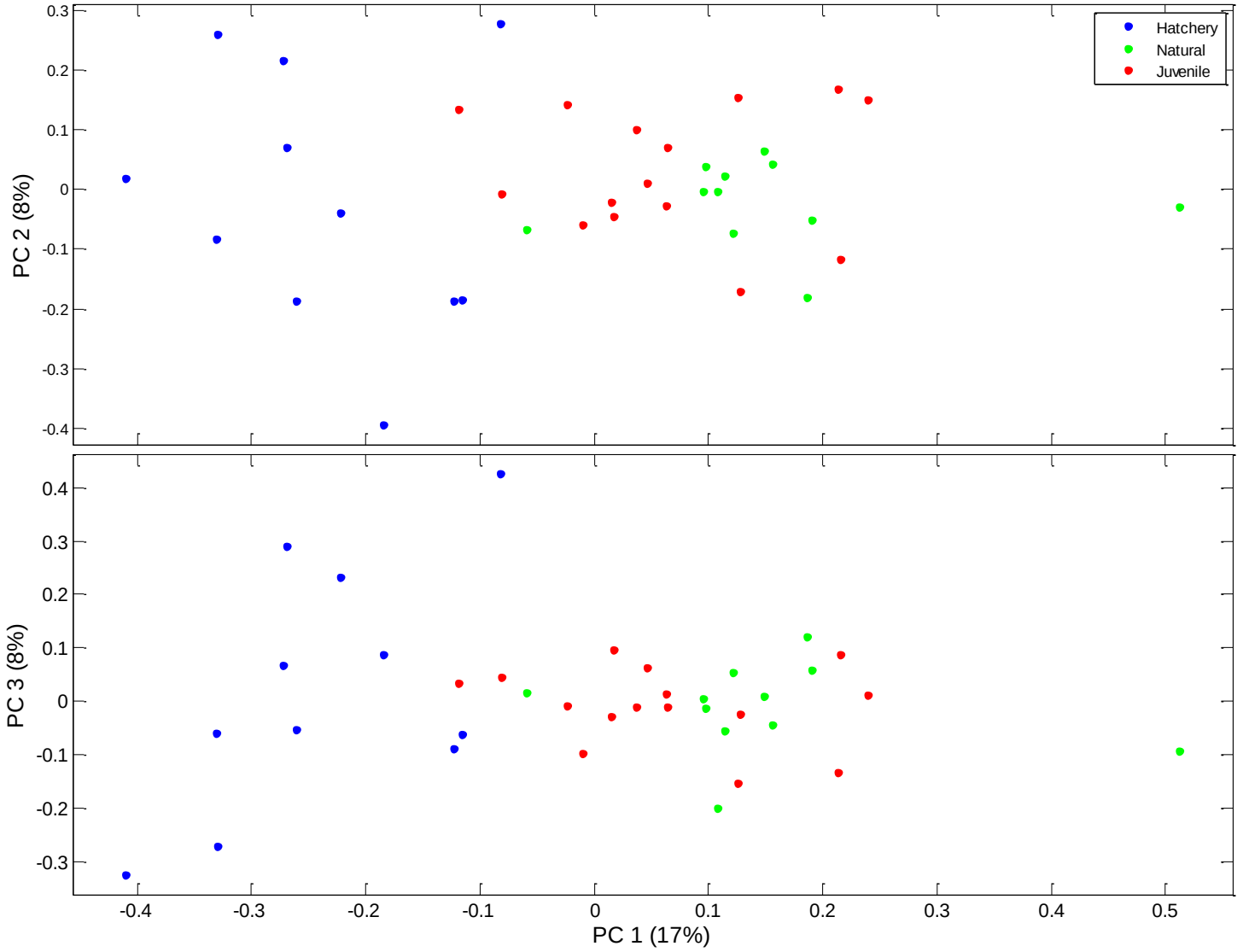


Figure 7. Pairwise Euclidian distances versus brood-year (top) and spawn-year (bottom), with zero distance equal to average distance across all pairwise distances. Blue lines are least-squares fits, which is not significant (slope = 0) for brood-year, but significant (slope > 0) for spawn-year.

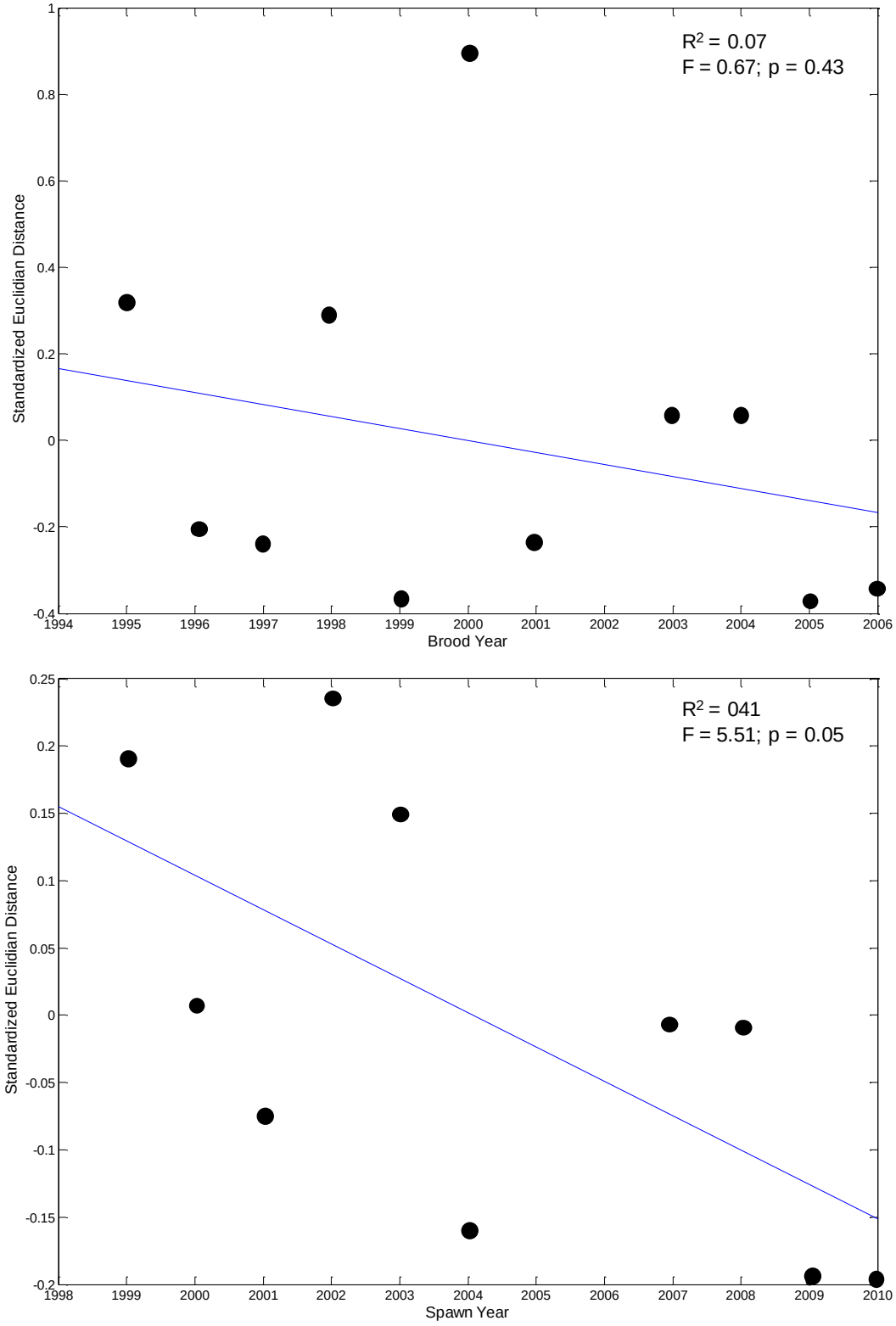


Figure 8. Effective population size estimates (N_b) from Wenatchee River adult hatchery-produced steelhead annual collections calculated using single sample methods implemented in the program LDNE (Waples and Do 2008). Each line connects annual estimates of N_b estimated with a different value of P_{crit}, the smallest allelic proportion allowed during analysis. With SNP data, omitting an allele omits the locus. Estimates of N_b changed very little when P_{crit} varied from 0.1 to 0.001. Setting $P_{crit} = 0.001$ forced the use of all available loci.

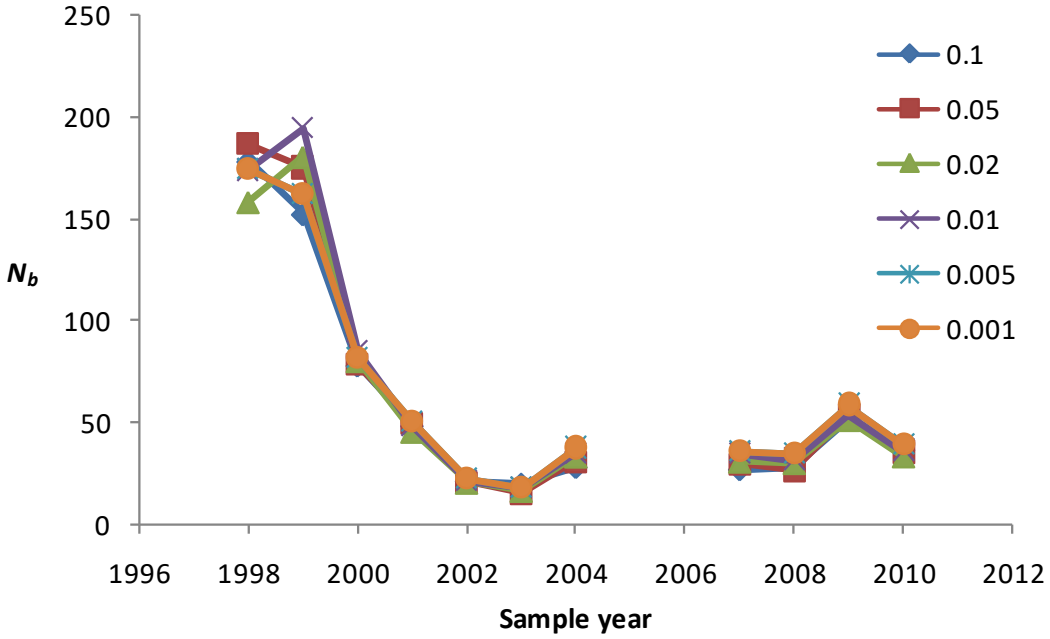


Figure 9. Estimates of Wenatchee River steelhead effective number of breeders (N_b) estimated using the single sample methods incorporated in the program LDNE (Waples and Do 2008). Estimates of N_b refer to parental (and even grantparental) generations. N_b data were plotted against their estimated parental brood year. We assumed a 5 year generation time for natural origin adults (NOR), a 4 year generation time for hatchery-produced adults (HOR) and an age of smolt outmigration of age 2 for smolt collections from Wenatchee River tributaries (Chiwawa River, Nason Creek, Peshastin Creek), the lower Wenatchee River, and the Entiat River. Bars represent the 95% confidence interval estimated by jackknife procedure. Bars that exceed the upper limit of the Y axis are labeled with the upper bound (Inf. = infinity).

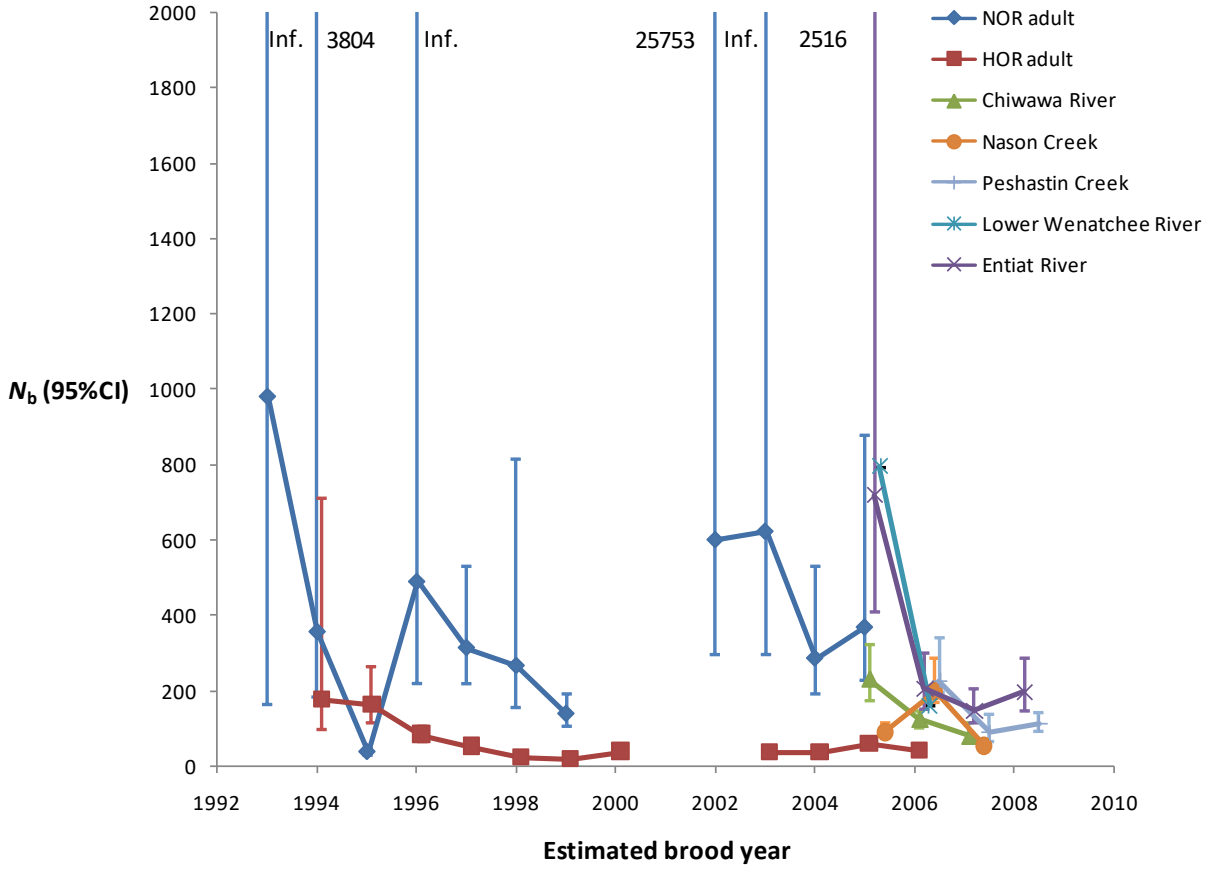


Figure 10. Estimates of N_b for collections of hatchery-produced (HOR) and natural origin (NOR) Wenatchee River summer steelhead grouped by brood year rather than spawn year. Brood year was estimated using scale-based age data. Error bars that extend past the top of the chart are all bounded by infinity.

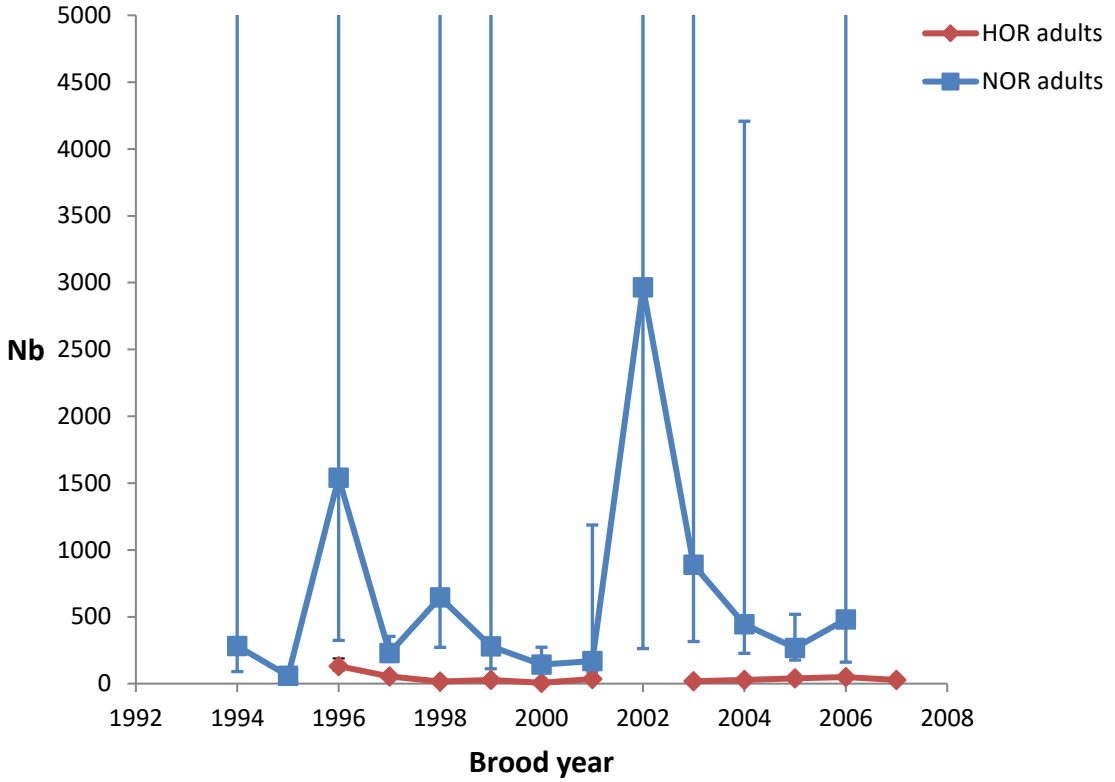


Figure 11. Estimates of N_b for combined annual adult hatchery-produced (HOR) and natural origin (NOR) steelhead and for HOR adults alone. The temporal patterns are similar, though estimates from combined collections are larger than those from HOR collections alone.

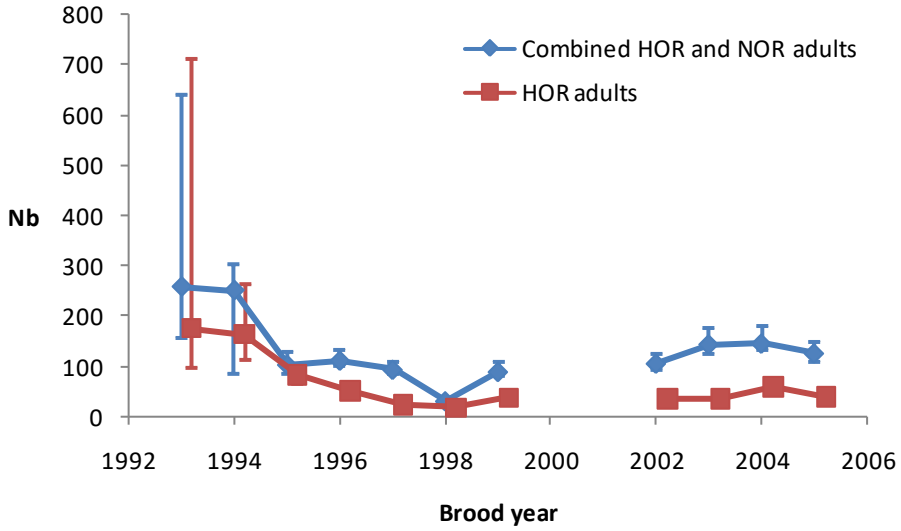


Figure 12. N_b/N ratios for hatchery-produced (HOR) and natural origin (NOR) adult Wenatchee River summer steelhead grouped by spawn year. The average N_b/N ratios are not different, though in later years NOR adults appear to have lower N_b/N ratios.

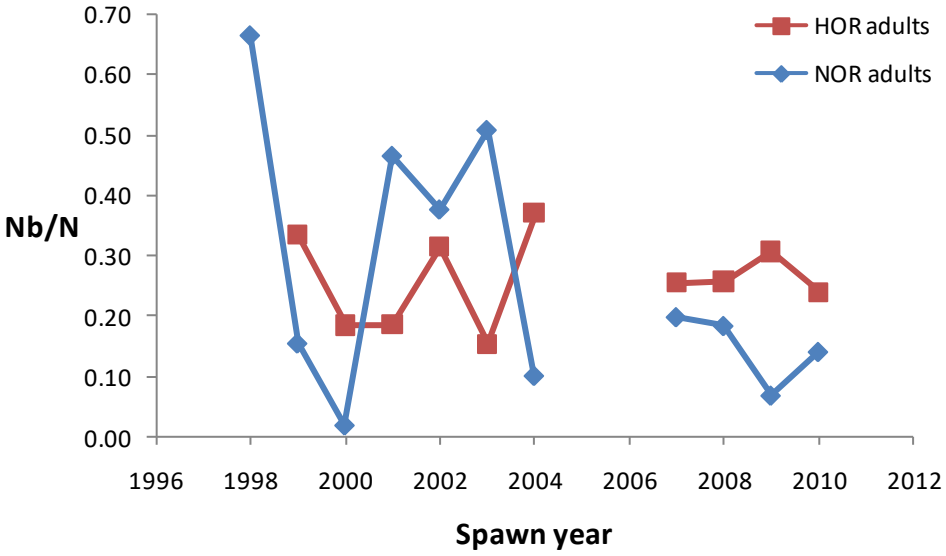
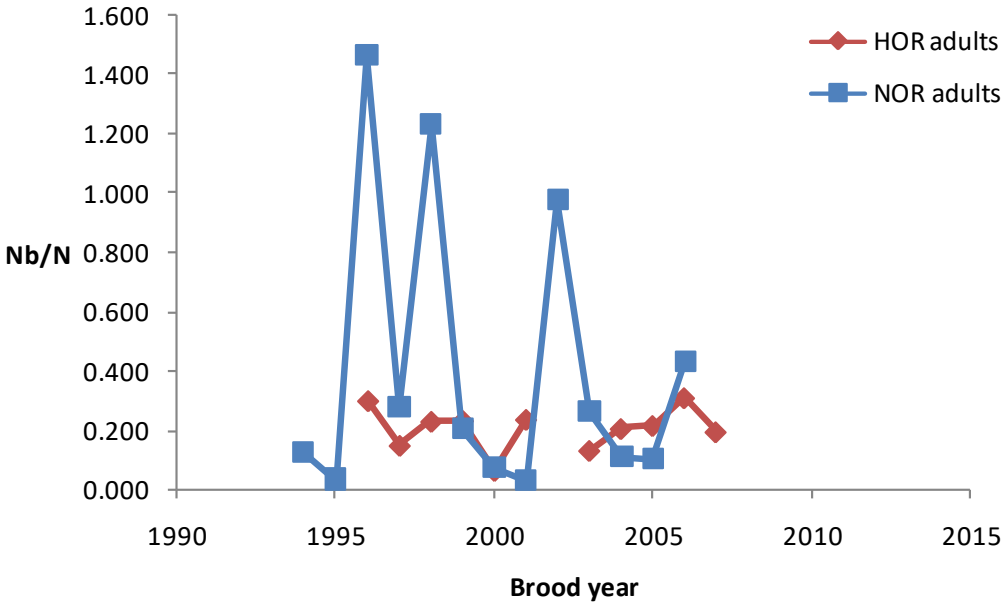


Figure 13. N_b/N ratios for hatchery-produced (HOR) and natural origin (NOR) adult Wenatchee River summer steelhead collections with individuals grouped in brood years rather than spawn years. Individual brood year was estimated using scale-based age data.



Tables

Table 1. Samples of adult steelhead collected for Wenatchee Program broodstock and used for genetic monitoring and evaluation.

Origin	Sampling Location	Year spawned	WDFW Collection code	Samples (N)	Unused Samples[a]
Hatchery	Dryden/Tumwater Dams	1998	98AE	32	4
		1999	98LJ	62	2
		2000	99NE	60	5
		2001	00DQ	99	1
		2002	01MS	64	
		2003	02NP	89	
		2004	03KW	61	
		2007	06CW	64	1
		2008	08AG	56	
		2009	09AV	74	
		2010	10FE	76	1
			Total	737	14
Natural	Dryden/Tumwater Dams	1998	98AF	30	5
		1999	99AA	51	1
		2000	99ND	33	3
		2001	00DP	50	
		2002	01MR	95	
		2003	02NO	50	
		2004	03KV	71	3
		2007	06CX	74	
		2008	08AF	74	1
		2009	09AU	82	2
		2010	10FD	90	2
			Total	700	17

[a]Samples were not used if they had incomplete (≤ 80% or 95 of 119 loci) or duplicate genotypes.

Table 2. Samples of natural origin juvenile steelhead and rainbow trout collected from four Wenatchee basin rivers or creeks and the Entiat River.

Sampling Location	Collection Year	WDFW Collection Code	Samples (N)	Unused samples[a]
Chiwawa River	2007	07AO	127	5
	2008	08CG	143	1
	2009	09NF	35	2
Entiat River	2007	07AL	134	4
	2008	08CI	82	4
	2009	09NC	74	1
	2010	10OX	82	1
Lower Wenatchee River	2007	07AM	139	5
	2008	08CE	98	2
Nason Creek	2007	07AN	81	4
	2008	08CF	133	6
	2009	09NG	103	2
Peshastin Creek	2008	08CH	142	2
	2009	09NE	34	1
	2010	10OY	94	1
		Total	1501	41

[a]Samples were not used if they were genetically identified as cutthroat trout or cutthroat/rainbow trout hybrids, or if they had incomplete (≤ 80% or 95 of 119 loci) or duplicate genotypes.

Table 3. List of 132 general use, diploid single nucleotide polymorphic (SNP) loci genotyped in Wenatchee River basin and Entiat River steelhead.

WDFW Name	Locus Name	Allele 1	Allele 2	Reference
AOmy005	Omy_aspAT-123	T	C	(Campbell et al. 2009)
AOmy014	Omy_e1-147	G	T	(Sprowles et al. 2006)
AOmy015	Omy_gdh-271	C	T	(Campbell et al. 2009)
AOmy016	Omy_GH1P1_2	C	T	(Aguilar and Garza 2008)
AOmy021	Omy_LDHB-2_e5	T	C	(Aguilar and Garza 2008)
AOmy023	Omy_MYC_2	T	C	(Aguilar and Garza 2008)
AOmy027	Omy_nkef-241	C	A	(Campbell et al. 2009)
AOmy028	Omy_nramp-146	G	A	(Campbell et al. 2009)
AOmy047	Omy_u07-79-166	G	T	WDFW - S. Young unpubl.
AOmy051	Omy_121713-115	T	A	(Abadía-Cardoso et al. 2011)
AOmy056	Omy_128693-455	T	C	(Abadía-Cardoso et al. 2011)
AOmy059	Omy_187760-385	A	T	(Abadía-Cardoso et al. 2011)
AOmy061	Omy_96222-125	T	C	(Abadía-Cardoso et al. 2011)
AOmy062	Omy_97077-73	T	A	(Abadía-Cardoso et al. 2011)
AOmy063	Omy_97660-230	C	G	(Abadía-Cardoso et al. 2011)
AOmy065	Omy_97954-618	C	T	(Abadía-Cardoso et al. 2011)
AOmy067	Omy_aromat-280	A	T	WSU - J. DeKoning unpubl.
AOmy068	Omy_arp-630	G	A	(Campbell et al. 2009)
AOmy071	Omy_cd59-206	C	T	WSU - J. DeKoning unpubl.
AOmy073	Omy_colla1-525	C	T	WSU - J. DeKoning unpubl.
AOmy079	Omy_g12-82	T	C	WSU - J. DeKoning unpubl.
AOmy081	Omy_gh-475	C	T	(Campbell et al. 2009)
AOmy082	Omy_gsdf-291	T	C	WSU - J. DeKoning unpubl.
AOmy089	Omy_hsp90BA-193	C	T	(Campbell and Narum 2009)
AOmy094	Omy_inos-97	C	A	WSU - J. DeKoning unpubl.
AOmy095	Omy_mapK3-103	A	T	CRITFC - N. Campbell unpubl.
AOmy096	Omy_mcsf-268	T	C	WSU - J. DeKoning unpubl.
AOmy100	Omy_nach-200	A	T	WSU - J. DeKoning unpubl.

AOmy107	Omy_Ots249-227	C	T	(Campbell et al. 2009)
AOmy108	Omy_oxct-85	A	T	WSU - J. DeKoning unpubl.
AOmy110	Omy_star-206	A	G	WSU - J. DeKoning unpubl.
AOmy111	Omy_stat3-273	G	Deletion	WSU - J. DeKoning unpubl.
AOmy113	Omy_tlr3-377	C	T	WSU - J. DeKoning unpubl.
AOmy117	Omy_u09-52-284	T	G	WDFW - S. Young unpubl.
AOmy118	Omy_u09-53-469	T	C	WDFW - S. Young unpubl.
AOmy120	Omy_u09-54.311	C	T	WDFW - S. Young unpubl.
AOmy123	Omy_u09-55-233	A	G	WDFW - S. Young unpubl.
AOmy125	Omy_u09-56-119	T	C	WDFW - S. Young unpubl.
AOmy129	Omy_BAMBI4.238	T	C	WDFW - S. Young unpubl.
AOmy132	Omy_G3PD_2.246	C	T	WDFW - S. Young unpubl.
AOmy134	Omy_Il-1b-028	T	C	WDFW - S. Young unpubl.
AOmy137	Omy_u09-61.043	A	T	WDFW - S. Young unpubl.
AOmy151	Omy_p53-262	T	A	CRITFC - N. Campbell unpubl.
AOmy173	BH2VHSVip10	C	T	Pascal & Hansen unpubl.
AOmy174	OMS00003	T	G	(Sánchez et al. 2009)
AOmy176	OMS00013	A	G	(Sánchez et al. 2009)
AOmy177	OMS00018	T	G	(Sánchez et al. 2009)
AOmy179	OMS00041	G	C	(Sánchez et al. 2009)
AOmy181	OMS00052	T	G	(Sánchez et al. 2009)
AOmy182	OMS00053	T	C	(Sánchez et al. 2009)
AOmy183	OMS00056	T	C	(Sánchez et al. 2009)
AOmy184	OMS00057	T	G	(Sánchez et al. 2009)
AOmy185	OMS00061	T	C	(Sánchez et al. 2009)
AOmy186	OMS00062	T	C	(Sánchez et al. 2009)
AOmy187	OMS00064	T	G	(Sánchez et al. 2009)
AOmy189	OMS00071	A	G	(Sánchez et al. 2009)
AOmy190	OMS00072	A	G	(Sánchez et al. 2009)
AOmy191	OMS00078	T	C	(Sánchez et al. 2009)
AOmy192	OMS00087	A	G	(Sánchez et al. 2009)

AOmy193	OMS00089	A	G	(Sánchez et al. 2009)
AOmy194	OMS00090	T	C	(Sánchez et al. 2009)
AOmy195	OMS00092	A	C	(Sánchez et al. 2009)
AOmy196	OMS00094	T	G	(Sánchez et al. 2009)
AOmy197	OMS00103	A	T	(Sánchez et al. 2009)
AOmy198	OMS00105	T	G	(Sánchez et al. 2009)
AOmy199	OMS00112	A	T	(Sánchez et al. 2009)
AOmy200	OMS00116	T	A	(Sánchez et al. 2009)
AOmy201	OMS00118	T	G	(Sánchez et al. 2009)
AOmy202	OMS00119	A	T	(Sánchez et al. 2009)
AOmy203	OMS00120	A	G	(Sánchez et al. 2009)
AOmy204	OMS00121	T	C	(Sánchez et al. 2009)
AOmy205	OMS00127	T	G	(Sánchez et al. 2009)
AOmy206	OMS00128	T	G	(Sánchez et al. 2009)
AOmy207	OMS00132	A	T	(Sánchez et al. 2009)
AOmy208	OMS00133	A	G	(Sánchez et al. 2009)
AOmy209	OMS00134	A	G	(Sánchez et al. 2009)
AOmy210	OMS00153	T	G	(Sánchez et al. 2009)
AOmy211	OMS00154	A	T	(Sánchez et al. 2009)
AOmy212	OMS00156	A	T	(Sánchez et al. 2009)
AOmy213	OMS00164	T	G	(Sánchez et al. 2009)
AOmy215	OMS00175	T	C	(Sánchez et al. 2009)
AOmy216	OMS00176	T	G	(Sánchez et al. 2009)
AOmy218	OMS00180	T	G	(Sánchez et al. 2009)
AOmy220	Omy_1004	A	T	(Hansen et al. 2011)
AOmy221	Omy_101554-306	T	C	(Abadía-Cardoso et al. 2011)
AOmy222	Omy_101832-195	A	C	(Abadía-Cardoso et al. 2011)
AOmy223	Omy_101993-189	A	T	(Abadía-Cardoso et al. 2011)
AOmy225	Omy_102505-102	A	G	(Abadía-Cardoso et al. 2011)
AOmy226	Omy_102867-443	T	G	(Abadía-Cardoso et al. 2011)
AOmy227	Omy_103705-558	T	C	(Abadía-Cardoso et al. 2011)

AOmy228	Omy_104519-624	T	C	(Abadía-Cardoso et al. 2011)
AOmy229	Omy_104569-114	A	C	(Abadía-Cardoso et al. 2011)
AOmy230	Omy_105075-162	T	G	(Abadía-Cardoso et al. 2011)
AOmy231	Omy_105385-406	T	C	(Abadía-Cardoso et al. 2011)
AOmy232	Omy_105714-265	C	T	(Abadía-Cardoso et al. 2011)
AOmy233	Omy_107031-704	C	T	(Abadía-Cardoso et al. 2011)
AOmy234	Omy_107285-69	C	G	(Abadía-Cardoso et al. 2011)
AOmy235	Omy_107336-170	C	G	(Abadía-Cardoso et al. 2011)
AOmy238	Omy_108007-193	A	G	(Abadía-Cardoso et al. 2011)
AOmy239	Omy_109243-222	A	C	(Abadía-Cardoso et al. 2011)
AOmy240	Omy_109525-403	A	G	(Abadía-Cardoso et al. 2011)
AOmy241	Omy_110064-419	T	G	(Abadía-Cardoso et al. 2011)
AOmy242	Omy_110078-294	A	G	(Abadía-Cardoso et al. 2011)
AOmy243	Omy_110362-585	G	A	(Abadía-Cardoso et al. 2011)
AOmy244	Omy_110689-148	A	C	(Abadía-Cardoso et al. 2011)
AOmy245	Omy_111005-159	C	T	(Abadía-Cardoso et al. 2011)
AOmy246	Omy_111084-526	A	C	(Abadía-Cardoso et al. 2011)
AOmy247	Omy_111383-51	C	T	(Abadía-Cardoso et al. 2011)
AOmy248	Omy_111666-301	T	A	(Abadía-Cardoso et al. 2011)
AOmy249	Omy_112301-202	T	G	(Abadía-Cardoso et al. 2011)
AOmy250	Omy_112820-82	G	A	(Abadía-Cardoso et al. 2011)
AOmy252	Omy_114976-223	T	G	(Abadía-Cardoso et al. 2011)
AOmy253	Omy_116733-349	C	T	(Abadía-Cardoso et al. 2011)
AOmy254	Omy_116938-264	A	G	(Abadía-Cardoso et al. 2011)
AOmy255	Omy_117259-96	T	C	(Abadía-Cardoso et al. 2011)
AOmy256	Omy_117286-374	A	T	(Abadía-Cardoso et al. 2011)
AOmy257	Omy_117370-400	A	G	(Abadía-Cardoso et al. 2011)
AOmy258	Omy_117540-259	T	G	(Abadía-Cardoso et al. 2011)
AOmy260	Omy_117815-81	C	T	(Abadía-Cardoso et al. 2011)
AOmy261	Omy_118175-396	T	A	(Abadía-Cardoso et al. 2011)
AOmy262	Omy_118205-116	A	G	(Abadía-Cardoso et al. 2011)

AOmy263	Omy_118654-91	A	G	(Abadía-Cardoso et al. 2011)
AOmy265	Omy_120255-332	A	T	(Abadía-Cardoso et al. 2011)
AOmy266	Omy_128996-481	T	G	(Abadía-Cardoso et al. 2011)
AOmy267	Omy_129870-756	C	T	(Abadía-Cardoso et al. 2011)
AOmy268	Omy_131460-646	C	T	(Abadía-Cardoso et al. 2011)
AOmy269	Omy_98683-165	A	C	(Abadía-Cardoso et al. 2011)
AOmy270	Omy_cyp17-153	C	T	WSU - J. DeKoning unpubl.
AOmy271	Omy_ftzf1-217	A	T	WSU - J. DeKoning unpubl.
AOmy272	Omy_GHSR-121	T	C	CRITFC - N. Campbell unpubl.
AOmy273	Omy_metA-161	T	G	CRITFC - N. Campbell unpubl.
AOmy274	Omy_UBA3b	A	T	(Hansen et al. 2011)

Primer and probe sequences for unpublished loci available by request.

Table 4. List of 20 species identification single nucleotide polymorphic (SNP) loci genotyped in Wenatchee River basin and Entiat River steelhead.

		Expected genotype			
WDFW Name	Locus Name	*O. mykiss*	*O. clarkii clarkii*	*O. clarkii lewisi*	Reference
ASpI001	Ocl_Okerca	T	C	C	(McGlauflin et al. 2010)
ASpI002	Ocl_Oku202	A	C	C	(McGlauflin et al. 2010)
ASpI003	Ocl_Oku211	G	T	T	(McGlauflin et al. 2010)
ASpI004	Ocl_Oku216	C	C	A	(McGlauflin et al. 2010)
ASpI005	Ocl_Oku217	C	C	A	(McGlauflin et al. 2010)
ASpI006	Ocl_SsaHM5	A	A	G	(McGlauflin et al. 2010)
ASpI007	Ocl_u800	T	C	C	(McGlauflin et al. 2010)
ASpI008	Ocl_u801	A	T	T	(McGlauflin et al. 2010)
ASpI009	Ocl_u802	C	C	T	(McGlauflin et al. 2010)
ASpI010	Ocl_u803	C	T	T	(McGlauflin et al. 2010)
ASpI011	Ocl_u804	G	G	C	(McGlauflin et al. 2010)
ASpI012	Omy_B9_228	A	A	C	(Finger et al. 2009)
ASpI013	Omy_CTDL1_243	C	A	A	(Finger et al. 2009)
ASpI014	Omy_F5_136	C	G	G	(Finger et al. 2009)
ASpI016	Omy_myclarp404-111	T	G	G	CRITFC - S. Narum - unpubl.
ASpI017	Omy_myclgh1043-156	C	T	T	CRITFC - S. Narum - unpubl.
ASpI018	Omy_Omyclmk436-96	A	C	C	CRITFC - S. Narum - unpubl.
ASpI019	Omy_RAG11_280	T	A	A	(Sprowles et al. 2006)
ASpI020	Omy_URO_302	T	C	C	(Finger et al. 2009)
ASpI021	Omy_BAC-F5.238	C	G	G	WDFW - S. Young unpubl.

Primer and probe sequences for unpublished loci available by request.

Table 5. Pairwise F_{ST} estimates for collections from Wenatchee River tributaries and the Entiat River (below diagonal) and associated bootstrap estimated *P*-values (above diagonal).

		Chiwawa River			Nason Creek			Peshastin Creek			Lower Wenatchee River		Entiat River			
Population	Year	2007	2008	2009	2007	2008	2009	2008	2009	2010	2007	2008	2007	2008	2009	2010
Chiwawa	2007		**0.000**	**0.003**	**0.000**	**0.000**	**0.000**	**0.000**	**0.002**	**0.000**	**0.001**	**0.001**	**0.000**	**0.001**	**0.000**	**0.000**
River	2008	0.004		**0.004**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
	2009	0.004	0.003		**0.000**	**0.001**	0.061	**0.000**	**0.001**	**0.000**	0.086	**0.050**	**0.022**	0.108	**0.005**	**0.045**
Nason	2007	0.011	0.010	0.007		**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
Creek	2008	0.007	0.007	0.005	0.009		**0.003**	**0.000**	**0.002**	**0.000**	0.079	**0.000**	**0.001**	**0.000**	**0.000**	**0.000**
	2009	0.007	0.007	0.003	0.014	0.006		**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
Peshastin	2008	0.010	0.011	0.008	0.013	0.010	0.013		**0.001**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
Creek	2009	0.005	0.005	0.006	0.010	0.007	0.008	0.003		**0.002**	**0.002**	**0.047**	**0.028**	**0.004**	**0.005**	**0.001**
	2010	0.010	0.011	0.008	0.015	0.008	0.011	0.003	0.003		**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
Lower Wenatchee	2007	0.003	0.003	0.000	0.005	0.008	0.007	0.009	0.010	0.008		0.112	**0.020**	**0.012**	**0.002**	**0.017**
River	2008	0.002	0.005	0.002	0.003	0.004	0.005	0.007	0.009	0.006	0.000		**0.049**	0.459	**0.047**	**0.002**
Entiat	2007	0.005	0.006	0.002	0.005	0.006	0.005	0.005	0.007	0.006	0.001	0.002		0.451	0.173	**0.000**
River	2008	0.004	0.004	0.000	0.007	0.005	0.007	0.008	0.009	0.011	0.002	0.001	0.000		0.644	**0.002**
	2009	0.005	0.006	0.002	0.003	-0.001	0.003	0.002	0.003	0.004	0.003	0.002	0.002	0.000		**0.028**
	2010	0.005	0.006	0.003	0.006	0.004	0.006	0.006	0.008	0.009	0.002	0.003	0.003	0.003	0.002	

P-values in bold were significant at $\alpha = 0.05$ after correcting for multiple tests using false discovery rate.

Appendix F

NPDES Hatchery Effluent Monitoring, 2016

NPDES MONITORING FOR WDFW FACILITIES

All WDFW hatcheries monitor their discharge in accordance with the National Pollutant Discharge Elimination System (NPDES) permit. This permit is administered in Washington by the Washington Department of Ecology under agreement with the United States Environmental Protection Agency. The previous permit was extended until March 31, 2016. The current permit was renewed effective April 1, 2016 and will expire March 31, 2021.

Facilities are exempted from sampling during any month that pounds of fish on hand fall below 20,000 lbs and pounds of feed used fall below 5,000 lbs, with the exception of offline settling basin discharges which are to be monitored once per month when ponds are in use and discharging to receiving waters. Inactive permitted facilities retain a permit but are not required to monitor discharges because the pounds of fish and pounds of feed remain below monitoring guideline set by the permit.

Sampling at permitted facilities includes the following parameters:

<FLOW	Measured in millions of gallons per day (MGD) discharge.
<SS EFF	Average net settleable solids in the hatchery effluent, measured in ml/L.
<TSS COMP	Average net total suspended solids, composite sample (6 x/day) of the hatchery effluent, measured in mg/L.
<TSS MAX	Maximum daily net total suspended solids, composite sample (6 x/day) of the hatchery effluent, measured in mg/L.
<SS PA	Maximum settleable solids discharge from the pollution abatement pond, measured in ml/L.
<SS %	Removal of settleable solids within the pollution abatement pond from inlet to outlet, measured as a percent. No longer required under permit effective June 1, 2000.
<TSS PA	Maximum total suspended solids effluent grab from the pollution abatement pond discharge, measured in mg/L.
<TSS %	Removal of suspended solids within the pollution abatement pond from inlet to outlet, measured as a percent. No longer required under permit effective June 1, 2000.
<SS DD	Settleable solids discharged during drawdown for fish release. One sample per pond drawdown, measured in ml/L.
<TRC	Total residual chlorine discharge after rearing vessel disinfection and after neutralization with sodium thiosulfate. One sample per disinfection, measured in ug/L.

In addition, at Similkameen Hatchery only, the following sampling was conducted at the request of Washington Department of Ecology, but is not required under NPDES permit:

<SS IW	Settleable solids influent grab taken as wastes are pumped into the pollution abatement pond, measured in mg/L. No longer monitored as of January 2008.
<TSS IW	Total suspended solids influent grab as wastes are pumped into the pollution abatement pond, measured in mg/L. No longer monitored as of January 2008.

Eastbank Hatchery

NPDES Permit Number WAG13-5011

		FLOW	SS EFF	TSS COMP	TSS MAX	FLOW PA	SS PA	SS %	TSS PA	TSS %	Lbs of Fish	Lbs of Feed
2016	JAN	29.72	0	0	0	5000	0.01		14.2		24405	6167
	FEB	29.72	0	0	0	7000	0.01		18		34129	6724
	MAR	31.02	0	0	0	15000	0		27.5		44129	7136
	APR	14.87	0	0.2	0.2	5000	0.01		6		34824	5588
	MAY	19.39	0	0.2	0.2	7500	0.01		13		28243	8931
	JUN	29.09	0	0.2	0.2	15000	0		14.4		36506	9347
	JUL	29.09	0	0.8	0.8	12000	0.01		30.2		42904	7331
	AUG	29.09	0	0.5	1	7500	0.01		12.6		38218	7227
	SEP	29.09	0	0	0	10000	0.01		19.8		35629	11396
	OCT	29.72	0	0.6	0.6	7000	0.6		21.2		46349	12083
	NOV	29.72	0	0	0	7000	0		17.2		46363	3241
	DEC	15.51	0	0	0	5000	0		27.3		18401	4101

Wells Hatchery

NPDES Permit Number WAG13-5009

		FLOW	SS EFF	TSS COMP	TSS MAX	FLOW PA	SS PA	SS %	TSS PA	TSS %	Lbs of Fish	Lbs of Feed
2016	JAN	17.38	0.01	0	0	**	**		**		68738	14203
	FEB	19.59	0.01	1.2	1.2	**	**		**		86459	18204
	MAR	24.67	0.01	1.4	1.4	**	**		**		102881	18878
	APR	6.62	0	-10.4	9.4	**	**		**		10038	286
	MAY	6.62	0	0.4	1.6	**	**		**		10708	1660
	JUN	6.62	-0.1	-0.2	8.4	**	**		**		15118	3432
	JUL	3.97	0.01	1	1	**	**		**		5613	2481
	AUG	4.19	0.01	0	0	**	**		**		9105	3393
	SEP	6.06	0	1.4	1.4	**	**		**		13849	4538
	OCT	7.39	0	0.8	0.8	9288	0.1		2.4		22216	5753
	NOV	8.61	0.03	3.4	3.4	15309	0.05		1.2		28056	9830
	DEC	8.68	0.02	1	1	17573	0.06		1.4		46313	13557

** PA pond - No discharge. PA pond system down during hatchery rebuild.

Chiwawa Ponds - Chiwawa River

NPDES Permit Number WAG13-5015

		FLOW	SS EFF	TSS COMP	TSS MAX	Lbs of Fish	Lbs of Feed	SS DD	TSS DD
2016	JAN	3.67	0	2	2	9716	353		
	FEB	2.87	0	-0.4	-0.4	9323	518		
	MAR	3.22	0	0	0	17838	2848	0.05	5.2
	APR	2.32	0	1	1	17477	1320	0.03	14.4
	MAY		No Monitoring			0	0		
	JUN		No Monitoring			0	0		
	JUL		No Monitoring			0	0		
	AUG		No Monitoring			0	0		
	SEP	4.6	0.03	-0.4	-0.4	6553	132		
	OCT	4.49	0	-2	-0.2	6553	619		
	NOV	4.22	0	0.4	0.4	7865	750		
	DEC	3.71	0	0.8	0.8	8288	241		

Chiwawa Ponds - Wenatchee River

NPDES Permit Number WAG13-5015

		FLOW	SS EFF	TSS COMP	TSS MAX	Lbs of Fish	Lbs of Feed	SS DD	TSS DD
2016	JAN	No Monitoring				0	0		
	FEB	No Monitoring				0	0		
	MAR	No Monitoring				0	0		
	APR	2.18	0	0.8	0.8	18309	2746		
	MAY	2.25	0			7500	0	0.05	50.6
	JUN		No Monitoring			0	0		
	JUL		No Monitoring			0	0		
	AUG		No Monitoring			0	0		
	SEP		No Monitoring			0	0		
	OCT		No Monitoring			0	0		
	NOV	3	0	-1.4	-1.4	11778	1316		
	DEC	6.91	0	0.2	0.2	14254	1150		

Methow Hatchery

NPDES Permit Number WAG13-5000

		FLOW	SS EFF	TSS COMP	TSS MAX	FLOW PA	SS PA	SS %	TSS PA	TSS %	Lbs of Fish	Lbs of Feed	SS DD	TSS DD
2016	JAN	7.98	0	0.2	0.2	14400	0.1		0.2		11800	850		
	FEB	7.98	0	0	0	14400	0.1		0		12400	925		
	MAR	6.4	0	0.5	1	14400	0.1		0.2		13000	970		
	APR	6.4	0	-1.6	-1.6	14400	0.1		0.2		15000	1000	0.1	7.6
	MAY	6.4	0	0	0	14400	0.1		0.2		16000	1100	0.1	1.2
	JUN	6.2	0	0.2	0.2	14400	0.1		0.4		4000	240		
	JUL	6.4	0	0	0	14400	0		0		4400	1700		
	AUG	6.4	0	0	0	14400	0		0.2		4900	2100		
	SEP	6.4	0	0.2	0.2	14400	0		0.4		6300	3150		
	OCT	5.83	0	0	0	14400	0		0		7200	1200		
	NOV	5.83	0	0	0	14400	0		0		9100	1560		
	DEC	9.86	0	0	0	14400	0		0		10300	1100		

Similkameen Hatchery

NPDES Permit Number WAG13-5007

		FLOW	SS EFF	TSS COMP	TSS MAX	FLOW PA	SS IW	TSS IW	Lbs of Fish	Lbs of Feed	SS DD	TSS DD
2016	JAN	6.62	0	-10.4	-10.4				10038	286		
	FEB	6.62	0	0.4	0.4				10708	1660		
	MAR	6.62	-0.1	-0.2	0.2				15118	3432		
	APR	6.62	0	-14.2	-14.2				17224	2322	0	13.8
	MAY			No Monitoring					0	0		
	JUN			No Monitoring					0	0		
	JUL			No Monitoring					0	0		
	AUG			No Monitoring					0	0		
	SEP			No Monitoring					0	0		
	OCT	6.48	0	-1	-1				5730	528		
	NOV	6.84	0	-1.6	-1.6				6624	1584		
	DEC	6.48	0	0.8	0.8				7548	0		

Chelan Hatchery

NPDES Permit Number WAG13-5006

		FLOW	SS EFF	TSS COMP	TSS MAX	FLOW PA	SS PA	SS %	TSS PA	TSS %	Lbs of Fish	Lbs of Feed
2016	JAN	5.2	0.05	0.4	0.4	68000	0.05		3.2		14000	5163
	FEB	7.2	0.05	0.2	0.2	68000	0.05		1		16000	7936
	MAR	7.2	0.05	1.2	1.2	68000	0.05		4.6		27000	6417
	APR	5.2	0.05	0.7	1	68000	0.05		2.6		10332	2324
	MAY	7.2	0.05	1.2	1.2	68000	0.05		7		5400	2076
	JUN	7.2	0.05	1.2	1.2	68000	0.05		2		4200	2105
	JUL	9.5	0.04	0.4	0.4	68000	0.05		2.8		4196	4137
	AUG	9.8	0.05	-0.8	-0.8	68000	0.05		2.2		5325	5766
	SEP	9.8	0.05	0.4	0.4	68000	0.05		1.8		9374	8256
	OCT	8.9	0.05	1.4	1.4	68000	0.05		2.8		32535	10733
	NOV	8.9	0.05	0	0	68000	0.05		1.8		20152	4236
	DEC	6.23	0.05	0.2	0.2	68000	0.05		1.6		9000	3420

Chelan Falls Hatchery

NPDES Permit Number WAG13-7019

		FLOW	SS EFF	TSS COMP	TSS MAX	FLOW PA	SS PA	SS %	TSS PA	TSS %	Lbs of Fish	Lbs of Feed
2016	JAN	12.8	0.05	-6.6	-6.6	857	0.05		0.8		23897	2475
	FEB	12.8	0.05	-2	-2	857	0.05		0.2		23595	1919
	MAR	12.8	0.05	-14	-14	857	0.05		0.8		24208	5895
	APR	12.8	0.05	-1.6	-1.6	857	0.05		1.2		27623	2409
	MAY	No Monitoring									0	0
	JUN	No Monitoring									0	0
	JUL	No Monitoring									0	0
	AUG	No Monitoring									0	0
	SEP	No Monitoring									0	0
	OCT	No Monitoring									0	0
	NOV	6.9	0.04	-0.6	-0.6	3000	0.05		0.6		25846	3779
	DEC	6.9	0.04	-0.4	-0.4	3000	0.05		1.4		28196	3344

Dryden Acclimation Pond

NPDES Permit Number WAG13-5014

		FLOW	SS EFF	TSS COMP	TSS MAX	Lbs of Fish	Lbs of Feed	SS DD	TSS DD
2016	JAN		No Monitoring			0	0		
	FEB		No Monitoring			0	0		
	MAR	14.2	0	0.2	0.2	35272	484		
	APR	14.08	0.01	-0.2	-0.2	43929	2024	-0.01	12.4
	MAY		No Monitoring			0	0		
	JUN		No Monitoring			0	0		
	JUL		No Monitoring			0	0		
	AUG		No Monitoring			0	0		
	SEP		No Monitoring			0	0		
	OCT		No Monitoring			0	0		
	NOV		No Monitoring			0	0		
	DEC		No Monitoring			0	0		

Priest Rapids

NPDES Permit Number WAG13-7013

		FLOW	SS EFF	TSS COMP	TSS MAX	FLOW PA	SS PA	TSS PA	Lbs of Fish	Lbs of Feed	SS DD	TSS DD
2016	JAN	22.8	0	0.9	1	**	**	**	5054	0		
	FEB	26.6	0	0.2	0.2	**	**	**	6759	539		
	MAR	40.73	0	-0.8	-0.8		0.01	55.2	15217	5674		
	APR	26.1	0	0.2	0.2		0	17	36203	21076		
	MAY	38.03	0	1.4	1.4		0	33.8	72648	33627		
	JUN	30.25	0	0.6	0.6		0	32	108095	37585	0	1.9
	JUL		No Monitoring						0	0		
	AUG		No Monitoring						0	0		
	SEP	57.24	0			**	**	**	3280	0		
	OCT	60.39	0			**	**	**	39030	0		
	NOV	62.67	0			**	**	**	25050	0		
	DEC	34.85	0	0.6	0.6	**	**	**	7062	0		

**PA pond - No discharge this month

Appendix G

Steelhead Stock Assessment at Priest Rapids Dam, 2014-2015

Priest Rapids Dam 2014-2015 Adult Upper Columbia River Steelhead Run-Cycle Stock Assessment Report

Introduction

Upper Columbia River (UCR) steelhead stock assessment sampling at Priest Rapids Dam (PRD) is authorized through the Endangered Species Act (ESA) Section 10 Permit 1395 (NMFS 2003). Permit authorizations include interception and biological sampling of up to 10 percent of the UCR steelhead passing PRD to determine upriver population size, estimate hatchery to wild ratios, determine age-class contribution and evaluate the need for managing hatchery steelhead consistent with ESA recovery objectives, which include fully seeding spawning habitat with naturally produced UCR steelhead supplemented with artificially propagated enhancement steelhead (NMFS 2003).

Stock Assessment

The 2014 steelhead sampling at Priest Rapids Dam began on 7 July and concluded 8 November. Sampling consisted of operating the Priest Rapids Off Ladder Fish Trap (OLAFT), located on the left-bank fishway at Priest Rapids Dam, 8 hours per day, up to three days per week, for a total of 53 sampling days. Steelhead were trapped, handled, and released in accordance with Section 2.1 and 2.2.1 of the National Marine Fisheries Service (NMFS) Biological Opinion for ESA Permit 1395 (NMFS 2003). The cumulative sample rate attained during 2014 totaled 17.3%.

The Washington Department of Fish and Wildlife (WDFW) sampled 3,428 steelhead of the 2014/2015 run-cycle passing PRD, totaling 19,766 steelhead, for an overall sampling rate of 17.3%. Of the 3,428 steelhead sampled, 2,262 (70.0%) were hatchery origin and 1,166 (30.0%) were wild origin. The estimated 2014-2015 run-cycle total wild steelhead return was 5,930 representing 207.2% of the 1986-2013 average and about 106.2% of the most recent 5-year average (Table 1).

Based on external marks and external and internal tags, 2,217 hatchery-origin steelhead were sampled at Priest Rapids Dam during the 2014 return cycle and included 30.4% Wenatchee hatchery-origin steelhead and 47.1% “above Wells Dam” hatchery-origin steelhead[1] (Table 2), while 11.0% of the hatchery-origin steelhead sampled could not be assigned to a specific hatchery program. Ringold FH origin steelhead represented about 11.5% of the hatchery sample (Table 2).

[1] Defined as “above Wells Dam” because hatchery origin, adipose-clipped steelhead released into the Methow and Okanogan rivers from the Wells FH and Winthrop NFH have the same marks and are indistinguishable from one another.

Table 1. Priest Rapids Dam adult steelhead returns and stock composition, 1974-2013.

Run-cycle[1/]	Hatchery	Wild	Wild percent	Total run
1974				2,950
1975				2,560
1976				9,490
1977				9,630
1978				4,510
1979				8,710
1980				8,290
1981				9,110
1982				10,770
1983				32,000
1984				26,200
1985				34,010
1986	20,022	2,342	10.5	22,364
1987	9,955	4,058	29.0	14,013
1988	7,530	2,670	26.2	10,200
1989	8,033	2,685	25.1	10,718
1990	6,252	1,585	20.2	7,837
1991	11,169	2,799	20.0	13,968
1992	12,102	1,618	11.8	13,720
1993	4,538	890	16.4	5,428
1994	5,880	855	12.7	6,735
1995	3,377	993	22.7	4,370
1996	7,757	843	9.8	8,600
1997	8,157	785	8.8	8,942
1998	4,919	928	15.9	5,847
1999	6,903	1,374	16.6	8,277
2000	9,023	2,341	20.6	11,364
2001	24,362	5,715	19.0	30,077
2002	12,884	2,983	18.8	15,867
2003	14,890	2,837	16.0	17,729
2004	15,670	2,985	16.0	18,655
2005	10,352	3,127	23.2	13,479
2006	8,738	1,677	16.1	10,415
2007	12,160	3,097	20.3	15,257
2008	13,528	3,030	18.3	16,558
2009	32,557	7,439	18.6	39,996
2010	18,784	7,647	28.9	26,431
2011	15,910	4,896	23.5	20,806
2012	13,908	3,284	19.1	17,192
2013	10,415	4,657	30.9	15,072
2014	13,836	5,930	30.0	19,766
1986-2013 average	**11,778**	**2,862**	**19.1**	**14,204**
2009-2013 average	**18,317**	**5,583**	**24.2**	**23,899**

[1/] A return cycle is the combined total of steelhead passing PRD from 1 June – 30 November during year (x), plus steelhead passing PRD between 15 April and 31 May on year (x+1).

Table 2. Origin classification of steelhead sampled at Priest Rapids Dam, 7 July – 8 November 2014.

Steelhead origin																						
Wild			Hatchery																			
Wild			Wenatchee						Above Wells				Ringold FH			Unk. Hat.						
Criteria			VIE						Criteria				Criteria			Criteria						
NS	NM	Total	LTGR	RTGR	RTOR	CWT	AD	Total	AD	Ped	LV	Total	AD	RV	Total	SD	NM	Total	Total Wild	Total Hatchery	Total
x	x	1,166	x					0	x			997	x	x	255	x	x	243	1,166	2,217	3,383
				x				0		x		11									
					x			0			x	36									
						x		141													
							x	534													
Total		**1,166**						**675**				**1,044**			**255**			**243**	**1,166**	**2,217**	**3,383**
% Hatchery								**30.4**				**47.1**			**11.5**			**11.0**		**100.0**	
% Total		**34.4**						**20.0**				**30.9**			**7.5**			**7.2**	**34.5**	**65.5**	**100.0**

Reconciliation of saltwater age of wild and hatchery steelhead sampled at Priest Rapids Dam during 2014 was accomplished through scale analysis. Salt-age analysis of the 2014 UCR steelhead run-cycle provides an estimated hatchery-origin return dominated by 1-salt and 2-salt age composition of 34.1% and 65.8%, respectively (Table 3). Natural-origin steelhead salt ages were 31.1% and 68.8% for salt ages-1 and 2, respectively. Three-salt age fish represented less than 0.1% of the combined hatchery/wild sample (Table 3).

Table 3. Salt-water age composition of 2014 – 2015 return cycle Upper Columbia River steelhead sampled at Priest Rapids Dam, corrected by scale age/origin determination.

	Origin					
	Hatchery		Wild		Combined	
Salt-age	***N***	**%**	***N***	**%**	***N***	**%**
1-salt	791	35.7	370	31.1	1161	34.1
2-salt	1,422	64.3	817	68.8	2239	65.8
3-salt	0	0.0	1	0.1	1	>0.1
4-salt	0	0.0	0	0.0	0	0.0
Total	**2,213**		**1,188**		**3,401**	

Freshwater residency of naturally produced Upper Columbia River steelhead present in the 2014-2015 run-cycle were dominated by age-2 freshwater fish (78.9%), and was only slightly lower than the 1986-2013 average of 74.2% (Table 4).

Table 4. 2014 return year freshwater age of wild Upper Columbia River steelhead sampled at Priest Rapids Dam during steelhead stock assessment activities, compared to July – November 1986-2013 average.

Freshwater age	**2014-2015 run cycle**		**1986-2013 proportion**	
	N	**%**	***N***	**%**
1.x	53	4.9	489	7.9
2.x	851	78.9	4,581	74.2
3.x	168	15.6	1,046	17.0
4.x	7	0.6	51	0.8
5.x	0	0.0	3	>0.1
Total	**1,079**		**6,170**	

Wild and hatchery-origin steelhead exhibited similar saltwater growth in the 2014 run-cycle. Wild 1and 2-salt adults were slightly larger than their hatchery cohorts (Table 5). Age-1 salt hatchery and age-1 and 2 salt wild steelhead observed in the 2014-2015 adult run-cycle return past PRD were comparable in size to the 1986-2013 run-cycle average (Table 5).

Table 5. Average fork length of 1-salt and 2-salt, Upper Columbia River steelhead sampled at Priest Rapids Dam during July – November 2014 and the period between 1986-2013.

	Average fork length (cm)			
	2014-2015 run cycle		1986-2013 run cycle	
Salt age	Wild	Hatchery	Wild	Hatchery
x.1	57.4	55.8	59.7	58.7
x.2	71.1	70.2	72.5	71.6

Appendix H

Wenatchee Sockeye Salmon Spawning Escapement, 2016

PUBLIC UTILITY DISTRICT NUMBER 1 OF CHELAN COUNTY
Natural Resource Division
Fish and Wildlife Department
327 N. Wenatchee Ave., Wenatchee WA 98801 (509) 663-8121

March 28, 2017

To: HCP Hatchery Committee

From: Catherine Willard and Scott Hopkins

Subject: 2016 Wenatchee Sockeye Mark/Recapture-Based Sockeye Escapement Estimates to Tributaries

Introduction

In 2016, the Chelan County Public Utility District (District) estimated sockeye escapement to tributaries based on mark-recapture methodology. The purpose of this document is to report the spawning escapement estimates for the Little Wenatchee and White River subbasins. This information is used to track and/or estimate viable salmonid population parameters (VSP): abundance, productivity, spatial structure, and diversity (McElhaney et al. 2000).

Methods

Mark-Recapture Method:

Detection efficiencies of the in-stream arrays were calculated for the Little Wenatchee River and White River in 2016. The in-stream arrays include a series of upstream and downstream coils (Figure 1). Combined, these coils represented the upstream and downstream detection arrays, respectively. Overall detection efficiency P_{all} of the arrays was calculated based on observed detection probabilities of individual arrays:

$$P_{all} = 1 - \left(1 - P_{array\,1}\right)\left(1 - P_{array\,2}\right)$$

where the probability of missing a fish on both the upstream P_{array1} and downstream P_{array2} arrays were combined for an overall efficiency P_{all} (Connolly et al. 2008).

Adult sockeye salmon were tagged at adult fishways within the Columbia River and at Tumwater Dam. Additionally, adult returns that were PIT tagged as juveniles were used in the analyses. Total passage of adult sockeye salmon through Tumwater Dam was obtained from Columbia River Data Access in Real Time (DART 2016). Resulting tag files were queried in PTAGIS (2016), providing detection histories for each study fish.

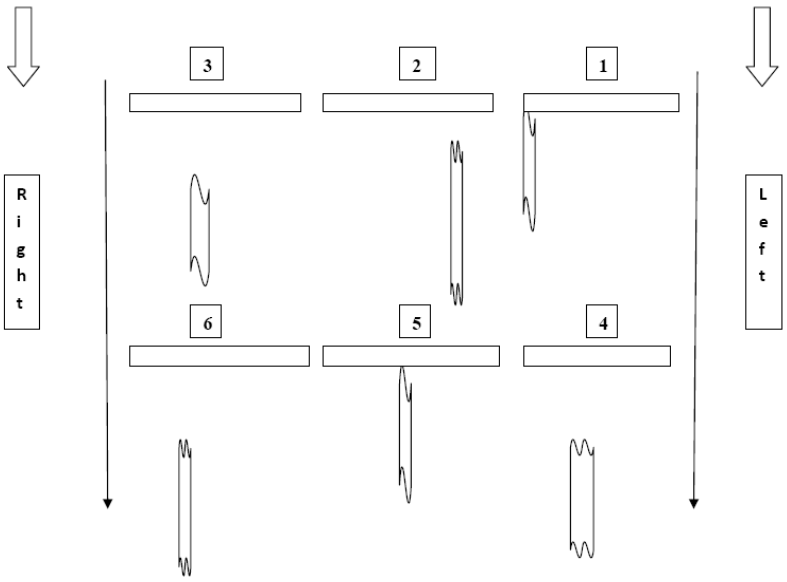


Figure 1. Schematic of a PIT array configuration.

Resulting data from passage at Tumwater Dam, mark and recapture using PIT tags, and detection efficiency estimates can provide estimation of escapement to spawning tributaries. Assumptions include: (1) the study population is "closed," i.e., no individuals die or emigrate between the initial mark and subsequent recaptures; (2) tags are not lost and detections are correctly identified; (3) all individuals have the same probability of being detected, and (4) the number of recapture events are proportional to the total population. Lastly, it was assumed that PIT-tagging efforts at Tumwater have negligible influence on fish behavior and tagged individuals behave similarly to untagged individuals. The resulting escapement rate, adjusted for detection efficiency, was then applied to the total population as such:

$$Escapement = \left(\frac{\left(\frac{Obs_{LWN}}{Eff_{LWN}} + \frac{Obs_{WTL}}{Eff_{WTL}} \right)}{PITs_{TUM}} \right) \times Counts_{TUM}$$

where the PIT tag detections (*Obs*) at the Little Wenatchee (*LWN*) and White River (WTL) were adjusted for detection efficiency (*Eff*), compared to the number released (*PITs*) at Tumwater Dam (*TUM*), and the resulting proportion was applied to the population observed (*Counts*) passing Tumwater Dam.

Results

Sockeye Salmon Mark-Recapture Method

Fishway enumeration at Tumwater Dam indicated that 73,697 adult sockeye salmon passed the facility during the 2016 migration, which was a sufficient return to open a recreational fishery in Lake Wenatchee for 2016. PIT tags were implanted in 790 fish at Tumwater and 630 fish were PIT-tagged before passing Tumwater; 130 fish were subsequently detected at the Little Wenatchee PIT tag array and 743 fish were subsequently detected at the White River PIT tag array (Table 1). Based on the recapture of PIT-tagged adult sockeye and assigned detection efficiency, total estimated escapement from Tumwater Dam to the Little Wenatchee River was 6,747 adult sockeye and 38,321 adult sockeye to the White River (Table 2).

Table 1. Number of adult sockeye salmon PIT-tagged, released, and detected upstream of Tumwater Dam in 2009 through 2016, and mark/recapture based tributary escapement estimates. Obs. = observed, D.E. = detection efficiency, Est = estimated (Obs./D.E.), and NA = not available.

Year	Number of PIT-tagged adults detected or tagged at Tumwater[1]	White River			Little Wenatchee River			Chiwawa River Obs.	Nason Creek Obs.
		Obs.	*D.E.* (p_{all})	*Est*	Obs.	*D.E.* (p_{all})	*Est*		
2009	1,085	381	0.406	*939*	38	0.971	*39*	37	7
2010	1,164	571	0.900[2]	*635*	67	1.000	*67*	3	1
2011	484	40	NA[3]	*NA*	84	--	*0*	0	0
2012	1,154	410	0.943	*435*	74	0.987	*75*	0	0
2013	719	152	NA[3]	*NA*	55	0.818	*67*	0	0
2014	1,729	848	0.999	*848*	76	1.000	*76*	0	3
2015[4]	950	371	0.999	*371*	50	1.000	*50*	69	4
2016	1,420	743	0.994	*738*	130	1.000	*130*	2	1

[1] Also includes fish detected downstream of release point (fallbacks).
[2] Detection efficiency p_{all} = 0.406 in 2009 was assigned from 2010 data.
[3] Technical difficulties with the White River PIT array prevented the calculation of detection efficiency and a mark-recapture based escapement estimate.
[4] In 2015, 45 sockeye salmon were detected in Chiwaukum Creek.

Table 2. Estimated escapement of adult sockeye salmon to Little Wenatchee and White rivers based on mark-recapture events, in-stream detection efficiency, and adult enumeration at Tumwater Dam, 2009-2016.

Year	Tumwater count	Recreational harvest	Little Wenatchee	White River	Combined	Escapement
2009	16,034	2,229	576	13,876	14,452	0.901
2010	35,821	4,129	2,062	19,542	21,604	0.603
2011[1]	18,634	0	2,431	14,582	17,013	0.913
2012	66,520	12,107	4,607	23,866	28,473	0.428
2013[1]	29,015	6,262	2,426	14,294	16,720	0.576
2014	99,898	16,281	4,319	49,021	53,340	0.534
2015	51,435	7,916	2,707	20,097	22,804	0.443
2016	73,697	14,630	6,747	38,321	45,068	0.612
Average	**48,882**	**7,944**	**3,234**	**24,200**	**27,434**	**0.626**

[1] Escapement was calculated using AUC counts for the Little Wenatchee River and a linear regression relationship to the Little Wenatchee River for the White River.

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Appendix I

Genetic Diversity of Wenatchee Sockeye Salmon

Assessing the Genetic Diversity of Lake Wenatchee Sockeye Salmon And Evaluating The Effectiveness Of Its Supportive Hatchery Supplementation Program

Developed for

Chelan County PUD

and the

Habitat Conservation Plan's Hatchery Committee

Developed by
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March 2008

Executive Summary

Nine spawning populations of sockeye (*Oncorhynchus nerka*) salmon have been identified in Washington, including stocks in the Lake Wenatchee basin (SaSI 5800) (Washington Department of Fisheries et al. 1993). Lake Wenatchee sockeye are classified as an Evolutionary Significant Unit (ESU), and consists of sockeye salmon that spawn primarily in tributaries above Lake Wenatchee (the White River, Napeequa River, and Little Wenatchee Rivers). Since 1990, the Wenatchee Sockeye Program has released juveniles into Lake Wenatchee to supplement natural production of sockeye salmon in the basin. The program's broodstock are predominantly natural-origin sockeye adults returning to the Wenatchee River captured at Tumwater Dam (Rkm 52.0), where a net-pen system is used to house both maturing adults and juveniles prior to release into Lake Wenatchee to over-winter.

Previous genetic studies have generally found a lack of concordance between population genetic relationships and their geographic distributions. These studies indicate that the nearest geographic neighbors of sockeye salmon populations are not necessarily the most genetically similar. Specifically for the Columbia River Basin, sockeye from Lake Wenatchee, Okanogan River, and Redfish Lake may be more closely related to a population from outside the Columbia River (depending on marker used) then to each other.

In this study we investigated the temporal and spatial genetic structure of Lake Wenatchee sockeye collections, without regard to sockeye populations outside of the Lake Wenatchee area. Our primary objective here was to determine if the Wenatchee Sockeye Program affected the natural Lake Wenatchee sockeye population. More specifically, we were tasked to determine if the genetic composition of Lake Wenatchee sockeye population had been altered by a supplementation program that was based on the artificial propagation of a small subset of that population. Using microsatellite DNA allele frequencies, we investigated population differentiation between temporally replicated collections of natural-origin Lake Wenatchee sockeye and program broodstock. We analyzed thirteen collections of Lake Wenatchee sockeye (Table 1), eight temporally replicated collections of natural-origin Lake Wenatchee sockeye (N=786) and five temporally replicated collections of Wenatchee Sockeye Program broodstock (N=248). Paired natural – broodstock collections were available from years 2000, 2001, 2004, 2006, and 2007.

Conclusions

We observed that allele frequency distributions were consistent over time, irrespective of collection origin, resulting in small and statistically insignificant measures of genetic differentiation among collections. We interpreted these results to indicate no year-to-year differences in allele frequencies among natural-origin or broodstock collections. Furthermore, there were no observed difference between pre- and post-supplementation collections. Therefore, we accepted our null hypothesis that the allele frequencies of the broodstock collections equaled the allele frequencies of the natural collections, which

equaled the allele frequency of the donor population. Given the small differences in genetic composition among collections, the genetic model for estimating N_e produced estimates with extremely large variances, preventing the observation of any trend in N_e.

Introduction

A report titled "Conceptual Approach to Monitoring and Evaluating the Chelan County Public Utility District Hatchery Programs" was prepared July 2005 by Andrew Murdoch and Chuck Peven for the Chelan PUD Habitat Conservation Plan's Hatchery Committee. This report outlined 10 objectives to be applied to various species assessing the impact (positive or negative) of hatchery operations mitigating the operation of Rock Island Dam. This current study pertains only to Lake Wenatchee sockeye and objective 3:

> *Determine if genetic diversity, population structure, and effective population size have changed in natural spawning populations as a result of the hatchery program. Additionally, determine if hatchery programs have caused changes in phenotypic characteristics of natural populations.*

In order to evaluate cause and effect of hatchery supplementation, WDFW Molecular Genetics Lab surveyed genetic variation of Lake Wenatchee sockeye. The conceptual approach for this project follows that of a parallel study regarding the Wenatchee River spring Chinook supplementation program (Blankenship et al. 2007). We determined the genetic diversity present in the Lake Wenatchee sockeye population by analyzing temporally replicated collections spanning 1989 – 2007, which included collections from before and following the inception of the Wenatchee Sockeye Program. Documenting the genetic composition of the Lake Wenatchee sockeye population is necessary to assess the effect of the hatchery program on the Lake Wenatchee population. In addition, this work provides a genetic baseline for future projects requiring genetic data. See study objectives below for specific details about how this project addresses Murdoch and Peven (2005) objective 3.

Lake Wenatchee Sockeye Salmon

Nine spawning populations of sockeye (*Oncorhynchus nerka*) salmon have been identified in Washington (Washington Department of Fisheries et al. 1993): 1) Baker

River, 2) Ozette Lake, 3) Lake Pleasant, 4) Quinault Lake, and 5) Okanogan River (classified as native stock); 6) Cedar River (classified as non-native stock); 7) Lake Wenatchee, classified as mixed stock); 8) Lake Washington/Lake Sammamish tributaries; and 9) Lake Washington beach spawners (classified as unknown origin). Chapman et al. (1995) listed four additional spawning aggregations of sockeye salmon that appear consistently in Columbia River tributaries: the Methow, Entiat, and Similkameen Rivers; and Icicle Creek in the Wenatchee River drainage.

Located in north central Washington, the Wenatchee River basin drains a portion of the eastern slope of the Cascade Mountains, including high mountainous regions of the Cascade crest. The headwater area of the Wenatchee River is Lake Wenatchee, a typical low productivity oligotrophic or ultra-oligotrophic sockeye salmon nursery lake (Allen and Meekin 1980, Mullan 1986, Chapman et al. 1995). Sockeye salmon bound for Lake Wenatchee enter the Columbia River in April and May and arrive at Lake Wenatchee in late July to early August (Chapman et al. 1995; Washington Department of Fisheries et al. 1993). The run timing of Lake Wenatchee sockeye salmon, classified as an Evolutionary Significant Unit (ESU), appears to have become earlier by 6 - 30 days during the past 70 years (Chapman et al. 1995; Quinn and Adams 1996). Additionally, scale pattern analysis suggests Wenatchee sockeye migrate past Bonneville Dam earlier than the sockeye bound for the Okanogan River (Fryer and Schwartzberg 1994). The Wenatchee population spawns from mid-September through October in the Little Wenatchee, White, and Napeequa Rivers above Lake Wenatchee (Washington Department of Fisheries et al. 1993), peaking in late September (Chapman et al. 1995). Limited beach spawning is believed to occur in Lake Wenatchee (L. Lavoy pers. com.; Mullan 1986), although Gangmark and Fulton (1952) reported two lakeshore seepage areas in Lake Wenatchee that were used by spawning sockeye salmon. Sockeye salmon fry enter Lake Wenatchee between March and May (Dawson et al. 1973), and typically rear in the lake for one year before leaving as smolts (Gustafson et al. 1997; Peven 1987).

Both the physical properties of the habitat and ecological/biological factors of the sockeye populations differ between the Lake Wenatchee ESU and the geographically

proximate Okanogan ESU. For example: 1) Different limnology is encountered by sockeye salmon in Lakes Wenatchee and Osoyoos; 2) Lake Wenatchee sockeye predominantly return at ages four and five (a near absence of 3-year-olds), where a large percentage of 3-year-olds return to the Okanogan population; and 3) the apparent one month separation in juvenile outmigration-timing between Okanogan- and Wenatchee-origin fish (Gustafson et al. 1997 and references therein).

Sockeye Artificial Propagation In Lake Wenatchee

The construction of Grand Coulee Dam completely blocked fish passage to the upper Columbia River, and 85% of sockeye salmon passing Rock Island Dam between 1935 and 1936 were estimated to be from natural stocks bound for areas up-river to Grand Coulee Dam (Mullan 1986; Washington Department of Fisheries et al. 1938). To compensate for loss of habitat resulting from Grand Coulee Dam, the federal government initiated the Grand Coulee Fish-Maintenance Project (GCFMP) in 1939 to maintain fish runs in the Columbia River above Rock Island Dam. Between 1939 and 1943, all sockeye salmon entering the mid-Columbia River were trapped at Rock Island Dam, and over 32,000 mixed Lake Wenatchee, Okanogan River, and Arrow Lake adult sockeye salmon were released into Lake Wenatchee (Gustafson et al. 1997 Appendix Table D-2). In addition to adult relocation, between 1941 and 1969 over 52.8 million fry descended from original spawners collected at Rock Island and Bonneville Dams, were released into Lake Wenatchee (Gustafson et al. 1997 Appendix Table D-2).

No releases of artificially-reared sockeye salmon occurred in the Wenatchee watershed during the years 1970 to 1989 (Gustafson et al. 1997 Appendix Table D-2). Since 1990, the Wenatchee Sockeye Program has released juveniles into Lake Wenatchee to supplement natural production of sockeye salmon in the basin. Sockeye adults returning to the Wenatchee River are captured at Tumwater Dam (Rkm 52.0) and transferred to Lake Wenatchee net pens until mature. The Wenatchee Sockeye Program goals are 260 adults with an equal sex ratio, <10% hatchery-origin returns (identified by coded wire tags), and the adults removed for broodstock account for <10% of the run size. Fish are spawned at Lake Wenatchee and their gametes are taken to Rock Island Fish Hatchery

Complex (i.e., Eastbank) for fertilization and incubation. Fry are returned to the Lake Wenatchee net -pens after they are large enough to be coded wire tagged, and are housed in the pens until fall (one year after spawning), when they are liberated into the lake to over-winter. For brood years 1991 – 2004 an average of 218,683 (std. dev. = 71,090) pen-reared Lake Wenatchee-origin juvenile sockeye salmon have been released yearly into Lake Wenatchee.

Previous Genetic Studies

Protein (allozyme) variation – Surveying genetic variation at 12 allozyme loci, Utter et al. (1984) reported moderate population structure among 16 sockeye collections from southeast Alaska through the Columbia River Basin, including Okanogan and Wenatchee stocks, with an apparent genetic association between upper Fraser River and Columbia River sockeye salmon. Winans et al. (1996) surveyed variation at 55 allozyme loci for 25 sockeye salmon and two kokanee collections from 21 sites in Washington, Idaho, and British Columbia, and reported the lowest level of allozyme variability of any species of Pacific salmon and a highest level of inter-population differentiation. Furthermore, these authors reported that there was no clear relationship between geographic and genetic differentiation among the populations within there study. Other studies corroborate the results of Winans et al. (1996), finding a lack of discernible geographic patterning for sockeye salmon populations in British Columbia, Alaska, and Kamchatka (Varnavskaya et al. 1994, Wood et al. 1994, Wood 1995). These studies indicate that the nearest geographic neighbors of sockeye salmon populations are not necessarily the most genetically similar, which contrasts with the other Pacific salmon species that exhibit concordance between geographic and genetic differentiation (Utter et al. 1989, Winans et al. 1994, Shaklee et al. 1991). As part of the comprehensive status review of west coast sockeye salmon (Gustafson et al. 1997), NMFS biologists collected new allozyme genetic information for 17 sockeye salmon populations and one kokanee population in Washington and combined these data for analysis with the existing Pacific Northwest sockeye salmon and kokanee data from Winans et al. (1996). Results of the updated study were consistent with Winans et al. (1996), with no clear concordance between geographic and genetic distances. Sockeye salmon from Lake Wenatchee, Redfish Lake,

Ozette Lake, and Lake Pleasant are very distinct from other collections in the study, and Columbia River populations were not necessarily most closely related to each other. Gustafson et al. (1997) also examined between-year variability within a collection location and found low levels of statistical significance among the five Lake Wenatchee collections included in the study (For 10 pair-wise comparisons using sum-G test, five were statistically significant). Lake Wenatchee brood year 1987 accounted for three of the significant comparisons, which were driven by unusually high frequencies of two allozyme alleles (ALAT*95 and ALAT*108) (Winans et al. 1996). Nevertheless, Gustafson et al. (1997) conclude that, in general, temporal variation at a locale was considerably less than between-locale variation.

Nucleic acid variation - Beacham et al. (1995) reported levels of variation in nuclear DNA of *O. nerka* using minisatellite probes. They analyzed 10 collections, including a sample from Lake Wenatchee. Cluster analysis showed the Lake Wenatchee sample was different from all the other collections, including those from the Columbia River. Using a similar molecular technique, Thorgaard et al. (1995) examined the use of multi-locus DNA fingerprinting (i.e., banding patterns) to discriminate among 14 sockeye salmon and kokanee populations. Dendrograms based on analysis of banding patterns produced different genetic affinity groups depending on the probes used. While none of the five DNA probes showed a close relationship between Lake Wenatchee and Okanogan River sockeye salmon, if information from all probes were combined, *O. nerka* from Redfish Lake, Wenatchee, and Okanogan were separate from kokanee of Oregon and Idaho and a sockeye salmon sample from the mid-Fraser River.

Study Objective

We documented temporal variation in genetic diversity (i.e., heterozygosity and allelic diversity), and investigated population differentiation between temporally replicated collections of natural-origin Lake Wenatchee sockeye and program broodstock, using microsatellite DNA allele frequencies. Temporally replicated collections from the same location can also be used to estimate effective population size (N_e). If populations are "ideal", the census size of a population is equal to the "genetic size" of the population.

Yet, numerous factors lower the "genetic size" below census, such as, non-equal sex ratios, changes in population size, and variance in the numbers of offspring produced from parent pairs. N_e is thought to be between 0.10 and 0.33 of the estimated census size (Bartley et al. 1992; RS Waples pers. comm.), although numerous observations differ from this general rule. N_e can be calculated directly from demographic data, or inferred from observed differences in genetic variance over time. Essentially, when calculated from genetic data, N_e is the estimated size of an "ideal" population that accounts for the genetic diversity changes observed, irrespective of abundance.

We will address the hypotheses associated with Objective 3 in Murdock and Peven (2005) using the following four specific tasks:

Task 1 - Document the observed genetic diversity.

Task 2 - Test for population differentiation among Lake Wenatchee collections and the associated supplementation program.

Task 2 was designed to address two hypotheses listed as part of Objective 3 in Murdoch and Peven (2005):

- Ho: Allele frequency $_{\text{Hatchery}}$ = Allele frequency $_{\text{Naturally produced}}$ = Allele frequency $_{\text{Donor pop}}$.
- Ho: Genetic distance between subpopulations $_{\text{Year x}}$ = Genetic distance between subpopulations $_{\text{Year y}}$

Murdoch and Peven (2005) proposed these two hypotheses to help evaluate supplementation programs through a "Conceptual Process" (Figure 5 in Murdoch and Peven 2005). There are two components to the first hypothesis, which must be considered separately for Lake Wenatchee sockeye. The first component involves comparisons between natural-origin populations from Lake Wenatchee to determine if there have been changes in allele frequencies through time starting with the donor population. Documenting a change does not necessarily indicate that the supplementation program has directly affected the natural-origin fish, as additional tests would be necessary to support that hypothesis. The intent of the second component is to determine if the hatchery produced populations have the same genetic composition as the naturally produced populations.

Task 3 - Calculate N_e using the temporal method for multiple samples from the same location to document trend.

Task 4 - Compare N_e estimates with trend in census size for Lake Wenatchee sockeye.

Methods and Materials

Sampling

Thirteen collections of Lake Wenatchee sockeye were analyzed, eight temporally replicated collections of natural Lake Wenatchee sockeye (N=786) and five temporally replicated collections of Wenatchee Sockeye Program broodstock (N=248) (Table 1). Paired natural – broodstock collections were available from years 2000, 2001, 2004, 2006, and 2007 (Table 1). All collections were made at Tumwater Dam on the Wenatchee River. Note that collections classified as broodstock were predominantly natural-origin sockeye. A majority of the genetic samples were from dried scales. The tissue collections from 2006 and 2007 were fin clips stored immediately in ethanol after collection. DNA was extracted from stored tissue using Nucleospin 96 Tissue following the manufacturer's standard protocol (Macherey-Nagel, Easton, PA, U.S.A.).

Laboratory Analysis

Polymerase chain reaction (PCR) amplification was performed using 17 fluorescently end-labeled microsatellite marker loci, *One* 2 (Scribner et al 1996) *One* 100, 101, 102, 105, 108, 110, 114, and 115 (Olsen et al. 2000), *Omm* 1130, 1135, 1139, 1142, 1070, and 1085 (Rexroad et al. 2001), *Ots* 3M (Banks et al. 1999) and *Ots* 103 (Small et al. 1998). PCR reaction volumes were 10 μL, with the reaction variables being 2 μL 5x PCR buffer (Promega), 0.6 μL $MgCl_2$ (1.5 mM) (Promega), 0.2 μL 10 mM dNTP mix (Promega), and 0.1 μL *Go Taq* DNA polymerase (Promega). Loci were amplified as part of multiplexed sets, so primer molarities and annealing temperatures varied. Multiplex one had an annealing temperature of 55°C, and used 0.09 Molar (M) *One* 108, 0.06 M *One* 110, and 0.11 M *One* 100. Multiplex two had an annealing temperature of 53°C, and used 0.08 M *One* 102, 0.1 M *One* 114, and 0.05 M *One* 115. Multiplex three had an annealing temperature of 55°C, and used 0.08 M *One* 105 and 0.07 M *Ots* 103. Multiplex four had

an annealing temperature of 53°C, and used 0.09 M *Omm* 1135 and 0.08 M *Omm* 1139. Multiplex five had an annealing temperature of 60°C, and used 0.2 M *Omm* 1085, 0.09 M *Omm* 1070, and 0.05 M *Ots* 3M. Multiplex six had an annealing temperature of 48°C, and used 0.06 M *One* 2, 0.08 M *Omm* 1142, and 0.08 M *Omm* 1130. *One* 101 was run in isolation with a primer molarity of 0.06. Thermal cycling was conducted on either PTC200 (MJ Research) or GeneAmp 9700 thermal cyclers as follows: 94°C (2 min); 30 cycles of 94°C for 15 sec., 30 sec. annealing, and 72°C for 1 min.; a final 72°C extension and then a 10°C hold. PCR products were visualized by denaturing polyacrylamide gel electrophoresis on an ABI 3730 automated capillary analyzer (Applied Biosystems). Fragment analysis was completed using GeneMapper 3.7 (Applied Biosystems).

Genetic data analysis

Assessing within collection genetic diversity - Heterozygosity measurements were reported using Nei's (1987) unbiased gene diversity formula (i.e., expected heterozygosity) and Hedrick's (1983) formula for observed heterozygosity. Both tests were implemented using the microsatellite toolkit (Park 2001). For each locus and collection FSTAT version 2.9.3.2 (Goudet 1995) was used to assess Hardy-Weinberg equilibrium, where deviations from the neutral expectation of random associations among alleles were calculated using a randomization procedure. Alleles were randomized among individuals within collections (4160 randomizations for this dataset) and the F_{IS} (Weir and Cockerham 1984) calculated for the randomized datasets were compared to the observed F_{IS} to obtain an unbiased estimation of the probability that the null hypothesis was true. The 5% nominal level of statistical significance was adjusted for multiple tests (Rice 1989). Genotypic linkage disequilibrium was calculated following Weir (1979) using GENETIX version 4.05 (Belkhir et al. 1996). Statistical significance of linkage disequilibrium results was assessed using a permutation procedure implemented in GENETIX for each locus by locus combination within each collection.

Assessing among collection genetic differentiation - The temporal stability of allele frequencies was assessed by the randomization chi-square test implemented in FSTAT version 2.9.3.2 (Goudet 1995). Multi-locus genotypes were randomized between

collections. The G-statistic for observed data was compared to G-statistic distributions from randomized datasets (i.e., null distribution of no differentiation between collections). Population differentiation was also investigated using pairwise estimates of F_{ST}. Multi-locus estimates of pairwise F_{ST}, estimated by a "weighted" analysis of variance (Weir and Cockerham, 1984), were calculated using GENETIX version 4.05 (Belkhir et al.1996). F_{ST} was used to quantify population structure, the deviation from statistical expectations (i.e., excess homozygosity) due to non-random mating between populations. To determine if the observed F_{ST} estimate was consistent with statistically expectations of no population structure, a permutation test was implemented in GENETIX (1000 permutations).

Effective population size (N_e) – Estimates of the effective population size were obtained using a multi-collection temporal method (Waples 1990a). The temporal method assumes that cohorts are used, but we did not decompose the collection year samples into their respective cohorts using age data. Therefore, N_e estimates that pertain to individual year classes of breeders are not valid; however the harmonic mean over all samples will estimate an N_e that pertains to the time period from which the collections are derived. Comparing samples from years *i* and *j*, Waples' (1990a) temporal method estimates the effective number of breeders ($\hat{N}_{b(i,j)}$) according to:

$$\hat{N}_{b(i,j)} = \frac{b}{2(\hat{F} - 1/\widetilde{S}_{i,j})}$$

The standardized variance in allele frequency ($\hat{F}$) is calculated according to Pollack (1983). The parameter b is calculated analytically from age structure information and the number of years between samples (Tajima 1992). The age-at-maturity information required to calculate b was obtained from ecological data (Hillman et al. 2007). The harmonic mean of sample sizes from years *i* and *j* is $\widetilde{S}_{i,j}$. The harmonic mean over all pairwise estimates of $\hat{N}_{b(i,j)}$ is $\widetilde{N}_b$. SALMONNb (Waples et al. 2007) was used to calculate $\widetilde{N}_b$.

Results and Discussion

In this section we combine our presentation and interpretations of the genetic analyses. Additionally, this section is organized based on the task list presented in the study plan.

Task 1 - Document the observed genetic diversity.

Substantial genetic diversity was observed over all Lake Wenatchee sockeye collections analyzed (Table 1), with heterozygosity estimates over all loci having a mean of 0.79. Genetic diversity was consistent with expected Hardy-Weinberg random mating genotypic proportions for all collections. The F_{IS} observed for each collection was not statistically significant given the distribution of F_{IS} generated using a randomization procedure. Additionally, there were no statistically significant associations observed between alleles across loci (i.e., linkage equilibrium) (data not shown). We concluded from these results that the genetic data from each collection was consistent with statistical expectations for random association of alleles within and between loci. In other words, each collection represents samples from a single gene pool (i.e., populations), and the genetic diversity observed has no detectable technical artifacts or evidence of natural selection.

Task 2 - Test for differentiation among Lake Wenatchee collections and the associated supplementation program.

We explicitly tested the hypothesis of no significant differentiation within natural-origin or broodstock collections from Lake Wenatchee using a randomization chi-square test. The null hypothesis for these tests was that the allele frequencies from two different populations were drawn from the same underlying distribution. We show the results for the pairwise comparisons among eight temporally replicated natural-origin collections from Lake Wenatchee (28 pairwise tests), and report all tests were non-significant (Table 2A). Similarly, for five temporally replicated broodstock collections, 10 of 10 pairwise tests were non-significant (Table 2B). We also tested if natural-origin and broodstock

collections were differentiated from each other over time, and report that 40 of 40 tests were non-significant (Table 2C). The nominal level of statistical significance ($\alpha = 0.05$) was adjusted for multiple comparisons using strict Bonferroni correction (Rice 1989). Yet, there are perhaps slight differences between paired natural-broodstock collections. Note that the p-values for comparisons regarding 2006 and 2007 paired collections are lower than for comparisons regarding 2000, 2001, and 2004. The small sample sizes for broodstock collections in 2006 and 2007 may not have been random samples from the Lake Wenatchee sockeye population.

Given the consistencies observed for allele frequency distributions over time, metrics of population structure were expected to be small. This was the case, as the estimated F_{ST} over all thirteen collections was 0.0003. This observed value fell within the distribution of F_{ST} values expected if there were no population structure present (permutation test p-value 0.12). Analysis of the paired natural-broodstock collections corroborated this result. Pairwise estimates of F_{ST} were 0.000 for years 2000, 2001, 2004, and 2007, and 0.002 for 2006. All five estimates were non-significant. Essentially, all 13 sockeye collections could be considered samples from the same population. Given these results, it is valid to combine all collections for statistical analysis. Therefore, we did not calculate genetic distances among any collections, as it is inappropriate to estimate distances that are effectively zero.

Conclusions

We interpret these data to indicate that there appears to be no significant year-to-year differences in allele frequencies among natural-origin or broodstock collections, nor are there observed differences between collections pre- and post-supplementation. As a result, we accept the null hypothesis that the allele frequencies of the broodstock collections equal the allele frequencies of the natural collections, which equals the allele frequency of the donor population. Furthermore, the observed genetic variance that can be attributed to among collection differences was negligible.

Task 3 - Calculate N_e using the temporal method for multiple samples from the same location to document trend.

The fundamental parameter for inferring N_e using genetic data is the standardized variance in allele frequency ($\hat{F}$) (Pollack 1983). Methods estimate N_e from observed changes in $\hat{F}$ over temporally replicated collections from the same location. Yet, as previously shown, there were no statistically significant differences detected in allele frequencies. The underlying model for estimating N_e produced estimates with extremely large variances, given small temporal differences in $\hat{F}$, which rendered any trend in N_e unobservable. Table 3 shows N_e estimates calculated using temporally replicated natural collections.

Task 4 - Compare N_e estimates with trend in census size for Lake Wenatchee sockeye.

See Task 3

Acknowledgements

We would like to thank Jeff Fryer (CRITFC) for providing critical collections of natural-origin sockeye from Lake Wenatchee. We would like to thank Norm Switzler for collection curation and Ken Warheit and Denise Hawkins for helpful comments regarding this project. This project was funded by Chelan County PUD and the Washington State General Fund.

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Table 1 Lake Wenatchee sockeye collections analyzed. MNA is the mean number of alleles per locus, Hz is unbiased heterozygosity, Obs Hz is observed heterozygosity, and HW is the p-value of the null hypothesis of random association of alleles (i.e., Hardy – Weinberg equilibrium). For reference, the nominal level of statistical significance at $\alpha = 0.05$ is 0.0002 after correction for multiple tests.

Year	Collection Code	Tissue Type	Source	N	MNA	Hz	Obs Hz	HW
1989	89[1]	Scales	Natural	96	14.35	0.792	0.791	0.424
1990	90[1]	Scales	Natural	96	13.19	0.793	0.779	0.131
2000	00AAE	Scales	Broodstock	96	12.31	0.787	0.776	0.213
2000	00[1]	Scales	Natural	96	11.76	0.801	0.826	0.868
2001	01AAS	Scales	Broodstock	53	9.47	0.788	0.793	0.392
2001	01[1]	Scales	Natural	96	14.35	0.786	0.794	0.456
2002	02[1]	Scales	Natural	96	14.53	0.794	0.777	0.780
2004	04[1]	Scales	Natural	96	14.65	0.798	0.803	0.704
2004	04AAV	Scales	Broodstock	43	14.35	0.796	0.795	0.051
2006	06CN	Tissue	Broodstock	38	14.59	0.793	0.785	0.688
2006	06CO	Tissue	Natural	96	14.53	0.806	0.803	0.408
2007	07EE	Tissue	Broodstock	18	14.00	0.790	0.790	0.221
2007	07EF	Tissue	Natural	96	14.35	0.789	0.800	0.347

[1] Samples taken from scale cards provided by Jeff Fryer (CRITFC)

Table 2 Allelic differentiation for Lake Wenatchee sockeye collections. A single analysis tested (pairwise) the allelic differentiation between all thirteen collections; however p-values for G-statistics are partitioned in the table by A) natural-origin, B) broodstock, and C) natural versus broodstock. Underlined values are for paired natural-broodstock collections from the same year. For reference, the nominal level of statistical significance at $\alpha = 0.05$ is 0.0006 after correction for multiple tests. No significant values were observed.

A) Natural-Origin Collections

	89	90	00	01	02	04	06CO	07EF
89		0.257	0.359	0.531	0.331	0.127	0.031	0.263
90			0.953	0.148	0.753	0.903	0.077	0.283
00				0.328	0.527	0.607	0.604	0.400
01					0.209	0.081	0.127	0.093
02						0.085	0.707	0.235
04							0.312	0.577
06CO								0.435
07EF								

B) Broodstock Collections

	00AAE	01AAS	04AAV	06CN	07EE
00AAE		0.189	0.090	0.008	0.058
01AAS			0.122	0.020	0.116
04AAV				0.008	0.031
06CN					0.326
07EE					

C) Natural vs. Broodstock

	89	90	00	01	02	04	06CO	07EF
00AAE	0.027	0.309	<u>0.572</u>	0.018	0.041	0.012	0.093	0.040
01AAS	0.115	0.471	0.160	<u>0.219</u>	0.519	0.049	0.654	0.133
04AAV	0.136	0.219	0.210	0.423	0.208	<u>0.328</u>	0.037	0.153
06CN	0.029	0.004	0.053	0.007	0.022	0.004	<u>0.019</u>	0.001
07EE	0.099	0.229	0.053	0.015	0.093	0.178	0.090	<u>0.037</u>

Table 3 Estimation of N_e for temporally replicated natural-original sockeye collections. Above the diagonal are pairwise estimates of N_e, where negative values mean sampling variance can account for genetic variance observed (i.e., genetic drift unnecessary). Below the diagonal are variances for pairwise estimates of N_e. Absent variance values (denoted by **-**) were too large for SalmonNb to display.

Collection	89	90	00	01	02	04	06CO	07EF
89		-3936.6	-1414	-2636.3	671.4	1871.1	1066.1	1951.2
90	2.59E+09		-1490.3	3649.1	-31144	-6808.4	817.6	93190.2
00	1.40E+09	4.45E+09		-592.2	-6842.2	-667.1	-1736.9	-1350.1
01	1.21E+09	1.47E+09	2.33E+09		977.1	6160.4	387.8	2531.5
02	1.91E+09	1.33E+09	1.16E+09	2.29E+09		1495.6	-848.5	3213.6
04	2.21E+09	3.62E+09	4.08E+09	1.27E+09	1.14E+09		896.6	2155.3
06CO	1.34E+09	1.39E+09	1.73E+09	-	4.51E+09	1.2E+09		3278.6
07EF	2.15E+09	1.51E+09	1.18E+09	1.68E+09	-	1.36E+09	2.65E+09	

Appendix J

Wenatchee Spring Chinook Redd Estimates, 2016

Spring Chinook Redd Estimates - 2016

Upper Wenatchee

Kevin See

December 22, 2016

Goals

Redd counts are an established method to provide an index of adult spawners (Gallagher et al. 2007). In the Wenatchee subbasin, spawning reaches are surveyed weekly during the spring Chinook spawning season (Jul 25, 2016 - Oct 03, 2016). The goals of this work are to:

- Estimate the true number of redds in each spawning reach with uncertainty.
- Summarize the number of redds at the tributary and population scale.

Methods

Data

Data were collected on the number of new redds during each survey (usually conducted about every week during the spawning season). Covariates such as surveyor experience, mean thalweg CV, and redd density (observed redds / km) were also collected on the reach scale to make predictions of surveyor error.

Surveyor Error

From the results of a previous study on spring Chinook, similar to the one outlined in Murdoch et al. (2014) for steelhead, we had a model that predicted surveyor net error (ratio of identified redds to true redds) based on covariates such as the surveyor's total experience with spawning ground surveys, the mean thalweg CV, and the observed redd density (redds/km). This model suggests that increasing experience and observed redd density lead to higher net error, while increasing the stream complexity (mean thalweg CV) leads to lower net error.

Because the net error model is a linear model, and therefore not constrained to be between 0 and 1 (less than 1 implies an underestimate of the number of redds, while net error greater than 1 implies an overestimate due to false identifications), we examined the values of the predictive covariates and compared them to the values used to fit the net error model. Several values were outside the range of the model dataset (See Figure 1). However,

using those more extreme values did not result in absurd predictions of observer error, so we did not alter or constrain them.

Values of the covariates for the net surveyor error model, colored by stream. Dashed lines depict the range of values from the data set used to develop the net error model.

Total Redds

Estimates of total redds were made for each reach using the Gaussian area under the curve (GAUC) model described in Millar et al. (2012). The GAUC model was developed with spawner counts in mind. As it is usually infeasible to mark every individual spawner, only total spawner counts can be used, and an estimate of average stream life must be utilized to translate total spawner days to total unique spawners. However, in adapting this for redd surveys, individual redds can be marked, and therefore we fit the GAUC model to new redds only. The equivalent of stream life thus becomes the interval between surveys. However, this year surveys were unable to be conducted during several weeks coinciding with peak spawning in the Chiwawa. Therefore, to fit the GAUC model, we used survey number instead of Julian day, and set the survey interval to one. We fit these models to reach-scale data, which did pose several challenges for a few reaches. We did not make

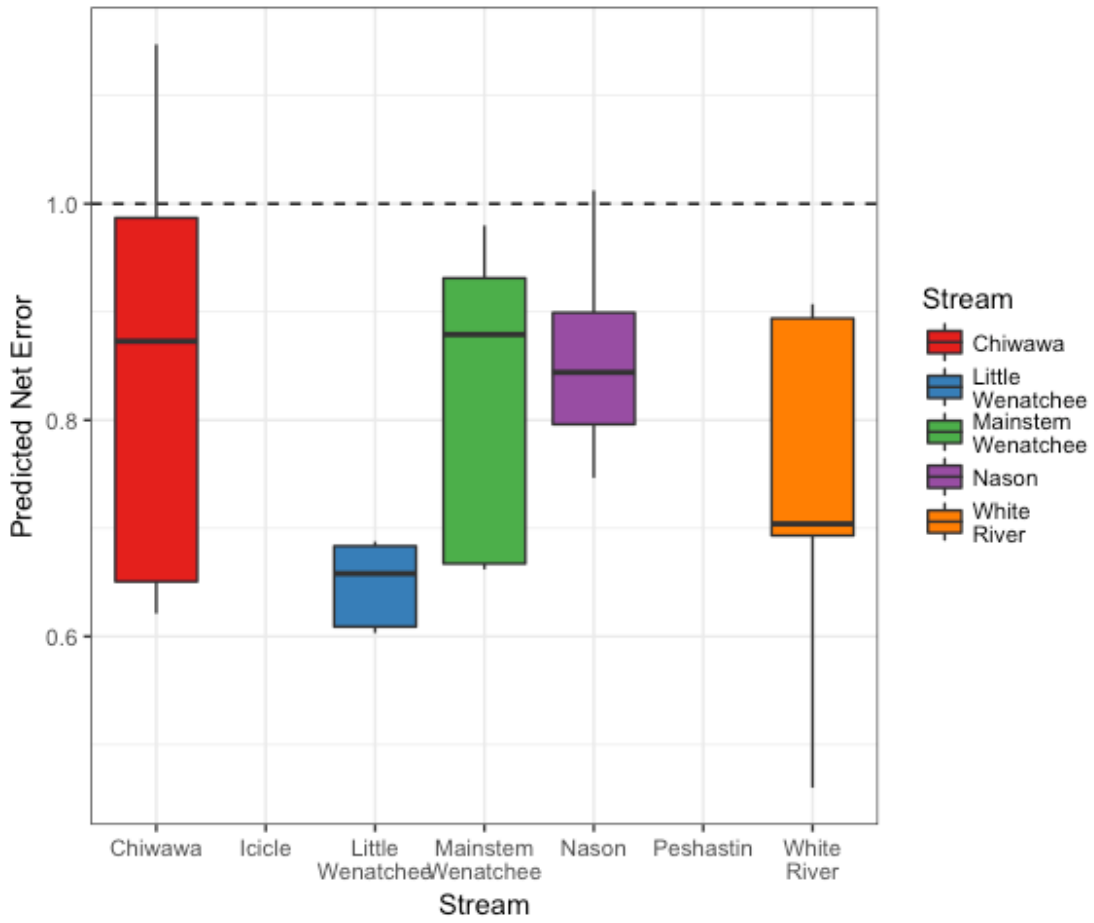
GAUC estimates for reaches that had fewer than 2 observed redds, or less than 3 weeks with at least one new redd observed.

When summing GAUC estimates at the reach-scale to obtain estimates at the stream scale, an attempt was made to incorporate the fact that the reaches within a stream are not independent. Estimates of correlation between the reaches within a stream were made based on weekly observed redds. This method may not be perfect, since spawners may use certain reaches preferentially at different times in the season, but it may be the best we can do. Because correlations are often quite high between reaches, this is a better alternative than to naively assume the standard errors between reaches are independent of one another. These estimates of correlation were combined with GAUC estimates of standard error for each reach to calculate a covariance matrix for the reaches within each stream, which was used when summing estimates of total redds to estimate the standard error at the stream-scale. Failure to incorporate the correlations between reaches would result in an underestimate of standard error at the stream scales. Different streams (and therefore reaches in different streams) were assumed to be independent.

Results

Surveyor Error

Predictions of net error are shown in Figure 2. Most predictions were less than one, implying some redds may have been missed. A few surveys had predictions of net error greater than one, implying some redds identified by surveyors were false redds.



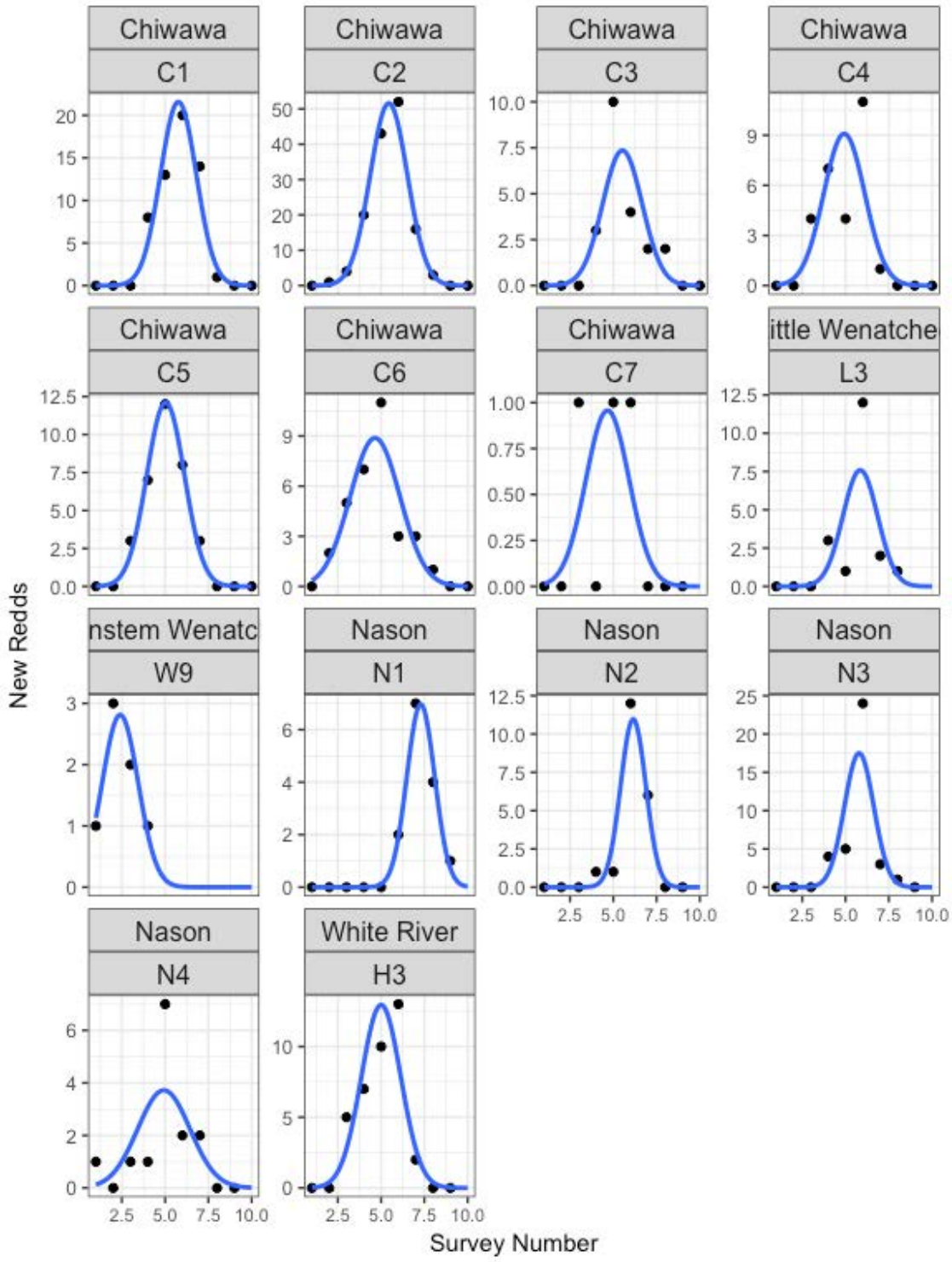
Boxplots showing predicted net error by stream. Dashed line shows no error.

Total Redds

Redds were estimated at the reach scale using the GAUC method whenever possible, and simply dividing the total number of observed redds by the predicted net error when not. For a few small tributary reaches, no estimates of observer error were made and instead the small number of observed redds was assumed to be observed without error. The estimates at the reach scale are displayed in Table 1. The curves that were fit in the GAUC process are shown in Figure 3. The results are summarized at the stream and population scale in Table 2.

Table 1: Estimates of total redds by reach.

Stream	Reach	Type	GAUC	Obs. Redds	Mean Net Error	Est. Redds	SE	CV
Chiwawa	C1	Major	Y	56	0.88	64	9.04	0.14
Chiwawa	C2	Major	Y	139	0.82	170	16.22	0.10
Chiwawa	C3	Major	Y	21	1.02	21	4.64	0.22
Chiwawa	C4	Major	Y	27	0.88	31	6.93	0.22
Chiwawa	C5	Major	Y	33	0.97	34	3.12	0.09
Chiwawa	C6	Major	Y	32	1.13	28	4.97	0.18
Chiwawa	C7	Major	Y	3	0.65	5	1.80	0.36
Chiwawa	K1	Minor	N	1	--	1	--	--
Chiwawa	R1	Minor	N	0	--	0	--	--
Chiwawa	S1	Minor	N	0	--	0	--	--
Icicle	I1	Minor	N	2	--	2	--	--
Icicle	I2	Minor	N	61	--	61	--	--
Icicle	I3	Minor	N	9	--	9	--	--
Little Wenatchee	L2	Major	N	3	0.69	4	1.33	0.33
Little Wenatchee	L3	Major	Y	19	0.61	31	13.43	0.43
Mainstem Wenatchee	A1	Minor	N	2	--	2	--	--
Mainstem Wenatchee	W10	Major	N	8	0.88	9	3.17	0.35
Mainstem Wenatchee	W9	Major	Y	7	0.67	11	2.30	0.21
Nason	N1	Major	Y	14	1.00	14	2.24	0.16
Nason	N2	Major	Y	20	0.85	23	5.94	0.26
Nason	N3	Major	Y	37	0.82	45	10.93	0.24
Nason	N4	Major	Y	14	0.76	18	7.17	0.40
Peshastin	D1	Minor	N	0	--	0	--	--
Peshastin	P1	Minor	N	0	--	0	--	--
Peshastin	P2	Minor	N	2	--	2	--	--
White River	H2	Major	N	4	0.69	6	1.86	0.31
White River	H3	Major	Y	37	0.85	43	8.14	0.19
White River	H4	Major	N	2	0.70	3	1.27	0.42
White River	Q1	Minor	N	1	--	1	--	--
White River	T1	Minor	N	0	--	0	--	--



Observed new redds by survey number and reach. Blue curve depicts the GAUC fitted curve.

Table 2: GAUC results at stream and population scale. Mean net error is the mean of net error estimates, weighted by the number of observed redds in each reach.

Stream	Obs. Redds	Mean Net Error	Est. Redds	Std. Err.	CV
Chiwawa	312	0.89	354	41.30	0.12
Icicle	72	--	72	0.00	0.00
Little Wenatchee	22	0.62	35	13.43	0.38
Mainstem Wenatchee	17	0.78	22	2.30	0.10
Nason	85	0.85	100	19.58	0.20
Peshastin	2	--	2	0.00	0.00
White River	44	0.83	53	8.14	0.15
Total	554	--	638	48.38	0.08

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Appendix K

Genetic Diversity of Chiwawa River Spring Chinook Salmon

Assessing the Genetic Diversity of Natural Chiwawa River Spring Chinook Salmon and Evaluating the Effectiveness of its Supportive Hatchery Supplementation Program

Developed for

Chelan County PUD

and the

Habitat Conservation Plan's Hatchery Committee

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Executive Summary

The main objective of this study was to determine the potential impacts of the Chiwawa River Supplementation Program on natural spring Chinook in the upper Wenatchee system. We did this by investigating population differentiation between temporally replicated Chiwawa River natural and hatchery samples from the Wenatchee River watershed using microsatellite DNA allele frequencies and the statistical assignment of individual fish to specific populations. Additionally, to assess the genetic effect of the hatchery program, we investigated the relationship between census and effective population sizes using collections obtained before and after the supplementation program. In this summary, we briefly describe the salient results contained within this report; however, each "Task" within the Results/Discussion section below contains extended coverage for each topic along with an expanded interpretation of each result.

Overall, we observed substantial genetic diversity within collections, with heterozygosities equal to roughly 80%, over thirteen microsatellite markers. Microsatellite allele frequencies among temporally replicated collections from the same population (i.e., location) were variable, resulting in significant genetic differentiation among these collections. However, these difference are likely the result of salmon life history in this area, as four-year-old Chinook comprise a majority of returns each year. That is, the genetic tests are detecting the differences of contributing parents from each cohort, rather than a hatchery effect.

Analysis of Chiwawa River Collections

To assess the multiple competing hypotheses regarding population differentiation within and among Chiwawa River collections, we found it necessary to organized the Chiwawa genetic data into three data sets: (1) fish origin (hatchery versus natural), (2) spawning location (hatchery broodstock versus in-river (natural) spawners), and (3) four "treatment" groups (1. hatchery-origin hatchery broodstock, 2. hatchery-origin natural spawner, 3. natural-origin natural spawner, and 4. natural-origin hatchery broodstock). We conducted separate analyses using each of the three data sets, with each analysis

touching on some aspect of the components necessary to move through the Conceptual Process outlined by Murdoch and Peven (2005).

Origin Dataset – We report that allele frequencies within and between natural- and hatchery-origin collections are significantly different, but there does not appear to be a robust signal indicating that the recent natural-origin collections have diverged greatly from the pre- or early post-supplementation collections. Genetic drift will occur in all populations, but does not appear to be a major factor affecting allele frequencies within the Chiwawa collections.

Spawning Location Dataset – There are significant allele frequency differences within and between hatchery broodstock and natural spawner collections. However, in recent years the allele frequency differences between the hatchery broodstock and natural spawner collections have declined. Furthermore, based on linkage disequilibrium, there is a genetic signal that is consistent with increasing homogenization of allele frequencies within hatchery broodstock collections, but a similar homogenization within the natural spawner collection is not apparent. These data suggest that there exists consistent year-to-year variation in allele frequencies among hatchery and natural spawning collections, but there is a trend toward homogenization of the allele frequencies of the natural- and hatchery-origin fish that compose the hatchery broodstock.

Four Treatment dataset – Although there are signals of allelic differentiation among Chiwawa River collections, there are no robust signs that these collections are substantially different from each other. We used two different analyses to measure the degree of genetic variation that exists among individuals and collections within the Chiwawa River. First, we conducted a principal component analysis using all Chiwawa samples with complete genotypes (i.e., no missing alleles from any locus). Although the first two principal component axes account for only 10.5% of the total molecular variance, a substantially greater portion of that variance is among individual fish, regardless of their identity, rather than among hatchery and natural collections. The

variances in principal component scores among individuals are 11 and 13 times greater than the variance in scores among collections.

Secondly, using an Analysis of Molecular Variance (AMOVA), we were able to determine how best to group populations, with "best" being defined as that grouping that accounts for the greatest proportion of among group (i.e., population) variance. Furthermore, by partitioning molecular variance into different hierarchical components, we are able to determine what level accounts for the majority of the molecular variance. The AMOVA results clearly show that nearly all molecular variation, no matter how the data are organized, resides within a collection. The percentage of total molecular variance occurring within collections ranged from 99.68% to 99.74%. These results indicate that the significant differences among collections of Chiwawa fish account for less than one percent of the total molecular variance, and these differences cannot be attributed to fish origin or spawning location.

Effective Population Size (N_e)

The contemporary estimate of N_e calculated using genetic data combined for Chiwawa natural-origin spawners (NOS) and hatchery-origin spawners (HOS) Chinook is N_e=386.8, which is slightly larger than the pre-hatchery N_e we estimated using demographic data from 1989 – 1992. Additionally, the N_e /N ratio calculated using 386.8 for N_e and the arithmetic mean yearly census of NOS and HOS Chinook from 1989 – 2005 for N is 0.40. These results suggest the N_e has not declined during the period of Chiwawa Hatchery Supplementation Program operation.

Analysis Of Upper Wenatchee Tributary Collections

We compared genetic data for spring Chinook collected from the major spawning aggregates of the Wenatchee River. We observed significant differences in allele frequencies among temporally replicated collections within populations, and among populations within the upper Wenatchee. However, these differences account for a very small portion of the overall molecular variance, and these populations overall are very similar to each other. Of all the populations within the Wenatchee River, the White River

appears to be the most distinct. Yet, this distinction is more a matter of detail than of large significance, as the median F_{ST} between White River collections and all other collections (except the Little Wenatchee collection; see Results/Discussion) is less than 1.5% among population variance. We consider the implications of these results in the Conclusion section that follows the Results/Discussion section. Additionally, there is no evidence that the Chiwawa River Supplementation Program has changed the allele frequencies in the Nason Creek and White River populations, despite the presence of hatchery-origin fish in both these systems.

Introduction

Murdoch and Peven (2005) outlined 10 objectives to assess the impact (positive or negative) of hatchery operations mitigating the operation of Rock Island Dam. Two objectives relate to monitoring the genetic integrity of populations:

Objective 3: Determine if genetic diversity, population structure, and effective population size have changed in natural spawning populations as a result of the hatchery program. Additionally, determine if hatchery programs have caused changes in phenotypic characteristics of natural populations.

Objective 5: Determine if the stray rate of hatchery fish is below the acceptable levels to maintain genetic variation between stocks.

This study addresses Objective 3 (above), and documents analyses and results WDFW completed for populations of spring Chinook (*Oncorhynchus tshawytscha*) in the Wenatchee River watershed. This study was not intended to specifically address Objective 5 (above); however, genetic data provide results relevant to Objective 5. The critical component of Objective 3 is to determine if hatchery supplementation has effected change. Furthermore, change in this context means altering census size and/or genetic marker allele frequencies; we did not attempt to measure changes in fitness. Perhaps a more meaningful rewording of Objective 3 is, "Did the hatchery supplementation program succeed at increasing the census size of a target population while leaving genetic integrity intact?" In order to evaluate cause and effect of hatchery supplementation, we surveyed and compared genetic variation in samples collected before and after potential effects from the Chiwawa Hatchery Supplementation Program. Samples were acquired from the primary spawning aggregates in the upper Wenatchee River watershed: Nason Creek, Little Wenatchee River, White River, and Chiwawa River. Hatchery samples were acquired from programs that could potentially affect genetic composition of Wenatchee stocks, the integrated Chiwawa River stock (local stock), Leavenworth National Fish Hatchery spring Chinook (Carson Stock – non local), and Entiat NFH (Carson Stock – non local). Additionally, the genetic markers used were the Genetic Analysis of Pacific Salmonids (GAPS) (Seeb et al. in review) standardized

microsatellites, so all data from the Wenatchee study will be available for inclusion in the GAPS Chinook coastwide microsatellite baseline.

History of Artificial Propagation

Artificial propagation in the upper Columbia River began in 1899 when hatcheries were constructed on the Wenatchee and Methow rivers (Mullan 1987). These initial operations were small, with the Tumwater Hatchery on the Wenatchee River releasing several hundred thousand fry, and the Methow River hatchery producing few Chinook salmon before it was closed in 1913 (Craig and Suomela 1941, Nelson and Bodle 1990). The Leavenworth State Hatchery operated in the Wenatchee River Basin between 1913 and 1931 using eggs from non-native stocks (Willamette River spring-run and lower Columbia Chinook hatchery fall-run). These early attempts at hatchery production were largely unsuccessful for spring-run Chinook (WDF 1934). Between 1931 and 1939, no Chinook salmon hatcheries were in operation above Rock Island Dam (Rkm 730).

In 1938, the last salmon was allowed to pass upstream through the uncompleted Grand Coulee Dam (Rkm 959). To mitigate the loss of habitat, adult Chinook salmon were trapped, under the auspices of the Grand Coulee Fish Maintenance Project (GCFMP), at Rock Island Dam beginning in May 1939, and relocated into three of the remaining accessible tributaries to the upper Columbia River: the Wenatchee, Entiat, and Methow Rivers. GCFMP transfers continued through the autumn of 1943. Spring- and summer/fall-run fish were differentiated at Rock Island Dam based on a 9 July cutoff date for Chinook arrivals at Rock Island Dam (Fish and Hanavan 1948). Spring-run adults collected at Rock Island Dam (pre 9 July fish) were either transported to Nason Creek on the Wenatchee River to spawn naturally (1939-43), or to the newly constructed Leavenworth NFH (1940) for holding and subsequent spawning (1940-43). Eggs were incubated on site or transferred to the Entiat NFH (1941) and Winthrop NFH (1941). In 1944 spring-run adults were allowed to freely pass Rock Island Dam. The GCFMP did not differentiate among late-run stocks (post 9 July fish) passing Rock Island Dam. Late-run offspring reared at the Leavenworth NFH, Entiat NFH, and Winthrop NFHs were an

amalgamation of summer and fall upper Columbia River populations (Fish and Hanavan 1948). Late-run fish were transplanted into the upper and lower Wenatchee, Methow, and Entiat Rivers.

After 1943, the Winthrop NFH continued to use local spring-run Chinook for hatchery production, while the other NFHs largely focused on summer-run Chinook salmon. Renewed emphasis on spring run production in the mid-1970s saw the inclusion of local and non-local eggs (Carson NFH stock, Klickitat River stock, and Cowlitz River stock) to the NFHs. In the early 1980s, imports of non-native eggs were reduced significantly, and thereafter the Leavenworth, Entiat, and Winthrop NFHs have relied on adults returning to their facilities for their egg needs (Chapman et al. 1995). Regarding late-run Chinook, due to the variety of methods employed to collect broodstock at dams, hatcheries, or the result of juvenile introductions into various areas, Chinook populations and runs (i.e., summer and fall) have been mixed considerably in the upper Columbia system over the past five decades (reviewed in Chapman et al. 1994).

Washington Department of Fish and Wildlife (WDFW) operates two facilities producing spring-run Chinook, the Methow Fish Hatchery (MFH) owned by Douglas County PUD that began operation in 1992 and Eastbank Fish Hatchery (EFH) owned by Chelan County PUD that began operation in 1989. Both programs were designed to implement supplementation (supportive breeding) programs for naturally spawning populations on the Methow and Wenatchee Rivers, respectively (Chapman et al. 1995). As part of the Rock Island Mitigation Agreement between Chelan County Public Utility District and the fishery management parties (RISPA 1989), a supplementation (supportive breeding) program was initiated in 1989 on the Chiwawa River to mitigate smolt mortality resulting from the operation of Rock Island Hydroelectric Project. EFH uses broodstock collected at a weir on the Chiwawa River, although in recent years hatchery fish have been collected at Tumwater Dam. Similarly, the MFHC uses returning adults collected at weirs on the Methow River and its tributaries, the Twisp and Chewuch Rivers (Chapman et al. 1995; Bugert 1998). Although low run size and trap efficiency has resulted in most broodstock being collected from the hatchery outfall or in some years Wells Dam,

progeny produced from these programs are reared at and released from satellite sites on the tributaries where the adults were collected. Numerous other facilities have reared spring-run Chinook salmon on an intermittent basis.

Previous Genetic Studies – Population differentiation

Waples et al. (1991a) examined 21 polymorphic allozyme loci in samples from 44 populations of Chinook salmon in the Columbia River Basin. These authors reported three major clusters of Columbia River Basin Chinook salmon: 1) Snake River spring- and summer-run Chinook salmon, and mid and upper Columbia River spring-run Chinook salmon, 2) Willamette River spring-run Chinook salmon, 3) mid and upper Columbia River fall- and summer-run Chinook salmon, Snake River fall-run Chinook salmon, and lower Columbia River fall- and spring-run Chinook salmon. Utter et al. (1995) examined allele frequency variability at 36 allozyme loci in samples of 16 upper Columbia River Chinook populations. Utter et al. (1995) indicated that spring-run populations were distinct from summer- and fall-run populations, where the average genetic distance between spring-run and late-run Chinook were about eight times the average of genetic distances between samples within each group. Additionally, allele frequency differences among spring-run populations were considerably greater than that among summer- and fall-run populations in the upper Columbia River. Utter et al. (1995) also reported hatchery populations of spring-run Chinook salmon were genetically distinct from natural spring-run populations, but hatchery populations of fall-run Chinook salmon were not genetically distinct from natural fall-run populations.

As part of an evaluation of the relative reproductive success for the Chiwawa River supplementation program, Murdoch et al. (2006), used eleven microsatellite loci to assess population differentiation among spring Chinook salmon population samples in the upper Wenatchee River. Murdoch et al. (2006) reported a >99% accuracy of correctly identifying spring-run and fall-run Chinook from the Wenatchee River. They also reported slight, but significantly different genetic variation among wild spring populations and between wild and hatchery stocks. Yet, since the spring-run populations

are genetically similar, identifying individuals genetically from the upper tributaries of the Wenatchee River was difficult. This result is exemplified in their individual assignment results, where < 8% of spring-run individuals, hatchery or wild, were correctly assigned using their criterion of an LOD (log of odds) score greater than 2. Murdoch et al. (2006) also reported contemporary natural spring Chinook show heterozygote deficit and low linkage disequilibrium (LD), while contemporary hatchery spring Chinook show heterozygote excess and high LD.

Williamson et al. (submitted) have continued the work of Murdoch et al. (2006) by analyzing Chiwawa River demographic data from 1989 – 2005 to estimate the proportions of recruits that were produced by Chinook with hatchery or wild origin. In an "ideal" population, the genetic size (i.e., effective size or N_e) and the census size are equal; however various demographic factors such as unequal sex ratios and variance in reproductive success among individuals reduces the genetic size below the census size. It is generally thought that the genetic size is approximately 10-33% the census size (Bartley et al. 1992; RS Waples pers. comm.), although values have been reported outside this range (Araki et al. 2007; Arden and Kapuscinski 2003; Heath et al. 2002). Despite being difficult to estimate, the effective population size in many respects is a more important parameter to know than census size, because N_e determines how genetic diversity is distributed within populations and how the forces of evolution (i.e., forces that change genetic diversity over time) will affect the genetic variation present.

Williamson et al. (submitted) used demographic data to 1) investigate the effect of unequal sex ratio on genetic diversity, 2) investigate the effect of variation in reproductive success on genetic diversity, 3) investigate the effect of fluctuations in population size on genetic diversity, and 4) estimate the effective population size, using the inbreeding method (Ryman and Laikre 1991). Most importantly, they use demographic data from 1989 – 2000 to assess the impact of the Chiwawa Hatchery Supplementation Program on the effective population size of natural-origin Chiwawa River spring Chinook. They estimate that the N_e of naturally spawning Chiwawa Chinook (i.e., both hatchery- and wild-origin fish on the spawning grounds) from 1989 –

1992 was N_e = 2683 and in 1997 – 2000 was N_e = 989. They compare spawning ground N_e to estimates calculated from combined broodstock and naturally spawning Chinook demographic data. The combined inbreeding N_e estimate from 1989 – 1992 was N_e = 147 and in 1997 – 2000 was N_e = 490. Williamson et al. (submitted) argue that since the combined N_e estimate is lower than the naturally spawning estimate, the supplementation program has had a negative impact on the Chiwawa River N_e.

Williamson et al. (submitted) also present genetic data for Chinook recovered on spawning grounds in upper Wenatchee River tributaries in 2004 and 2005. These genetic data are derived from the Murdoch et al. (2006) study. They compare samples collected from Chiwawa River (i.e., hatchery and wild), White River, Nason Creek, and Leavenworth Hatchery. Additionally, they include a 1994 Chiwawa River wild smolt sample for comparison with the 2004 brood year. Williamson et al. (submitted) report statistically significant genetic differentiation among Chiwawa River, White River and Nason Creek. Additionally, they report that the 1994 and 2004 Chiwawa River wild samples are not statistically different, but the 2004 Chiwawa wild and hatchery collections are statistically different.

Study Objectives

This study investigated within and among population genetic diversity to assess the effect of the Chiwawa Hatchery's supplemental program on the natural Chiwawa River spring Chinook population. Differences among temporal population samples, the census size, heterozygosity, and allelic diversity were documented. We investigated population differentiation between the Chiwawa River natural and hatchery samples, and among all temporally replicated samples from the Wenatchee River watershed using microsatellite DNA allele frequencies and the statistical assignment of individual fish to specific populations. To assess the genetic effect of the hatchery program, correlation between census and effective population sizes were investigated using temporally replicated samples obtained before and after the supplementation program operation. To address the hypotheses associated with Objective 3 in Murdock and Peven (2005) we developed

eleven specific "Tasks" (Blankenship and Murdoch 2006), to which we analyzed specific genetic data. We present the results from these analyses specific to each individual Task.

Methods and Materials

Tissue collection and DNA extraction

We analyzed thirty-two population collections of adult spring Chinook salmon (*Oncorhynchus tshawytscha*) obtained from the Wenatchee River between 1989 and 2006 (Table 1). Nine collections of natural Chinook adults from the Chiwawa River (n=501), and nine collections of Chiwawa Hatchery Chinook (n=595) were collected at a weir located in the lower Chiwawa River. The 1993 and 1994 Chiwawa Hatchery samples are smolt samples from the 1991 and 1992 hatchery brood years, respectively. Additional samples were collected from upper Wenatchee River tributaries, White River, Little Wenatchee River, and Nason Creek. Six collections of natural White River Chinook (n=179), one collection from the Little Wenatchee (n=19), and six collections from Nason Creek (n=268) were obtained. Single collections were obtained for Chinook spawning in the mainstem Wenatchee River and Leavenworth National Fish Hatchery. An additional out-of-basin collection from Entiat River was also included in the analysis. Samples collected in 1992 or earlier are scale samples. All other samples were either fin clips or operculum punches, stored immediately in ethanol after collection. DNA was extracted from stored tissue using Nucleospin 96 Tissue following the manufacturer's standard protocol (Macherey-Nagel, Easton, PA, U.S.A.).

Laboratory analysis

We performed polymerase chain reaction (PCR) amplification on each fish sample using the 13 fluorescently end-labeled microsatellite marker loci standardized as part of the GAPS project (Seeb et al. in review). GAPS genetic loci are: *Ogo*2, *Ogo*4 (Olsen et al. 1998); *Oki*100 (unpublished); *Omm*1080 (Rexroad et al. 2001); *Ots*201b (unpublished); *Ots*208b, *Ots*211, *Ots*212, and *Ots*213 (Grieg et al. 2003); *Ots*3M, *Ots*9 (Banks et al.

1999); *Ots*G474 (Williamson et al. 2002); *Ssa*408 (Cairney et al. 2000). PCR reaction volumes were 10 μL, and contained 1 μL 10x PCR buffer (Promega), 1.0 μL MgCl2 (1.5 mM final) (Promega), 0.2 μL 10 mM dNTP mix (Promega), and 0.1 units/mL Taq DNA polymerase (Promega). Loci were amplified as part of multiplexed sets, so primer molarities and annealing temperatures varied. Multiplex one had an annealing temperature of 50°C, and used 0.37 Molar (M) *Oki*100, 0.35 M *Ots*201b, and 0.20 M *Ots*208b, and 0.20 M *Ssa*408. Multiplex two had an annealing temperature of 63°C, and used 0.10 M *Ogo*2, and 0.25 M of a non-GAPS locus (*Ssa* 197). Multiplex three had an annealing temperature of 56°C, and used 0.18 M *Ogo*4, 0.18 M *Ots*213, and 0.16 M *Ots*G474. Multiplex four had an annealing temperature of 53°C, and used 0.26 M *Omm*1080, and 0.12 M *Ots*3M. Multiplex five had an annealing temperature of 60°C, and used 0.30 M *Ots*212, 0.20 M *Ots*211, and 0.10 M *Ots*9. Thermal cycling was conducted on either a PTC200 thermal cycler (MJ Research) or GeneAmp 9700 (Applied Biosystems) as follows: 95°C (2 min); 30 cycles of 95°C for 30 sec., 30 sec. annealing, and 72°C for 30 sec.; a final 72°C extension and then a 10°C hold. PCR products were visualized by electrophoresis on an ABI 3730 automated capillary analyzer (Applied Biosystems). Fragment analysis was completed using GeneMapper 3.7 (Applied Biosystems). Standardization of genetic data to GAPS allele standards was conducted following Seeb et al. (in review).

Genetic data analysis

Assessing within population genetic diversity - Heterozygosity measurements are reported using Nei's (1987) unbiased gene diversity formula (i.e., expected heterozygosity) and Hedrick's (1983) formula for observed heterozygosity. Both tests are implemented using the microsatellite toolkit (Park 2001). We used GENEPOP version 3.4 (Raymond and Rousset 1995) to assess Hardy-Weinberg equilibrium (HWE), where deviations from the neutral expectation of random associations among alleles are calculated using a Markov chain method (5000 iterations in this study) to obtain unbiased estimates of Fisher's exact test. Global estimates of F_{IS} according to Weir and Cockerham (1984) were calculated using GENEPOP version 3.4. Genotypic linkage disequilibrium was calculated following Weir (1979) using GENEPOP version 3.4.

Linkage results for population collections are reported as the proportion of pairwise (locus by locus) tests that are significant (alpha = 0.01). Linkage disequilibrium is considered statistically significant if more than 5% of the pairwise tests based on permutation are significant for a collection.

Within- and among-population genetic differentiation – The temporal stability of allele frequencies within populations, and pairwise differences in allele frequencies among populations were assessed using several different procedures. First, we tested for differences in allele frequencies among populations defined in Table 1 using a randomization chi-square test implemented in GENEPOP version 3.4 (Raymond and Rousset 1995). This procedure tests for differences between pairs of populations where alleles are randomized between the populations (i.e., genic test). The null hypothesis for this test is that the allele frequency distributions between two populations are the same. A low p-value should be interpreted as the allele frequency distributions being compared are unlikely to be samples drawn from the same underlying distribution.

Second, to graphically describe allele frequency differences among populations we conducted a nonmetric multidimensional scaling analysis using allele-sharing distance matrices from two different data sets. Pairwise allele-sharing distances are calculated as 1 – (mean over all loci of the sums of the minima of the relative frequencies of each allele common to a pair of populations). To calculate the allele-sharing distances for each pair of populations we used PowerMarker v3.25 (Liu and Muse 2005). Nonmetric multidimensional scaling is a technique designed to construct an n-dimensional "map" of populations, given a set of pairwise distances between populations (Manly 1986). The output from this analysis is a set of coordinates along n-axes, with the coordinates specific to the number of n-dimensions selected. To simplify our analysis we selected a 2-dimensional analysis to represent the relative positions of each population in a typical bivariate plot. The goodness of fit between the original allele-sharing distances and the pairwise distances between all populations along the 2-dimensional plot is measured by a "stress" statistic. Kruskal (in Rohlf 2002) developed a five-tier guide for evaluating stress levels, ranging from a perfect fit (stress=0) to a poor fit (stress=0.40). We

conducted the nonmetric multidimensional scaling analysis for one data set containing Chiwawa natural- and hatchery-origin collections, and another data set containing Chiwawa broodstock and in-river spawner collections. We used the mdscale module in MATLAB R2006b (The Mathworks 2006) to generate the nonmetric multidimensional scaling coordinates.

We examined the geographic and temporal structure of populations in the upper Wenatchee (Chiwawa River, Nason Creek, and White River, only) using a series of analyses of molecular variance (AMOVAs). Here, we defined an AMOVA as an analysis of variance of allele frequencies, as originally designed by Cockerham (1969), but implemented in Arlequin v2.1 (Schneider et al. 2000). These analyses permit populations to be aggregated into groups, and molecular variance is then partitioned into within collections, among collections, but within groups, and among group components. With this approach, we were able to determine how best to group populations, with "best" being defined as that grouping that accounts for the greatest proportion of among group variance. Furthermore, by partitioning molecular variance into three different hierarchical components, we are able to determine what level accounts for the majority of the molecular variance.

Finally, we explored the partitioning of molecular variance between among-individuals and among-populations using a principal component analysis and multi-locus estimates of pairwise F_{ST}, estimated by a "weighted" analysis of variance (Weir and Cockerham, 1984). Principal component analysis is a data-reduction technique whereby the correlation structure among variables can be used to combine variables into a series of multivariate components, with each original variable receiving a weighted value for each component based on its correlation with that component. Here, we used a program written by Warheit in MATLAB R2006b (The Mathworks 2006) that treats each allele for each locus as a single variable (13 loci = 26 alleles or variables), and these 26 "variables" were arranged into 26 components, with each component accounting for a decreasing amount of molecular variance. Estimates of F_{ST} were calculated using GENETIX version 4.05 (Belkhir et al.1996). To determine if the F_{ST} estimates were

statistically different from random (i.e., no structure), 1000 permutations were implemented in GENETIX version 4.05 (Belkhir et al.1996).

Effective population size (N_e) – Estimates of the effective population size were obtained using two methods, a multi-collection temporal method (Waples 1990), and a single-collection method (Waples 2006) using linkage disequilibrium data. The temporal method assumes that cohorts are used, but we did not decompose the collection year samples into their respective cohorts using age data. Therefore, N_e estimates that pertain to individual year classes of breeders are not valid; however the harmonic mean over all samples will estimate the contemporary N_e. Comparing samples from years *i* and *j*, Waples' (1990) temporal method estimates the effective number of breeders ($\hat{N}_{b(i,j)}$) according to:

$$\hat{N}_{b(i,j)} = \frac{b}{2(\hat{F} - 1/\hat{S}_{i,j})}$$

The standardized variance in allele frequency ($\hat{F}$) is calculated according to Pollack (1983). The parameter b is calculated analytically from age structure information and the number of years between samples (Tajima 1992). The age-at-maturity information required to calculate b was obtained from Murdoch et al. (2006) for this analysis. They observed for Chiwawa Hatchery Chinook that 8.6% matured at age 2, 4% at age 3, 87% at age 4, and 0.4% at age 5. For Chiwawa natural Chinook, Murdoch et al. (2006) observed that 1.8% matured at age 3, 81.6% at age 4, and 16.7% at age 5. The harmonic mean of sample sizes from years *i* and *j* is $\tilde{S}_{i,j}$. Over all pairwise comparisons the harmonic mean of all $\hat{N}_{b(i,j)}$ is $\tilde{N}_b$, the contemporary estimate of the effective population size (N_e). SALMONNb (Waples et al. 2007) was used to calculate $\tilde{N}_b$. As suggested by authors, alleles with a frequency below 0.05 were excluded from the analysis to reduce potential bias.

The method of Waples (2006) uses linkage disequilibrium (i.e., mean squared correlation of allele frequencies at different gene loci) as a means of estimating effective population size (N_e) from a single sample. While this method is biased in some cases where N_e /N

ratio is less the 0.1 and the sample size is less than the true N_e, it has been shown to produce comparable results to the temporal method. Burrows' delta method is used to estimate LD, and a bias corrected estimate of N_e is calculated after eliminating alleles with frequency less than 0.05. This test was implemented using LDN_e (Do and Waples unpublished). In age-structured species, N_e estimates based on LD are best interpreted as the effective number of breeders (N_b) that produced the sample (Waples 2006). N_b should be multiplied by the mean generation length (i.e., 4 in this case) to obtain an overall estimate of N_e based on an N_b estimate. We analyzed collections categorized by spawning location (i.e., hatchery broodstock or in-river) and did not analyze collections categorized by origin (i.e., hatchery or natural). Waples' (2006) method estimates N_e from observed LD, therefore the corresponding N_e estimates for the hatchery collections would be low and the estimates for the natural collections would be high. Yet, since the supplementation program is integrated, and hatchery fish can spawn naturally, we feel it inappropriate to analyze the hatchery and natural samples as if they were separate, which would essentially partition all the LD into the hatchery samples.

Each collection has an N_b estimate and an associated confidence interval. If the confidence interval includes infinity, it means that sampling error accounts for all the LD observed (i.e., empirical LD is less than expected LD). The usual interpretation is that there is no evidence for any disequilibrium caused by genetic drift in a finite number of parents. Since the LD method estimates the number of breeders that contributed to the sample being analyzed, in order to calculate an N_e /N ratio, the appropriate census size must be used. The census size used to derive a ratio was the estimate four years prior to the collection analyzed using LD, which assumed a strict four-year-old lifecycle, although the observed proportion of four-year-olds was approximately 85% each year. The census numbers (Table 2) used to calculate the ratios for Chiwawa broodstock and in-river spawners were combined NOS (natural-origin spawners) and HOS (hatchery-origin spawners) census estimates.

Individual assignment – A population baseline file was constructed containing all 1704 individual Chinook from 34 population collections (Table 1; Chiwawa origin data set

plus all samples from other populations). All individuals in the baseline had geneotypes that included nine or more loci. Individual Chinook were assigned to their most likely population of origin based on the partial Bayesian criteria of Rannala and Mountain (1997), using a "jack-knife" procedure, where each individual to be assigned was removed from the baseline prior to the calculation of population likelihoods. This procedure was implemented in a program written by Warheit in MATLAB R2006b (The Mathworks 2006). Two assignment criteria were used, 1) the population with the largest posterior probability for an individual was the "most-likely" population of origin (i.e., all individuals assigned to a collection), and 2) an assignment was consider valid only if the posterior probability was greater than or equal to 0.9. Please note that while the analysis used 34 population collections to assign Rannala and Mountain likelihoods for each individual, these likelihoods were aggregated based on "population" (i.e., Chiwawa, Nason, White, and so on) and posterior probabilities were calculated for population location, rather than individual collections.

Results and Discussion

In this section we combine our presentation and interpretations of the genetic analyses. Additionally, this section will be organized based on the task list presented in the study plan. Overall conclusions are provided following this section.

Task 1: Determine trend in census size for Chiwawa River spring Chinook.

Census data from 1989 – 2005 are provided in Table 2 for the Chiwawa Hatchery broodstock and spring Chinook present in the Chiwawa River. The demographic data for naturally spawning Chinook are based on redd sampling and carcass surveys, while broodstock data are based on Chiwawa hatchery records. As the supplementation program is integrated by design, we also present the proportion of natural-origin broodstock (pNOB) incorporated into the hatchery, in addition to the number of natural-origin (NOS) and hatchery-origin (HOS) spawners present in Chiwawa River. The

census size fluctuated yearly, and a general reduction in census size was observed in the mid to late 1990's. This trend was apparent in both the broodstock and in the river. The arithmetic mean census size from 1989 – 2005 for the Chiwawa Hatchery (i.e., broodstock) was N=87.5 per year. The arithmetic mean census size from 1989 – 2005 for the Chiwawa River (i.e., NOS and HOS combined) was N=961.9 per year. For collection years when adult Chiwawa hatchery-origin fish would have been absent in the Chiwawa River (1989 – 1992), the arithmetic mean of natural Chiwawa Chinook census size is N=962.7. We will use this number as the baseline census size to assess if census size has changed. We used two different values for the contemporary census size in the Chiwawa River, NOS only and NOS + HOS. Additionally, we used collection years 2002 – 2005 for the contemporary NOS and HOS estimates, as these are the most recent data and the number of years included for estimation is the same as the pre-hatchery estimate above (i.e., four years). For NOS only, the arithmetic mean census size from 2002 – 2005 was N=536.0. For total census size (i.e., NOS and HOS combined), the arithmetic mean census size from 2002 – 2005 was N=1324.0. For the demographic data presented here, the contemporary census size is larger than the census estimate derived from the years prior to hatchery operation.

Task 2: Document the observed genetic diversity.

Genetic Diversity Categorized By Origin

For Chiwawa River collections categorized by origin (Table 1A), substantial genetic diversity was observed, with heterozygosity estimates over all loci, having a mean of 0.80. Genetic diversity was consistent with expected Hardy-Weinberg random mating genotypic proportions for ten of the eighteen collections. Eight of the nine Chiwawa natural collections were consistent with HWE, and two of nine Chiwawa Hatchery collections were consistent with HWE. F_{IS} is observed to be slight for all Chiwawa population collections, suggesting individuals within collections do not show excessive homozygosity.

The deviations from HWE observed were generally associated with hatchery collections. The two smolt collections (i.e., 1993 and 1994) showed significant deviations from HWE, which may be a function of non-random hatchery practices involving the contributing natural-origin parental broodstocks (i.e., 1991 and 1992 cohort). Deviations from HWE in the remaining hatchery collections may be the result of few individuals being represented in the broodstock (see below).

Additionally, linkage disequilibrium (LD) was also common for Chiwawa hatchery-origin collections and minimal for Chiwawa natural-origin collections. The random association of alleles between loci (i.e., linkage equilibrium) is expected under ideal conditions. LD is observed when particular genotypes are encountered more than expected by chance. Laboratory artifacts (e.g. null alleles) or physical linkage of loci on the same chromosome can cause LD, but the LD we observed was not associated with certain locus combinations, which you would expect if either artifacts or physical linkage were the cause of LD. LD was observed for seven of the nine hatchery-origin collections. As with the deviations from HWE, the high LD in the 1993 and 1994 hatchery-origin collections may be a result of non-random hatchery practices. The substantial LD observed in the hatchery-origin adult collections (collection years 2000, 2001, 2004, and 2006) might be the result of small parental broodstock sizes contributing to those returning adults. During the mid 1990's, the Chiwawa broodstock size was low, with zero individuals collected in 1995 and 1999; so fewer individuals would be contributing to the hatchery adult returns than the natural. This idea is corroborated by the lower LD observed for the 2005 hatchery-origin collection, which had a contributing parental broodstock size in 2001 (i.e., the major contributing parental generation) approximately eight times as large as the previous few collection years (Table 2). LD reappears in the 2006 Chiwawa hatchery-origin collection, which had a contributing parental broodstock size (i.e., for the most-part, the 2002 hatchery brood year) five times lower (Table 2) than that of the 2005 collection.

While seven of nine hatchery-origin collections showed significant LD, only one natural origin collection showed LD, and for this collection, only 10% of the loci-pairs were in

disequilibrium (Table 1). The fact that LD predominated in the hatchery samples, suggests that variance in reproductive success (i.e., overrepresentation of particular parents) is higher in the hatchery-origin than in natural-origin collections.

Genetic Diversity Categorized By Spawning Location

For upper Wenatchee River collections categorized by spawning location (Table 1B), substantial genetic diversity was observed, with heterozygosity estimates over all loci, having a mean of 0.79 and ranging from a low of 0.69 (1993 White River) to 0.85 (1993 Little Wenatchee). Genetic diversity was consistent with HWE for nineteen of twenty-nine population collections. For the collections that departed from HWE, seven were from the Chiwawa River, one was from Leavenworth Hatchery, one was the Wenatchee mainstem collection of hatchery-origin – naturally spawning fish, and one was from the White River. F_{IS} is observed to be slight for all population collections except the 1993 White River collection (10% heterozygote deficit) (Table 1B). Collections deviating with HWE generally correlated with collections having high LD. Twelve population collections showed a proportion of pairwise linkage disequilibrium tests (across all loci) greater than 5% (Table 1B), eight of which were Chiwawa collections.

Starting in 1996, spawning location collections are composed of both natural- and hatchery-origin samples. The LD seen in the later spawning location collections may be caused by an admixing effect (i.e., mixing two populations), where random mating has not had the chance to freely associate alleles into genotypes. Interestingly, there appears to be a trend of reducing LD through time within the broodstock collections (Table 1B), which suggests that a "homogenizing" effect is taking place within the Chiwawa River. This observation is discussed more fully in Task 3 below.

Task 3: Test for population differentiation among collections within the Chiwawa River and associated supplementation program.

Introduction

Task 3 was designed to address two hypotheses listed as part of Objective 3 in Murdoch and Peven (2005):

- Ho: Allele frequency $_{\text{Hatchery}}$ = Allele frequency $_{\text{Naturally produced}}$ = Allele frequency $_{\text{Donor pop}}$.
- Ho: Genetic distance between subpopulations $_{\text{Year x}}$ = Genetic distance between subpopulations $_{\text{Year y}}$

Murdoch and Peven (2005) proposed these two hypotheses to help evaluate the Chiwawa supplementation program through the "Conceptual Process" (Figure 5 in Murdoch and Peven 2005; repeated here as Figure 1). There are two components to the first hypothesis, which must be considered separately. The first component involves comparisons between natural-origin populations in the Chiwawa to determine if there have been changes in allele frequencies or genetic distances, through time starting with the donor population. Documenting a change does not necessarily indicate that the supplementation program has directly affected the natural origin fish, as additional tests would be necessary to support that hypothesis. The intent of the second component is to determine if the hatchery produced populations have the same genetic composition as the naturally produced populations.

Although on the surface these two components and their associated comparisons may appear simple, from a hypothesis-testing perspective the analyses are complicated by the fact that natural-origin fish may have had hatchery-origin parents, and hatchery-origin fish may have had natural-origin parents. As such, we organized the Chiwawa genetic data into three data sets: (1) fish origin (hatchery versus natural), (2) spawning location (hatchery broodstock versus in-river (natural) spawners), and (3) four "treatment" groups (1. hatchery-origin hatchery broodstock, 2. hatchery-origin natural spawner, 3. natural-origin natural spawner, and 4. natural-origin hatchery broodstock). We conducted separate analyses using each of the three data sets, with each analysis touching on some aspect of the components necessary to move through the Conceptual Process (Figure 1).

Hatchery- Versus Natural-Origin

We address the following questions with the origin data set:

1. Are there changes in allele frequencies and allele sharing distances in the natural-origin collections from pre-supplementation to today?
2. Are there changes in allele frequencies and allele sharing distances in the hatchery-origin collections from early supplementation to today?
3. Are there significant differences in allele frequencies and large allele sharing distances between hatchery- and natural-origin adults from a collection year, and has this pattern changed through time?

Genic Differentiation Tests – We explicitly tested the hypothesis of no significant differentiation within natural- or hatchery-origin collections from the Chiwawa River using a randomization chi-square test. We show the results for the pairwise comparisons among natural-origin collections from the Chiwawa River populations in the first block of the second page of Table 3. Ten of the 36 (28%) pairwise comparisons have highly significant allele frequency differences, while only 12 of the 36 comparisons (33%) showed no significant differences. Eight of these 12 comparisons involved the 1996 collection, which included only eight samples and therefore provided little power to differentiate allele frequencies. If we exclude the 1996 collection, only 14% of the pairwise comparisons showed no significant differences, and here all but one of these comparisons involved the 1989 collection. The 1989 collection appeared to be the least differentiated collection in the natural-origin data set in that all pairwise comparisons were either not significant, or only mildly significant at the nominal critical value. No comparisons involving the 1989 collection were significant using a Bonferroni-corrected critical value, and 1989 is the only natural-origin collection in our data set that can be classified as "pre-supplementation."

We can interpret these results to indicate that although there appears to be significant year-to-year differences in allele frequencies among post-supplementation collections, the allele frequencies between each post-supplementation collection and the 1989 pre-supplementation collection are not greatly different. However, the level of differentiation

does increase from the early post-supplementation years to the more recent years (2001, 2004-2006), although the statistical level of this significance never exceeds the Bonferroni-corrected critical value. Finally, sample sizes were also small for the 1989 collection (n = 36) and we cannot eliminate a reduction in power as a contributing factor for the lack of significance for these tests.

As with the hatchery-origin collections, most pairwise comparisons of allele frequencies between hatchery-origin samples were significant (Table 3, first page, upper block). Out of the 36 pairwise comparisons, all but three are significant at some level, and most comparisons are highly significant. Similar to the natural-origin analysis, the non-significant results were limited to comparisons involving the 1996, which included only eight samples.

As a result of this analysis *we reject the hypothesis that there was no significant differentiation among natural- or hatchery-origin collections from the Chiwawa River*. Furthermore, the allele frequencies of the hatchery-origin collections are significantly different from those of natural-origin collections (Table 3, first page, second block). For those fish collected in the same year, allele frequencies are significantly different between hatchery- and natural-origin collections, although in 2005 the level of significance was below the Bonferroni critical value (Table 3). The next step is to examine the pattern of allelic differentiation to discover first if there is a trend among the data, and second, if this trend suggests that the allele frequency differences among Chiwawa River natural-origin fish collections has been affected by the hatchery-origin fish.

Allele-sharing and Nonmetric Multidimensional Scaling – We constructed a pairwise allele-sharing distance matrix for all hatchery- and natural-origin collections from the Chiwawa River and subjected this matrix to a nonmetric multidimensional scaling analysis, restricting the analysis to two dimensions (Figure 2). The stress statistic for this analysis is 0.09, a value Kruskal (in Rohlf 2002) listed as a good to excellent fit between the actual allele-sharing distances and the Euclidean (straight-line) distances in the plot.

In other words, Figure 2 is a good visual representation of the allele sharing distance matrix; collections with a high percentage of alleles shared will be closer to each other than collections with a lower percentage of alleles shared.

With the exception of the two outlier years (1996 and 1998) the Chiwawa natural-origin collections form a tight cluster indicating an overall common set of shared alleles among these collections. Even if we ignore the 1996 and 1998 hatchery-origin collections, there appears to be a greater variance in shared alleles among the Chiwawa hatchery-origin collections than the natural-origin collections (Figure 2). In fact, the median percentage of alleles shared among the Chiwawa natural-origin collections is 76% compared with 69% alleles shared among the Chiwawa hatchery-origin collections.

Also, there appears to be a convergence in allele sharing distances (i.e., a decrease in allele frequency differences) between the hatchery- and natural-origin fish from the late 1980s/early 1990s to 2006. The series of red arrows in Figure 2 represent the progression of change in hatchery-origin allele sharing distances from 1996 (first adult hatchery origin fish in our analysis) to 2006 and this progression is decidedly in the direction of the natural-origin cluster. However, the most recent natural-origin collections (2001, 2004-2006) appear to have pulled closer to the hatchery-origin collections, compared with the 1989 natural-origin collection (note the close proximity of the 2000 and 1989 natural-origin collections). Nevertheless, the cluster of natural-origin collections adjacent to the hatchery-origin collections in Figure 2 also includes the 1993 natural-origin collection. Qualitatively, it appears that the initial hatchery-origin and natural-origin collections were more different from each other in terms of the percentage of shared alleles than are the most recent hatchery- and natural-origin collections. This may have been a result of a non-random sample of natural-origin fish that was used as broodstock in the initial years of the supplementation program (see discussion in Task 2 concerning deviations from HWE and linkage disequilibrium).

That being said, we do need to emphasize that Figure 2 is dominated by five outlier collections (two each from the 1996 and 1998 collections, and the 1994 smolt collection).

The 1996 and 1998 collections are characterized by small samples sizes, and the 1994 smolt collection has nearly all pairs of loci in linkage disequilibrium (Table 1). If we eliminate these five outlier groups, both the hatchery- and natural-origin collections form a relatively tight cluster. Excluding the five outliers, the median percentage of shared alleles among all pairwise combinations of Chiwawa hatchery versus Chiwawa natural collections is 76%. This compares with a median pairwise percentage of 79% among only Chiwawa natural-origin collections. That is, there are nearly as many alleles shared between the hatchery-origin and natural-origin collections as there are among the natural-origin collections themselves. There is also a narrowing of differences between natural- and hatchery-origin fish from the same collection years from 1993 (76% shared alleles) through 2006 (83% shared alleles).

If allelic differentiation among collections is a function of genetic drift, we would expect a positive correlation between the number of years between two collections and the allele sharing distance. That is, if genetic drift is the primary cause of allele frequency differences between two collections, the greater the number of years between the two collections the larger the allele-sharing distance. For both the natural- and hatchery-origin collections we examined the relationship between the number of years between a pair of collections and the collections' allele-sharing distance (Figure 3). Although the relationship between time interval and allele distance appears to be a positive function in the natural collections, the slope of the regression line is 0.0017, and is not significantly different from zero. Furthermore, the correlation coefficient (r^2) equals 0.1068, which means that the time interval between collections accounts for only 10% of the pairwise differences in allelic distance. The hatchery-origin collections do show a significantly positive slope (0.0037; $p = 0.0254$) and a regression coefficient nearly three times greater than that for the natural-origin collections. However, the correlation coefficient is still relatively small ($r^2 = 0.3290$), indicating that the time interval between collections accounts for one-third of the pairwise differences in allelic distance. The results suggest that if genetic drift is a factor in allelic differentiation between collections, it is only a minor factor, and appears to have affected the hatchery-origin collections more than the natural-origin collections.

If four-year-old fish dominate each collection year, we would expect a closer relationship among collections that are spaced at intervals of four years. The average percentage of alleles shared between two natural-origin collections that are separated by four years or a multiple of four years is 81%, compared with 78% for natural-origin collections separated by years that are not divisible by four. Likewise, for hatchery-origin collections the average percentage of alleles shared is 80% and 75% for collections separated by years divisible and not divisible by four, respectively. Although the percent differences described above are relatively small, they are consistent with the idea that allelic differences between collections are a function of year-to-year variability among different cohorts of four year-old fish.

Summary – The allele frequencies within and between natural- and hatchery-origin collections are significantly different, but there does not appear to be a robust signal indicating that the recent natural-origin collections have diverged greatly from the pre- or early post-supplementation collections. Genetic drift will occur in all populations, but does not appear to be a major factor with the Chiwawa collections. We propose that the differences among collections are a function of differences in allele frequencies among cohorts of the four year-old fish that dominate each collection.

Hatchery Broodstock Versus Natural (In-River) Spawners

We address the following questions with the spawner data set:

1. Are there changes in allele frequencies and allele sharing distances in the natural spawning collections from pre-supplementation to today?
2. Are there changes in allele frequencies and allele sharing distances in the hatchery broodstock collections from early supplementation to today?
3. Are there significant differences in allele frequencies and large allele sharing distances between hatchery and natural spawning adults from a collection year, and has this pattern changed through time?

Genic Differentiation Tests – For the most part there are significant differences in allele frequencies among collections for both the hatchery broodstock and natural spawners (Table 4), and these differences are consistent with the origin data set (Table 3). There are four collection years with paired samples (2001, 2004-2006) where we can compare allele frequency differences between the hatchery broodstock and natural spawners, within the same year. The 2001 hatchery broodstock and natural spawner collections have significantly different allele frequencies, but the level of significance decreased from 2001 to 2004, and become non-significant in 2005 and 2006 (Table 4). This indicates that by 2005, the hatchery broodstock and natural spawners collections were effectively sampling from the same population of fish. Additionally, the percentage of alleles shared between the hatchery broodstock and the natural spawners increased from 76% in 2001 to 86% in 2006 (allele sharing distance matrix, not shown). From this analysis, we conclude that although there are year-to-year differences in allele frequencies within the natural and hatchery spawner collections, *there appears to be a convergence of allele frequencies within collection-year, between the natural and hatchery spawner populations*.

Linkage Disequilibrium – Linkage disequilibrium is the correlation of alleles between two loci, and can occur for several reasons. If two loci are physically linked on the same chromosome, than alleles from each of these loci should be correlated. However, linkage between two loci can occur as a result of population bottlenecks, small population sizes, and natural selection. If any of these conditions had occurred or were occurring within the Chiwawa River system, we would expect to find substantial linkage disequilibrium in many or perhaps all Chiwawa collections. However, many Chiwawa collections, especially the natural-origin collections, do not show linkage disequilibrium (Table 1), and it would appear that the linkage disequilibrium within certain Chiwawa collections is not a function of the processes listed above. Linkage disequilibrium can also result if the collection is composed of an admixture. That is, if two or more reproductively isolated populations are combined into a single collection, the collection will show linkage disequilibrium. Each broodstock and natural spawning collection is composed of natural- and hatchery-origin fish. If these hatchery- and natural-origin fish are drawn from the

same population, the spawning collections should not show substantial linkage disequilibrium. However, if the hatchery- and natural-origin fish are from different populations (i.e., full hatchery – natural integration has not been achieved), the spawning collections should show substantial linkage disequilibrium.

There are only three Chiwawa spawning collections that are not composed of both hatchery- and natural-origin samples: 1989 (natural-origin, natural spawner), 1993 (natural-origin, hatchery broodstock), and 2001 (natural-origin, natural spawner). Of the 10 spawning collections with both hatchery- and natural-origin fish, seven show significant linkage disequilibrium. Two of the three collections that did not show linkage disequilibrium are the 1996 and 1998 hatchery broodstock collections, which are composed of only seven natural- and six hatchery-origin fish, and two natural- and 19 hatchery-origin fish, respectively. Within the hatchery broodstock collections with linkage disequilibrium, the percent of loci pairs showing linkage decreased from 32% in 2000 to 13% in 2001 and 2004, to only 1% and 5% in 2005 and 2006, respectively (Table 1). If the homogenization of allele frequencies of natural- and hatchery-origin fish was increasing from 2000 to 2006, we would expect a decrease in linkage disequilibrium among the broodstock collections. This is what occurred within the hatchery broodstock collections, but did not occur within the natural spawner collections, where the percent of loci pairs showing linkage was 18% in 2004, 6% in 2005, and 10% in 2006 (Table 1). Furthermore, the 2001 natural spawner collection, with no hatchery-origin component showed linkage disequilibrium with 9% of loci pairs.

There is no correlation between percent of loci pairs showing linkage disequilibrium and percent of broodstock composed of hatchery-origin fish ($r^2 = 0.0045$). Furthermore, the natural spawner and hatchery broodstock collections were each composed of roughly the same average percentage of hatchery-origin fish (57% and 53%, respectively). If the decrease in linkage disequilibrium among the hatchery broodstock collections from 2000 to 2006 was a result of a homogenization of allele frequencies of natural- and hatchery-origin fish in the broodstock, the same degree of homogenization did not occur within the

natural spawner collections. This would occur if natural- and hatchery-origin fish spawning within the river remain segregated, either by habitat or by fish behavior.

Summary – As with the origin data set, there are significant allele frequency differences within and between hatchery broodstock and natural spawner collections. However, in recent years the allele frequency differences between the hatchery broodstock and natural spawner collections has declined. Furthermore, based on linkage disequilibrium, there is a genetic signal that is consistent with increasing homogenization of allele frequencies within hatchery broodstock collections, but a similar homogenization within the natural spawner collection is not apparent. These data suggest that there exists consistent year-to-year variation in allele frequencies among hatchery and natural spawning collections, but there is a trend toward homogenization of the allele frequencies of the natural- and hatchery-origin fish that compose the hatchery broodstock.

Four Treatment Groups

Analyses of genetic differences between hatchery (broodstock) and natural spawner collections is confounded by the fact that each these two groups are composed of fish of natural- and hatchery-origin. To understand the effects of hatchery supplementation on *natural-origin fish that spawn naturally*, we needed to divide the Chiwawa data set into four mutually exclusive groups: (1) hatchery-origin hatchery broodstock, (2) hatchery-origin natural spawner, (3) natural-origin hatchery broodstock, and (4) natural-origin natural spawner, with each group consisting of multiple collection years, for a total of 25 different groups.

Allele-sharing and Nonmetric Multidimensional Scaling –As with previous analyses discussed above, we constructed a pairwise allele-sharing distance matrix for all collections from each of these treatment groups and subjected this matrix to a nonmetric multidimensional scaling analysis, restricting the analysis to two dimensions. Figure 4 shows that five outlier groups dominate the allele-sharing distances within this data set. These outlier groups are also present in Figure 2, as discussed above, and Figure 2 and 4 resemble each other because the same fish are included in each analysis. The difference

between Figures 2 and 4 is that in Figure 4 the fish are grouped into collection year and the four treatment groups, rather than collection year and two treatment groups (hatchery- versus natural-origin).

Figure 4 does not provide useful resolution of the groups within the polygon, because the outlier groups dominate the allele sharing distances. We removed the five outlier groups from Figure 4, recalculated the allele sharing distances and subjected this new matrix to a multidimensional scaling analysis (Figure 5). Figure 5 shows separation among the 2001, 2004-2006 collections, but this separation does not necessarily indicate that within-year collections are more similar to each other than any collection is to a collection from another year. For example, the 2006 natural-origin natural spawner and the 2005 natural-origin hatchery broodstock collections share 81% alleles, while the 2006 natural-origin natural spawner and 2006 hatchery-origin hatchery broodstock collections share 75% alleles. There does not appear to be any discernable pattern of change in allele-sharing distance among the collections relevant to pre- or post-supplementation. Although the 1989 pre-supplementation natural-origin collection appears distinct (Figure 5), the 1993 natural-origin hatchery broodstock collection appears quite similar to the 2005 and 2006 natural-origin collections (Figure 5). The 1993 natural-origin hatchery broodstock collection, although not technically pre-supplementation, is composed of fish whose ancestry cannot be traced to any Chiwawa hatchery fish. Therefore, there is no clear pattern of allele sharing change from pre-supplementation to recent collections.

There does appear to be some change in the average percentage of alleles shared within the 2001 to 2006 collections, with an increase from 74% in 2001 and 2004 to 78% and 79% in 2005 and 2006, respectively. The results provided by this analysis are consistent with the results presented in the origin and spawner data sets. That is, there are allele frequency and allele sharing differences among the collections, but analyses do not strongly suggest that these differences are a function of the supplementation program. Furthermore, there is also a weak signal that the hatchery and natural collections within the most recent years are more similar to each other than in the previous years.

Overall Genetic Variance – Although there are signals of allelic differentiation among Chiwawa River collections, there are no robust signs that these collections are substantially different from each other. We used two different analyses to measure the degree of genetic variation that exists among individuals and collections within the Chiwawa River. First, we conducted a principal component analysis using all Chiwawa samples with complete genotypes (i.e., no missing alleles from any locus). Although the first two principal component axes account for only 10.5% of the total molecular variance, a substantially greater portion of that variance is among individual fish, regardless of their identity, rather than among hatchery and natural collections (Figure 6). The variances in principal component scores among individuals are 11 and 13 times greater than the variance in scores among collections, along the first and second axes, respectively.

Second, we conducted a series of analyses of molecular variance (AMOVA) to ascertain the percentage of molecular variance that could be attributed to differences among collections. We organized these analyses to test also for differences in the hierarchical structure of the data. That is, we tested for differences among collections using the following framework:

- No organizational structure – all 25 origin-spawner collections considered separately
- Origin-spawner collections organized into 10 collection year groups
- Origin-spawner collections organized into 2 breeding location groups (hatchery versus natural)
- Origin-spawner collections organized into 2 origin groups (hatchery versus natural)
- Origin-spawner collections organized into the 4 origin-spawner groups

It is clear from this analysis that nearly all molecular variation, no matter how the data are organized, resides within a collection (Table 5). The percentage of total molecular variance occurring within collections ranged from 99.68% to 99.74%. The among group variance component was limited to less than 0.26% and in all organizational structures,

except "no structure," the among group percentage was not significantly greater than zero. Furthermore, none of the organizational structures provided better resolution than "no structure" in terms of accounting for molecular variance within the data set. *These results indicate that if there are significant differences among collections of Chiwawa fish, these differences account for less than one percent of the total molecular variance, and these differences cannot be attributed to fish origin or spawning location.*

Summary and Conclusions

We reject the null hypothesis that the allele frequencies of the hatchery collections equal the allele frequencies of the natural collections, which equals the allele frequency of the donor population. Furthermore, because the allele-sharing distances are not consistent within and among collections years, we also reject the second stated hypothesis discussed above. However, there is an extremely small amount of genetic variance that can be attributed to among collection differences. The allelic differentiation that does exist among collections does not appear to be a function of fish origin, spawning location, genetic drift, or collection year. Figure 5 and related statistics does suggest that hatchery and natural collections in 2005 and 2006 are more similar to each other than previous years' collections, and this would be expected in a successful integrated hatchery supplementation program.

Since each of these collection years are generally composed of four-year-old fish, the differentiation among these collections for the most part is differentiation among specific cohorts. The slightly greater percentage of alleles shared among collections that are separated in time by multiples of four years, compared with collections that are not separated in time as such, suggests that cohort differences may be the most important factor accounting for differences in allele frequencies among collections.

Task 4: Develop a model of genetic drift.

See Task 3

Task 5: Analyze spring Chinook population samples from the Chiwawa River and Chiwawa Hatchery from multiple generations.

See Task 3

Task 6: Analyze among population differences for upper Wenatchee spring Chinook.

Supplementation of the Chiwawa River spring Chinook population may affect populations within the Wenatchee River watershed other than the Chiwawa River stock. If the stray rate for Chiwawa hatchery-origin fish is greater than that for natural-origin fish, an increase in gene flow from the Chiwawa population into other populations may result. If this gene flow is high enough, Chiwawa River fish may alter the genetic structure of these other populations. Records from field observations indicate that hatchery-origin fish are present in all major spawning aggregates (A.R Murdoch, unpublished data), and these fish are successfully reproducing (Blankenship et al 2006). The intent of this task is to investigate if there have been changes to the genetic structure of the spring Chinook stocks within upper Wenatchee tributaries during the past 15-20 years, and if changes have occurred, are they a function of the Chiwawa River Supplementation Program? Therefore, we ask the following two questions:

1. Are allele frequencies within populations in the upper Wenatchee stable through time? That is, is there significant allelic differentiation among collections within upper Wenatchee populations?
2. Are the recent collections from the upper Wenatchee populations more similar to the Chiwawa population than earlier collections from the same populations?

For this task we analyzed natural spawning collections from the White River (natural-origin), Little Wenatchee River (natural-origin), Nason Creek (natural-origin), and

Wenatchee mainstem (hatchery-origin), and hatchery collections from Leavenworth NFH and Entiat River NFH (Table 1). We also included in the analysis the natural- and hatchery-origin collections from the Chiwawa River. There are no repeated collections from Leavenworth, Entiat, Little Wenatchee, and Wenatchee mainstem (Table 1), so for many of the analyses we have limited our discussion to the Chiwawa River, White River, and Nason Creek collections. Furthermore, genetic structure of the Little Wenatchee collection, which consisted of only 19 samples, was unexpectedly quite different from the other collections. For example, the F_{ST} statistic measures the percent of total molecular variation that can be attributed to differences between populations. The median F_{ST} for all pairwise combinations of collections from all populations, except Little Wenatchee (33 populations, 528 individual F_{ST} statistics) equals 0.010 (1%), with a range of 0.000 to 0.037 (Table 6). The median F_{ST} for the Little Wenatchee paired with all other collections (33 individual F_{ST} statistics) equals 0.106 (10.6%), with a range of 0.074 to 0.121. The ten-fold increase in the F_{ST} statistic indicates that either the Little Wenatchee spring Chinook is unique among the upper Wenatchee River stocks, or this 1993 collection is somehow aberrant. Therefore, we exclude the Little Wenatchee collection from many other analyses.

Population Differentiation – Table 3 provides the levels of significance for all pairwise genic differentiation tests. Most between-collection comparisons are highly significant, with no pattern of increasing or decreasing differentiation with time, and no differences when comparisons are made with Chiwawa hatchery- versus Chiwawa natural-origin fish. For example, excluding the outlier 1996 and 1998 Chiwawa hatchery- and natural-origin collections, Nason Creek showed highly significant allele frequency differences between the Chiwawa hatchery- and natural-origin collections at 100% and 86% of the comparisons, respectively. The same comparisons with the White River produced 100% and 93% highly significant allele frequency comparisons, respectively. Allele frequencies between Nason Creek and White River were likewise differentiated from each other.

The collection allele frequencies within the upper Wenatchee system are significantly different, and these differences do not appear to change as a function of time (Table 3). Nason Creek shows greater within-population year-to-year variation in allele frequencies than does the White River, with 47% of the pairwise comparisons showing highly significant differences, compared with only 13% for the White River. However, the 2005 and 2006 collections from the White River appear to be somewhat more differentiated from not only each other, but from the earlier collections from the White River.

Despite the high degree of temporal and spatial structure suggested by the genic differentiation tests, as described above for within-Chiwawa analysis (Task 3), most of the genetic variation within this data set occurs within populations, rather than between populations (Table 6). The F_{ST} values for most population comparisons are between 0.01 and 0.02, indicating 1% to 2% among-population variance, with the remaining 98% to 99% variance occurring within populations. The White River shows the highest median F_{ST} among the natural-origin collections, equal to 0.014, compared with 0.009 for both the Nason Creek and Chiwawa natural-origin collections. The median F_{ST} for the Chiwawa hatchery-origin collections (0.012) was higher than that for the Chiwawa natural-origin collections.

Table 7 summarizes the information from the F_{ST} analyses, under five different temporal and spatial scenarios. Under all scenarios, over 99% of the molecular variance is within populations. There is significantly greater spatial structure among populations ("Origin") in 2005 and 2006 than from 1989 to 1996. That is, there appears to be more spatial structure among the Chiwawa hatchery-origin, Chiwawa natural-origin, White River, and Nason Creek now, than in 1989 to 1996, despite the potential homogenizing and cumulative effect of hatchery strays. However, we stress that the amount of molecular variance associated with the among population differences, despite being significantly greater than 0.00%, is limited to only 0.43%.

Allele-sharing and Nonmetric Multidimensional Scaling – As in the Chiwawa River data discussed above, we constructed an allele-sharing distance matrix and then subjected

that matrix to a multidimensional scaling analysis (Figure 7). Consistent with all previously discussed multidimensional scaling analyses, the 1996 and 1998 adult, and the 1994 smolt collections are outliers. There is clear separation between the White River collections and all other natural-origin and Chiwawa hatchery-origin collections, indicating that there are more alleles shared among the Nason Creek and Chiwawa collections, than with the White River collections. Furthermore, there is a slight separation between the Chiwawa natural-origin natural spawner collections and Nason Creek collections, suggesting different groups of shared alleles between these populations. There is more variation in the allele-sharing distances among collections involved with the Chiwawa hatchery (origin or broodstock) than any of the natural-origin collections, even if we exclude the 1994, 1996, and 1998 collections. This suggests that there is more year-to-year variation in the composition of hatchery-origin and hatchery broodstock than within natural-origin populations throughout the upper Wenatchee. All Wenatchee mainstem fish are hatchery-origin, and if these fish are from the Chiwawa Supplementation Program (rather than from Leavenworth), it is not unexpected that this collection would be plotted within the Chiwawa polygon (Figure 7).

Assignment of Individual to Populations – Finally, we conducted individual assignment tests whereby we assigned each individual fish to a population, based on a procedure developed by Rannala and Mountain (1997) (Table 8 and 9). Individual fish may be correctly assigned to the population from which they were collected, or incorrectly assigned to a different population. Incorrect assignments may occur if the fish is an actual migrant (i.e., source population different from population where collected), or because the genotype for that fish matches more closely with a population different from its source. If there are many individuals from a population incorrectly assigned to populations other than its source population, that original population is either unreal (i.e., an admixture), or there is considerable gene flow between that population and other populations. Furthermore, in assigning individuals to populations, we can either accept the assignment with the highest probability, regardless of how low that probability may be, or we can establish a more stringent criterion, such as to not accept an assignment unless the posterior probability is equal to or greater than 0.90. This value is roughly

equal to having the likelihood of the most-likely population equal to 10 times that of the second most-likely population.

We provide a summary of the assignments in Tables 8 and 9. On average, nearly 50% of the fish are assigned incorrectly if we accept all assignments (Table 8), but the incorrect assignment rate drops to roughly 10% when we accept only those assignments with probabilities greater than 0.90. However, with this more stringent criterion, nearly 64% of the fish go unassigned. These results indicate that the allele frequency distributions for these populations are very similar, and it would be very difficult to assign an individual fish of unknown origin to the correct population. If all fish are assigned, there is a 50% chance, overall, of a correct assignment. If you accept only those assignment with the 0.90 criterion, nearly two-thirds of the fish would be unassigned, but there is a 90% chance of correctly assigning those fish that are indeed assigned.

Of all the populations in the data set, there are fewer errors associated with assigning fish to the White River. If all fish are assigned (Table 8), 72% of those fish assigned to the White River, are actually from the White River (115 fish out of a total of 159 fish assigned to the White River). This compares to a rate of only 52% and 53% for Nason Creek and Chiwawa natural-origin, respectively, and 60% for the Chiwawa hatchery-origin collections. With the 0.90 criterion (Table 9), 89% of the fish assigned to the White River, are actually from the White River, compared with 70% and 65% for Nason Creek and Chiwawa natural origin, respectively, and 81% for the Chiwawa hatchery origin.

When all fish are assigned, most of the incorrectly assigned fish from Nason Creek and White River are assigned to Chiwawa River, at roughly equal frequencies to the hatchery- and natural-origin populations. Incorrectly assigned fish to other populations occur at a slightly higher rate in Nason Creek than in the White River. However, when only those fish meeting the 0.90 criterion are assigned (Table 9), incorrectly assigned fish from Nason Creek are distributed among White and Chiwawa Rivers, as well as Leavenworth NFH, and the Entiat NFH. Mis-assignment to the Chiwawa hatchery-origin was the

highest among the Nason Creek collections, equal to nearly 14%. This contrasts with the White River where mis-assignments do not exceed 7% anywhere, and there is a roughly even distribution of mis-assignments among Nason Creek and Chiwawa River collections.

Summary and Conclusions – There is little geographic or temporal structure among populations within the upper Wenatchee systems. Among population molecular variance is limited to 1% or less. The little variance that can be attributed to among populations indicates that the White River is more differentiated from the Chiwawa and Nason populations than these populations are from each other. Furthermore, although we cannot rule out a hatchery effect on the Nason Creek and White River populations, there is no indication there has been any temporal changes in allele frequencies within these populations that can be attributed directly to the Chiwawa River Supplementation Program. In fact, Table 7 weakly suggests that there is more differentiation among these populations now, than there was before or at the early stages of Chiwawa supplementation.

Therefore, returning to our two original questions, there are significant differences in allele frequencies among collections within populations, and among populations within the upper Wenatchee spring Chinook stocks. However, these differences account for a very small portion of the overall molecular variance, and these populations overall are very similar to each other. There is no evidence that the Chiwawa River Supplementation Program has changed the allele frequencies in the Nason Creek and White River populations, despite the presence of hatchery-origin fish in both these systems. Finally, of all the populations within the Wenatchee River, the White River appears to be the most distinct. Yet, this distinction is more a matter of detail than of large significance, as the median F_{ST} between White River collections and all other collections (except the Little Wenatchee) is less than 1.5% among population variance.

Task 7: Calculate the inbreeding effective population size using demographic data for each sample year, and document the ratio of census to effective size.

This analysis was completed by Williamson et al. (submitted).

Task 8: Calculate LD N_b using genetic data for each sample year, and document the ratio of census to effective size.

We report N_e estimated for the Chiwawa River collections based on the bias correction method of Waples (2006) implemented in LDNe (Do and Waples unpublished). N_e estimates based on LD are best interpreted as the effective number of breeders (N_b) that produced the sample (Waples 2006).

For collections categorized by spawning location (i.e., hatchery broodstock or natural), estimates of N_b are shown in Table 10. Considering the hatchery broodstock, N_b estimates range from 30.4 (1996) to 274.3 (2005). To obtain N_e /N ratios, the N_b estimate is multiplied by four (i.e., mean generation length) and divided by the total in river (i.e., NOS [natural-origin spawners] plus HOS [hatchery-origin spawners]) census data from four years prior (i.e., major cohort; see Table 2). The observed N_e /N ratios for the broodstock collections range from 11% to 54% of the census estimate, excluding the 2000 collection which is 106%. A ratio greater than one is possible under special circumstances, and certain artificial mating schemes within hatcheries can inflate N_e above N; yet, it is unknown if this is the case for this collection. While no direct comparisons are possible, the N_b estimates reported by Williamson et al. (submitted) for Chiwawa broodstock collections from 2000 – 2003 are similar in magnitude to our estimates. For Chiwawa natural spawner collections, the N_b estimates range from 5.2 (1989) to 231.5 (2005), with observed N_e /N ratios of 22% - 48% of the census estimate.

Task 9: Calculate N_b using the temporal method for multiple samples from the same location.

Estimates of effective number of breeders (N_b) derived from Waples' (1990) temporal method are shown in Tables 11-13. Eight collection years were used for the Chiwawa broodstock collections (Table 11). The harmonic mean of all pairwise estimates of N_b ($\widetilde{N}_b$) was 269.4. This estimate is the contemporary N_e for Chiwawa broodstock collections. For the five collection years of Chiwawa in-river spawners (Table 12), the estimated $\widetilde{N}_b$ = 224.2. This estimate is the contemporary N_e for Chiwawa River natural spawner collections. Since the Chiwawa Supplementation Program is integrated by design, we also performed another estimation of N_e using composite hatchery and natural samples. There are paired samples from 2004-2006. We combined genetic data for hatchery (HOS) and natural (NOS) origin fish from 2004 – 2006 to create a single Chiwawa River natural spawner sample for each year. The three composite samples from 2004 – 2006 were then analyzed using the temporal method (Table 13), resulting in a $\widetilde{N}_b$ = 386.8. This estimate is the contemporary N_e for Chiwawa River.

Williamson et al. (submitted) estimated N_e using Waples' (1990) temporal method for Chinook captured in 2004 and 2005, and used age data to decompose brood years into consecutive cohorts from 2000 – 2003. They report for Chiwawa broodstock a $\widetilde{N}_b$ = 50.4. This estimate is not similar to our Chiwawa broodstock estimate. However, if we analyze the hatchery-origin Chinook only, our estimate is $\widetilde{N}_b$ = 80.1 for collection years 1989 – 2006 (data not shown). Williamson et al. (submitted) report for Chiwawa naturally spawning Chinook a $\widetilde{N}_b$ = 242.7, which is slightly higher than our estimate for in-river spawners from 1989 – 2006, but lower than our estimate from combined NOS and HOS Chinook from 2004 – 2006 collection years.

Task 10: Use available data and the Ryman-Laikre and Wang-Ryman models to determine the expected change of N_e for natural spring Chinook salmon in the Wenatchee River due to hatchery operation.

N_e is generally thought to be between 0.10 and 0.33 of the estimated census size (Bartley et al. 1992; RS Waples pers. comm.). We used this range to generate an estimate of N_e for Chiwawa natural spawners prior to hatchery operation. For brood years 1989 – 1992, the arithmetic mean census size was N=962.7 (Table 2), resulting in an estimated N_e ranging from 96.3 – 317.7. The contemporary estimate of N_e calculated using genetic data for the Chiwawa in-river spawners is N_e=224.2 (Table 12), falling in the middle of the pre-hatchery range. The N_e /N ratio calculated using 224.2 and the arithmetic census of NOS Chinook from 1989 – 2005 is 0.42. A more appropriate contemporary N_e to compare with the pre-hatchery estimate (i.e., 96.3 – 317.7) is the combined NOS and HOS estimate from natural spawners, since the supplementation program is integrated. As discussed above, the contemporary estimate of N_e calculated using genetic data for Chiwawa NOS and HOS Chinook is N_e=386.8 (Table 13), which is slightly larger than the pre-hatchery range, suggesting the N_e has not declined during the period of hatchery operation. The N_e /N ratio calculated using 386.8 and the arithmetic census of NOS and HOS Chinook from 1989 – 2005 is 0.40. These results suggest the Chiwawa Hatchery Supplementation Program has not resulted in a smaller N_e for the natural spawners from the Chiwawa River.

Williamson et al. (submitted) argued that since their combined (i.e., broodstock and natural) N_e estimate was lower than the naturally spawning estimate, the supplementation program likely had a negative impact on the Chiwawa River N_e. We disagree with this interpretation of these data. Since the natural spawning component is mixed hatchery and natural ancestry, the N_e estimates from natural spawning data are the results that bear on possible hatchery impacts. The census data show the population declined in the mid 1990's and rebounded by 2000 (Table 2). This trend is reflected in the N_e results, as shown above, and Williamson et al. (submitted) clearly show in their Table 4 the N_e was lower in 2000 (N_e = 989) than it was in 1992 (N_e = 2683). Yet, the important comparison

they make in our view was the natural spawning N_e versus the natural only component N_e (i.e., hypothetically excluding hatchery program). Williamson et al. (submitted) report the 1989 – 1992 N_e estimated from naturally spawning Chinook (i.e., NOS and HOS integrated) was essentially the same as the natural only component estimate, 2683 and 2776, respectively. This result is not surprising since no HOS fish were present between 1989 – 1992. They also report that the 1997 – 2000 N_e estimated from naturally spawning Chinook (i.e., NOS and HOS integrated) was N_e =989, while the natural-origin estimate of N_e in 1997 – 2000 was N_e = 629. Since the natural-origin estimate of 629 is lower than 989, the N_e estimate from all in-river spawners, we argue that their analysis of demographic data show the N_e estimated from naturally spawning Chinook (i.e., NOS and HOS integrated) is larger only if the hatchery Chinook in the river are ignored.

Task 11: Use individual assignment methods to determine the power of self-assignment for upper Wenatchee River tributaries.

See "Assignment of Individual to Populations" in Task 6

Conclusions

Has the Chiwawa Hatchery Supplementation Program succeeded at increasing the census size of the target population while leaving genetic integrity intact? This is an important question, as hatcheries can impact natural populations by reducing overall genetic diversity (Ryman and Laikre 1991), reducing the fitness of the natural populations through relaxation of selection or inadvertent positive selection of traits advantageous in the hatchery (Ford 2002; Lynch and O'Hely 2001), and by reducing the reproductive success of natural populations (McLean et al. 2003). The census data presented here show that the current natural spawning census size is similar to the pre-supplementation census size. Despite large numbers of hatchery-origin fish on the Chiwawa River spawning grounds, the genetic diversity of the natural-origin collections appear unaffected by the supplementation program; heterozygosities are high, and contemporary N_e is similar (perhaps slightly higher) than pre-supplementation N_e. We did find

significant year-to-year differences in allele frequencies in both the origin and spawner datasets, but these differences do not appear to be related to fish origin, spawning area, or genetic drift. However, we do suggest that cohort differences may be the most important factor accounting for differences in allele frequencies among collections.

The main objective of this study was to determine the potential impacts of the hatchery program on natural spring Chinook in the upper Wenatchee system. We did this by analyzing temporally replicated collections from the Chiwawa River, and by comparing genetic diversity prior to the presumed effect of the Chiwawa Hatchery Supplementation Program, with contemporary collections. We report that the genetic diversity present in the Chiwawa River is unchanged (allowing for differences among cohorts) from 1989 – 2006, and the contemporary estimate of the effective population size (N_e) using genetic data is approximately the same as the N_e estimate extrapolated from 1989 – 1992 census data (i.e., pre-hatchery collection years). We observed substantial genetic diversity, with heterozygosities ~80% over thirteen microsatellite markers. Yet, temporal variation in allele frequencies was the norm among temporal collections from the same populations (i.e., location). The genetic differentiation of replicated collections from the same population is likely the result of salmon life history in this area, as four-year-old Chinook comprise a majority of returns each year. The genetic tests are detecting the differences of contributing parents for each cohort. An important point related to the temporal variation, is that the hatchery broodstock is composed in part of the natural origin Chinook from the Chiwawa River. When we compared the genetic data (within a collection year) for Chinook brought into the hatchery as broodstock with the Chinook that remained in the river (years 2001, 2004 – 2006), there was a trend of decreasing statistical differences in allele frequencies from 2001 to 2004, and no differences were detected for 2005 and 2006. While the replicated collections may have detectable differences in allele frequencies, those differences reflect actual differences in cohorts, not the result of hatchery operations, and the hatchery broodstock collection method captures the differences in returning Chiwawa River spring adults each year. We conclude from these results that the genetic diversity of natural spring Chiwawa Chinook has been maintained during the Chiwawa Hatchery Supplementation Program.

We observe slight, but statistically significant population differentiation between Chiwawa River, White River, and Nason Creek collections. Murdoch et al (2006) and Williamson et al. (submitted) also observed population differentiation between Chiwawa River, White River, and Nason Creek collections. Yet, 99.3% of the genetic variation observed was within samples, very little variance could be attributed to population differences (i.e., population structure). The AMOVA analysis and poor individual assignment results suggest the occurrence of gene flow among Wenatchee River locations or a very recent divergence of these groups. While Murdoch et al. 2006 did not perform an AMOVA analysis, their F_{ST} results provide comparable data to our among-population results. Murdoch et al. 2006 report F_{ST} ranging from 2%-3% for pairwise comparisons between of Chiwawa, White, and Nason River collections. Since F_{ST} is an estimate of among-sample variance, these results also imply a majority of the genetic variance (i.e., 97%-98%) resides within collections. To provide further context for the magnitude of these variance estimates, we present the among-group data from Murdoch et al. 2006 comparing summer-run and spring-run Chinook from the Wenatchee River. They report that approximately 91% of observed genetic variance is within-collection for comparisons between collections of summer- and spring-run Chinook. Ultimately, the information provided by this and other reports will be incorporated into the management process for Wenatchee River Chinook. However, we would like to emphasize that the application of these genetic data to management is more about the goals related to the distribution of genetic diversity in the future than specific data values reported. If Chinook are collected at Tumwater Dam instead of within the upper Wenatchee River tributaries, a vast majority of the genetic variation present in the basin would be captured, although any differences among tributaries would be mixed. Alternatively, management policies could be crafted to promote and maintain the among-group genetic diversity that genetic studies consistently observe to be non-zero within the Wenatchee River.

We agree with Murdoch et al. (2006) that it appears hatchery Chinook are not contributing to reproduction in proportion to their abundance. Additionally, if the total census size (i.e., NOS and HOS combined) within the Chiwawa River does not continue

to increase, genetic diversity may decline within this system, given the smaller N_e within the hatchery-origin collections compared with the natural-origin collections.

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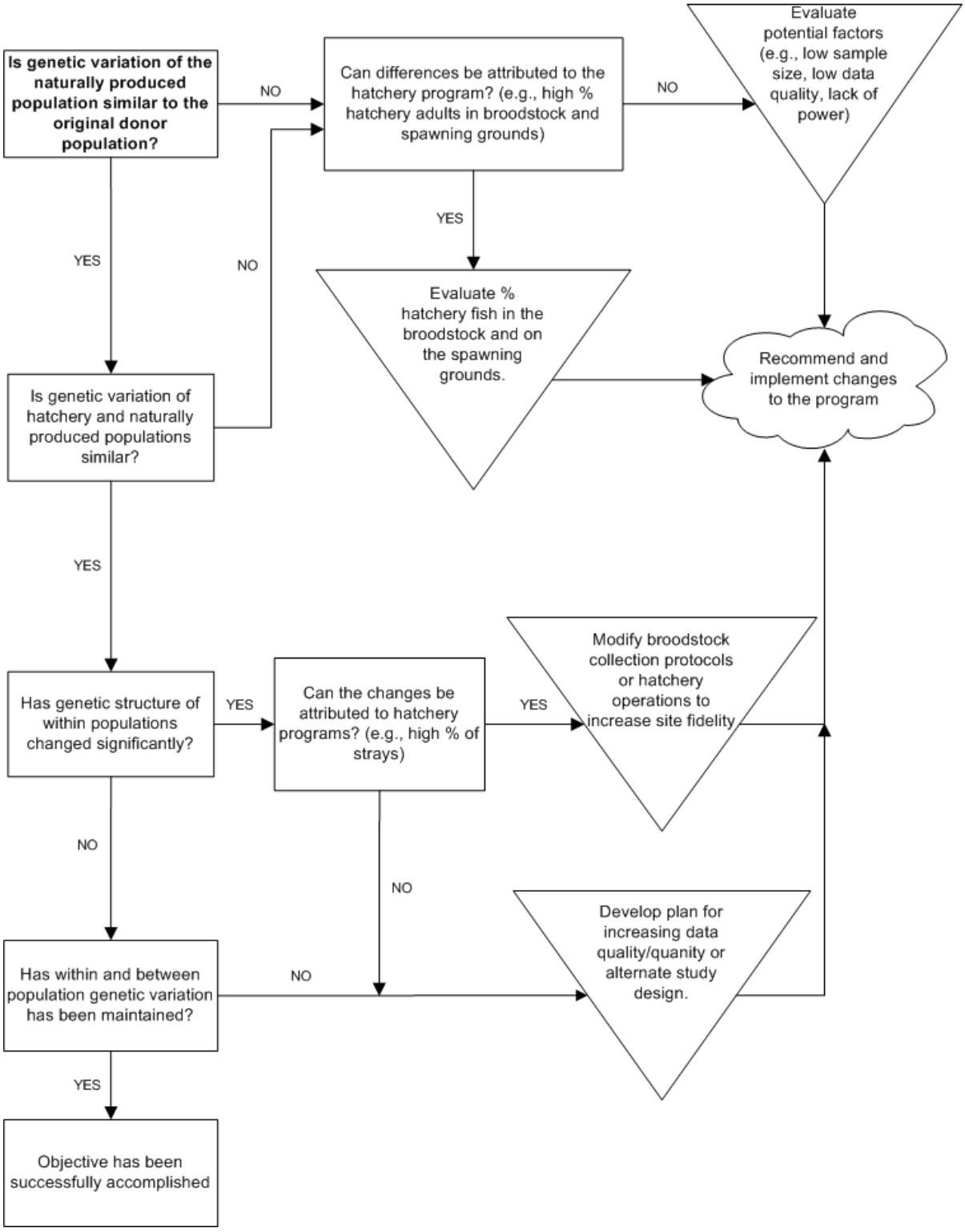

Figure 1. Conceptual process for evaluating potential changes in genetic variation in the Chiwawa naturally produced populations as a result of the supplementation hatchery programs (From Murdoch and Peven 2005).

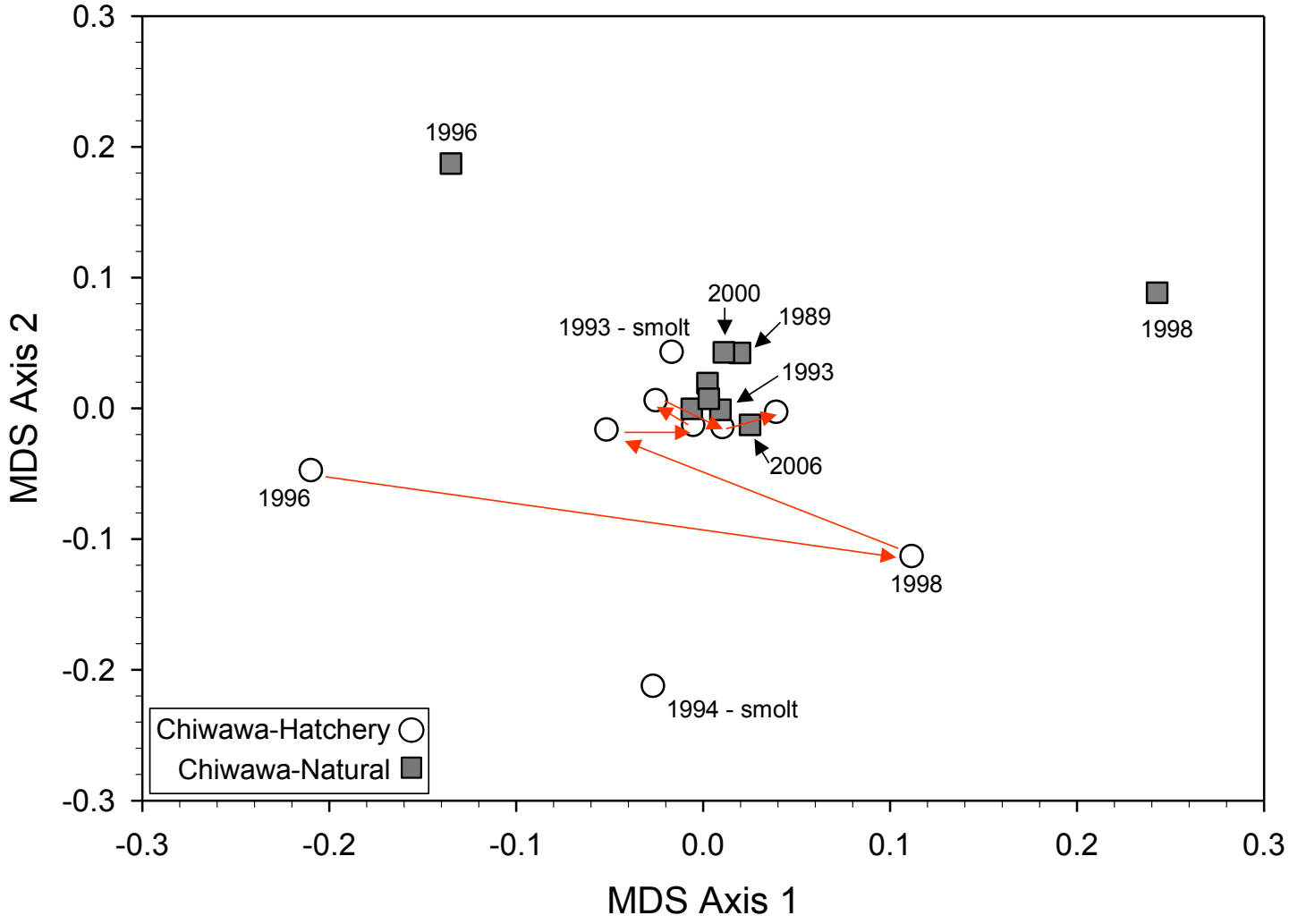

Figure 2. Multidimensional scaling plot from an allele-sharing distance matrix calculated from the Chiwawa data set organized by fish origin (i.e., hatchery versus natural). The red arrows connect consecutive hatchery-origin collections starting with the first adult collection (1996) and ending with the 2006 collection (see Table 1 for collection years).

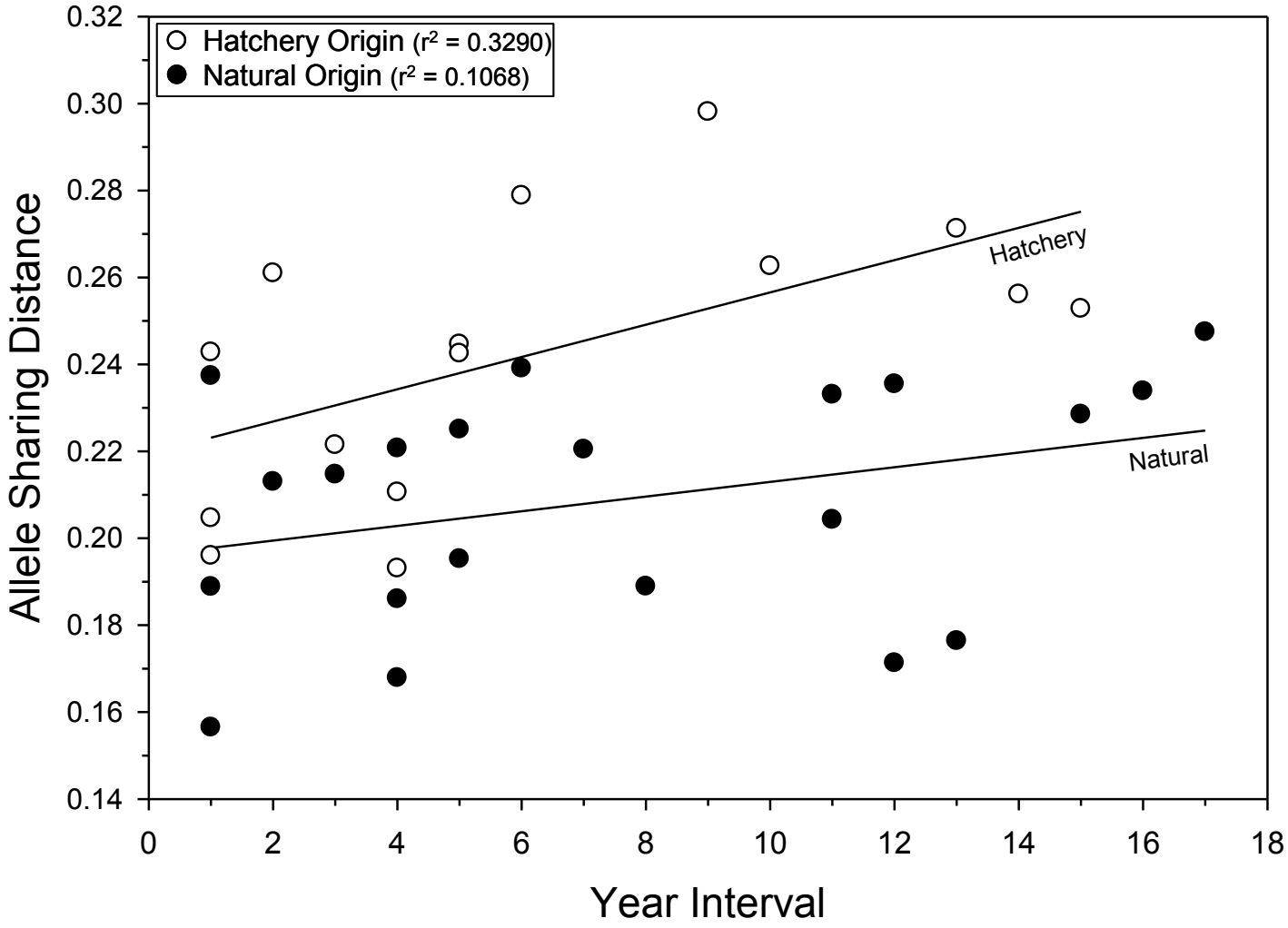

Figure 3. Relationships between the time interval in years and allele sharing distances, with each circle representing the pairwise relationship between two Chiwawa collections. Separate regression lines for the natural- and hatchery-origin collections. The slope for the natural-origin collection is not significantly different from zero (p=0.1483), while the slope for hatchery-origin collection is significantly greater than zero (p=0.0254) indicating a positive relationship between time interval and allele sharing distance.

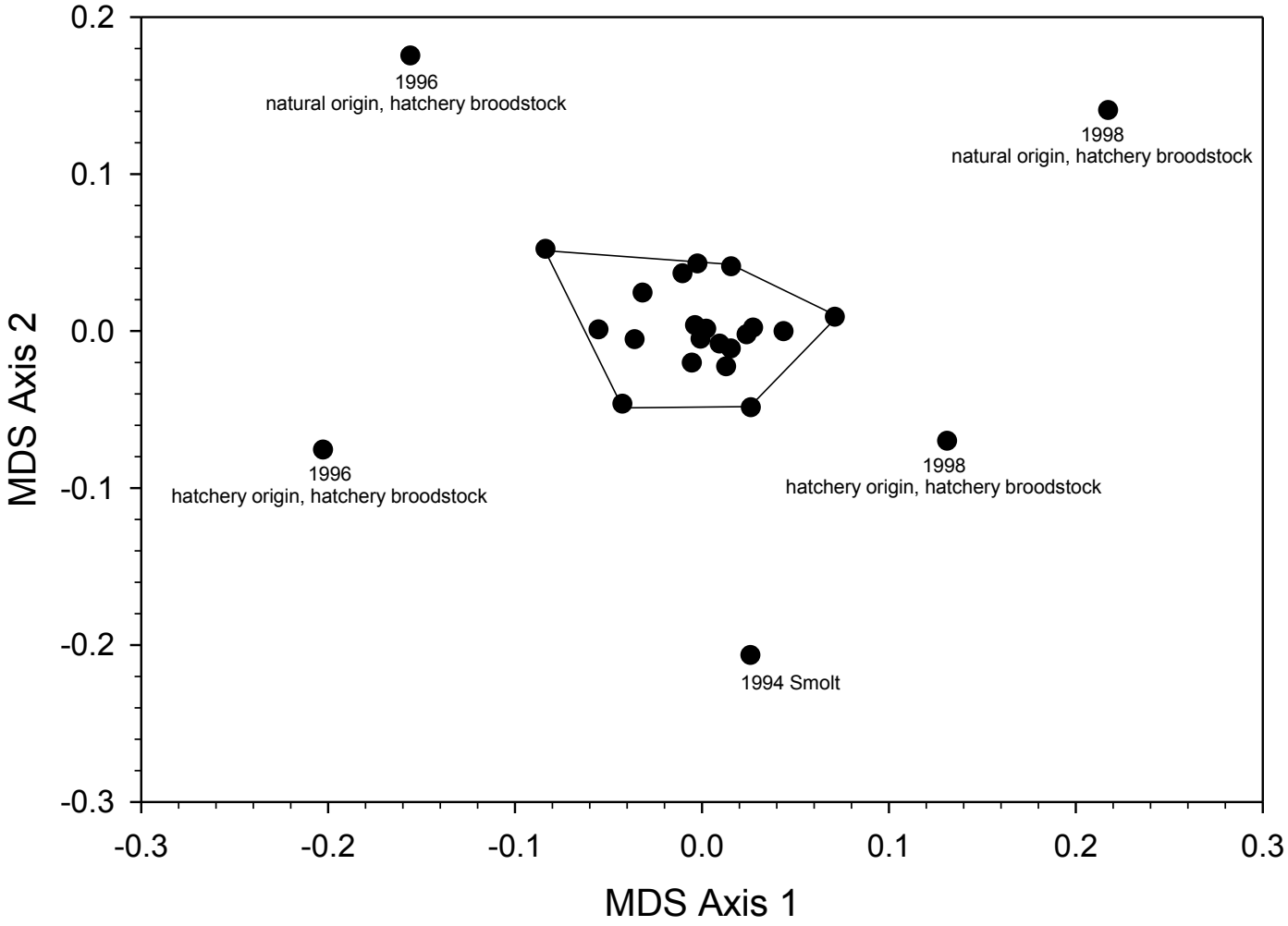

Figure 4. Multidimensional scaling plot from an allele-sharing distance matrix calculated from the Chiwawa data set organized by four treatment groups, as discussed in the text. Each circle represents a single collection within each of the four treatment groups, and the polygon encloses all groups that are not outliers. Each outlier group is specifically labeled.

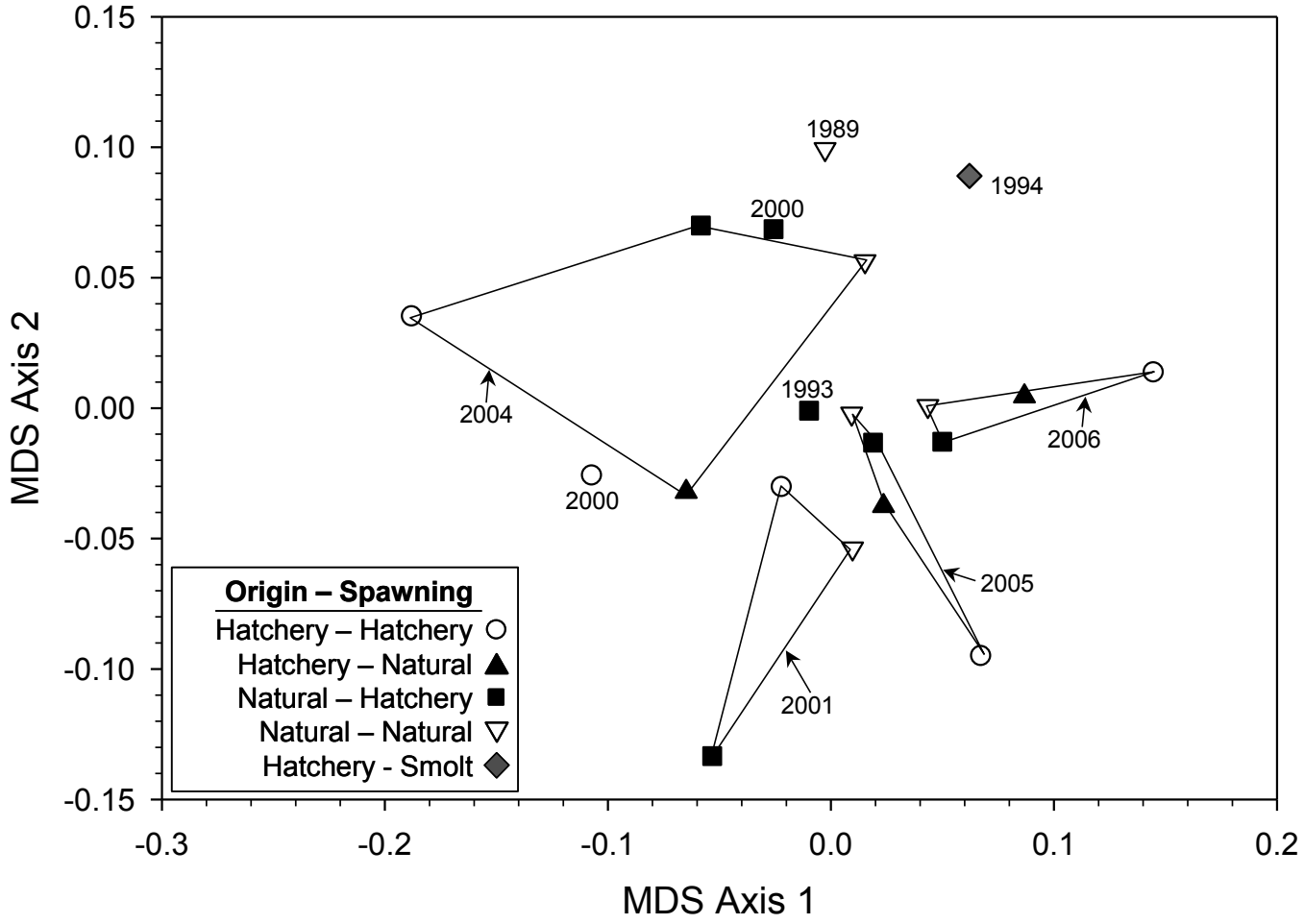

Figure 5. As in Figure 4, but allele-sharing distance matrix recalculated without the five outlier groups shown in Figure 4. Polygons group together treatment groups from the same collection year. Dates associated with symbols also refer to collection year. Collection years 2004-2006 included all four treatment groups, while collection year 2001 did not include a hatchery-origin natural spawner group. Legend is read as follows: Open circles refer to hatchery-origin hatchery spawner group, while filled box refers to natural-origin hatchery spawner group, and so on.

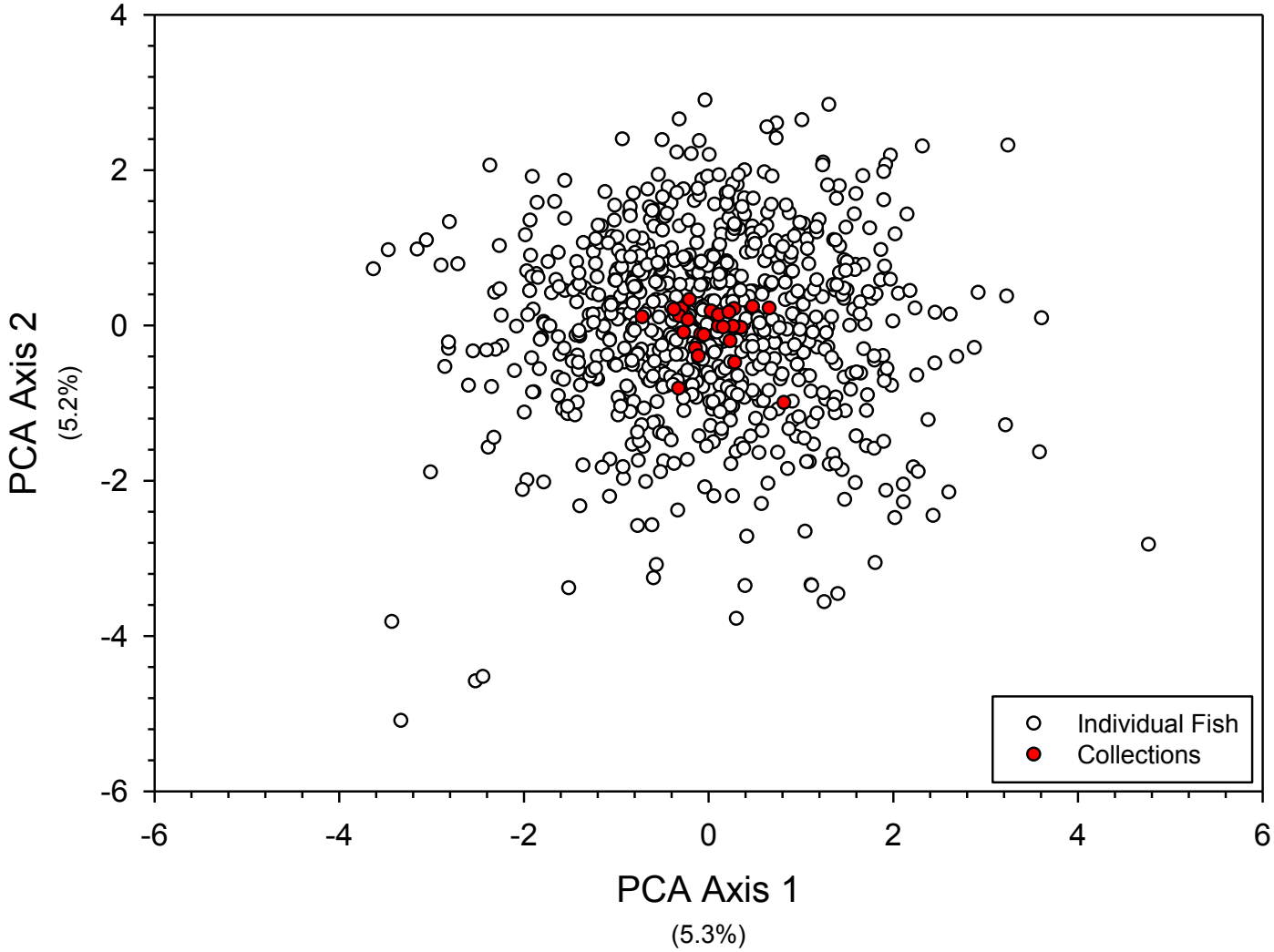

Figure 6. Principal component (PC) analysis of individual fish from the Chiwawa River. Only fish with complete microsatellite genotypes were included in the analysis (n = 757). Open circles are the PC scores for individual fish, and the filled circles are the centroids (bivariate means) for each of the 25 groups discussed in the text. PC axes 1 and 2 account for only 10.5% of the total molecular variance.

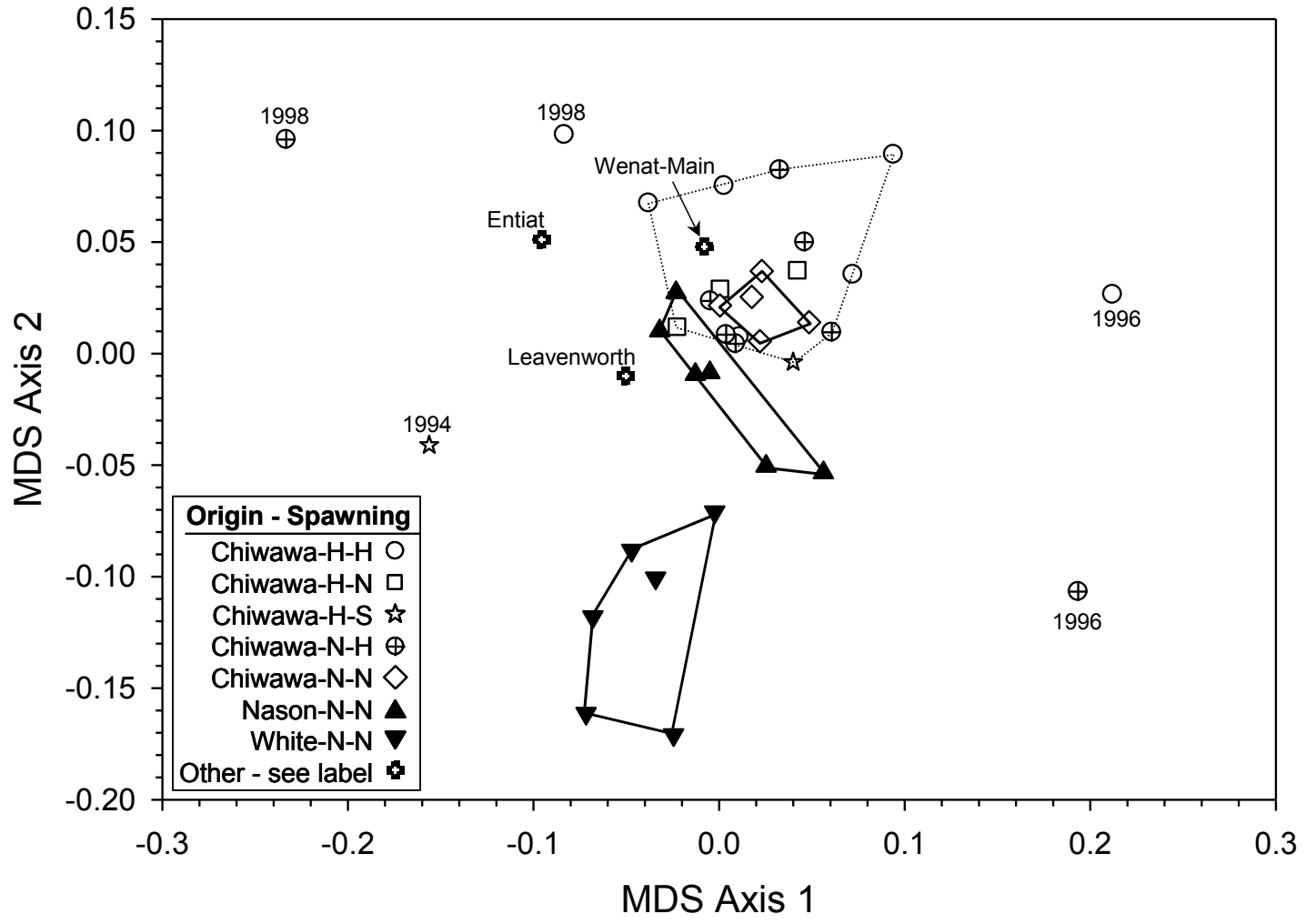

Figure 7. Multidimensional scaling plot from an allele-sharing distance matrix calculated from the Chiwawa origin data set and all other non-Chiwawa collections, except Little Wenatchee River. Legend is read with abbreviations beginning with origin and then spawning location. H=hatchery, N=natural, and S=smolts. Polygons with solid lines enclose the natural-origin natural spawner collections from each population (i.e., river). The polygon with the dotted lines enclose all Chiwawa collections, except for the five outlier collections, as discussed in text.

Table 1 Summary of within population genetic data. Chiwawa collection data are summarized in A) by origin of the sample (i.e., clipped vs. non-clipped). All collection data are summarized in B) by spawning location (i.e., hatchery broodstock or on spawning grounds). Hz is heterozygosity, HWE is the statistical significance of deviations from Hardy-Weinberg expectations (* = 0.05, ** = 0.01, and *** = 0.001), LD is the proportion of pairwise locus tests (across all populations) exhibiting linkage disequilibrium (bolded values are statistically significant), and the last column is mean number of alleles per locus.

Collection	Sample size	Gene Diversity	Observed Hz	HWE	F_{IS}	LD	Mean # Alleles
A) Origin							
1993 Chiwawa Hatchery	95	0.77	0.79	***	-0.02	**0.86**	14.00
1994 Chiwawa Hatchery	95	0.76	0.77	***	-0.01	**0.91**	11.38
1996 Chiwawa Hatchery	8	0.75	0.81	-	-0.01	0.00	8.23
1998 Chiwawa Hatchery	27	0.81	0.82	-	0.00	0.04	12.62
2000 Chiwawa Hatchery	43	0.75	0.78	***	-0.01	**0.19**	12.46
2001 Chiwawa Hatchery	69	0.77	0.80	***	-0.02	**0.14**	15.31
2004 Chiwawa Hatchery	72	0.77	0.77	***	0.01	**0.45**	15.92
2005 Chiwawa Hatchery	91	0.79	0.82	*	-0.03	**0.05**	16.15
2006 Chiwawa Hatchery	95	0.80	0.84	***	-0.05	**0.49**	15.85
1989 Chiwawa Natural	36	0.76	0.78	-	0.01	0.00	12.77
1993 Chiwawa Natural	62	0.78	0.81	-	-0.02	0.04	15.85
1996 Chiwawa Natural	8	0.72	0.78	-	-0.02	0.00	7.54
1998 Chiwawa Natural	10	0.78	0.84	-	0.00	0.00	8.23
2000 Chiwawa Natural	39	0.78	0.79	***	0.00	**0.10**	14.00
2001 Chiwawa Natural	75	0.78	0.80	-	-0.03	0.03	15.31
2004 Chiwawa Natural	85	0.78	0.77	-	0.02	0.01	15.77
2005 Chiwawa Natural	90	0.79	0.79	-	0.01	0.01	16.15
2006 Chiwawa Natural	96	0.80	0.81	-	-0.01	0.01	16.46

Table 1 Within population genetic data analysis summary continued.

Collection	Sample size	Gene Diversity	Observed Hz	HW	F_{IS}	LD	Mean # Alleles
B) Spawning Location							
1993 Chiwawa Broodstock	62	0.78	0.81	-	-0.02	0.00	15.85
1996 Chiwawa Broodstock	16	0.75	0.79	-	-0.02	0.00	10.92
1998 Chiwawa Broodstock	37	0.82	0.83	-	0.00	0.01	14.38
2000 Chiwawa Broodstock	82	0.78	0.78	***	0.00	**0.32**	15.62
2001 Chiwawa Broodstock	89	0.78	0.80	*	-0.02	**0.13**	15.77
2004 Chiwawa Broodstock	61	0.77	0.76	*	0.02	**0.13**	14.92
2005 Chiwawa Broodstock	75	0.79	0.78	*	0.02	0.01	15.85
2006 Chiwawa Broodstock	89	0.80	0.83	-	-0.03	**0.05**	16.46
1989 Chiwawa River	36	0.76	0.78	-	0.01	0.00	12.77
2001 Chiwawa River	55	0.78	0.80	-	-0.02	**0.09**	14.00
2004 Chiwawa River	96	0.78	0.78	*	0.01	**0.18**	17.23
2005 Chiwawa River	106	0.79	0.82	*	-0.02	**0.06**	16.69
2006 Chiwawa River	102	0.80	0.83	***	-0.03	**0.10**	16.77
1989 White River	48	0.75	0.75	-	0.01	0.01	12.85
1991 White River	19	0.76	0.76	-	0.03	0.00	10.92
1992 White River	22	0.75	0.79	-	-0.02	0.01	11.00
1993 White River	21	0.75	0.69	*	0.10	0.00	10.15
2005 White River	29	0.75	0.77	-	-0.01	0.03	12.23
2006 White River	40	0.76	0.76	-	0.01	0.04	13.38

Table 1 Within population genetic data analysis summary continued.

Collection	Sample size	Gene Diversity	Observed Hz	HW	F_{IS}	LD	Mean # Alleles
1993 Little Wenatchee R.	19	0.84	0.85	-	0.02	0.00	11.23
1993 Nason Creek	45	0.78	0.80	-	-0.01	0.01	13.77
2000 Nason Creek	51	0.76	0.78	-	-0.02	**0.13**	13.92
2001 Nason Creek	41	0.79	0.81	-	-0.01	**0.08**	14.23
2004 Nason Creek	38	0.76	0.76	-	0.02	0.03	13.23
2005 Nason Creek	45	0.78	0.82	-	-0.04	0.03	14.92
2006 Nason Creek	48	0.80	0.82	-	-0.01	0.00	15.77
2001 Wenatchee River	32	0.79	0.80	*	0.00	0.04	12.85
2000 Leavenworth NFH	73	0.80	0.82	*	-0.02	**0.15**	16.23
1997 Entiat NFH	37	0.81	0.83	-	-0.01	**0.06**	14.38

Table 2 Demographic data for Chiwawa Hatchery and Chiwawa natural spring Chinook salmon. BS is census size of hatchery broodstock, pNOB is the proportion of hatchery broodstock of natural origin, NOS is the census size of natural-origin spawners present in Chiwawa River, HOS is the census size of hatchery-origin spawners present in Chiwawa River, Total is NOS and HOS combined, and pNOS is the proportion of spawners present in Chiwawa River of natural origin.

	Hatchery		In River			
Brood Year	BS	pNOB	NOS	HOS	Total	pNOS
1989	28	1	1392	0	1392	1.00
1990	18	1	775	0	775	1.00
1991	32	1	585	0	585	1.00
1992	78	1	1099	0	1099	1.00
1993	94	1	677	491	1168	0.58
1994	11	0.64	190	90	280	0.68
1995	0	0	8	50	58	0.14
1996	18	0.44	131	51	182	0.72
1997	111	0.29	210	179	389	0.54
1998	47	0.28	134	45	178	0.75
1999	0	0	119	13	132	0.90
2000	30	0.3	378	310	688	0.55
2001	371	0.3	1280	2850	4130	0.31
2002	71	0.28	694	919	1613	0.43
2003	94	0.44	380	223	603	0.63
2004	215	0.39	820	788	1608	0.51
2005	270	0.33	250	1222	1472	0.17

Table 3 Levels of significance for pairwise tests of genic differentiation among all hatchery- and natural-origin collections used in this analysis. HS = highly significant (P < 0.000095; the Bonferroni corrected p-value for an alpha = 0.05); * = P < 0.05 (nominal critical value for most statistical test); - = P > 0.05 (not significant). A significant result between pairs of populations indicates that the allele frequencies between the pair are significantly different. Results are read by comparing the collections along the rows to collections along columns. The top block for each section is a symmetric matrix, as it compares collections within the same group.

		Chiwawa – Hatchery Origin								
		1993	**1994**	**1996**	**1998**	**2000**	**2001**	**2004**	**2005**	**2006**
Chiwawa – Hat. Origin	**1993**		HS	*	HS	HS	HS	HS	HS	HS
	1994	HS		HS	HS	HS	HS	HS	HS	HS
	1996	*	HS		*	-	*	-	-	*
	1998	HS	HS	*		HS	HS	HS	HS	HS
	2000	HS	HS	-	HS		HS	*	HS	HS
	2001	HS	HS	*	HS	HS		HS	*	HS
	2004	HS	HS	-	HS	*	HS		HS	HS
	2005	HS	HS	-	HS	HS	*	HS		HS
	2006	HS	HS	*	HS	HS	HS	HS	HS	
Chiwawa – Natural Origin	**1989**	HS	HS	-	HS	HS	*	HS	HS	HS
	1993	HS	HS	-	HS	HS	-	HS	*	HS
	1996	*	HS	-	*	-	-	-	-	-
	1998	HS	HS	-	-	HS	*	*	*	-
	2000	HS	HS	-	HS	HS	HS	*	HS	HS
	2001	HS	HS	-	HS	HS	HS	HS	*	HS
	2004	HS	HS	-	HS	HS	HS	HS	HS	HS
	2005	HS	HS	-	HS	HS	*	HS	*	HS
	2006	HS	HS	-	*	HS	HS	HS	HS	HS
Nason	**1996**	HS	HS	-	HS	HS	HS	HS	HS	HS
	2000	HS	HS	*	HS	HS	HS	HS	HS	HS
	2001	HS	HS	-	HS	HS	HS	HS	HS	HS
	2004	HS	HS	-	HS	HS	HS	HS	HS	HS
	2005	HS	HS	-	HS	HS	HS	HS	HS	HS
	2006	HS	HS	-	*	HS	HS	HS	HS	HS
White	**1989**	HS	HS	HS	HS	HS	HS	HS	HS	HS
	1991	HS	HS	-	HS	HS	HS	HS	HS	HS
	1992	HS	HS	*	HS	HS	HS	HS	HS	HS
	1993	HS	HS	*	HS	HS	HS	HS	HS	HS
	2005	HS	HS	-	HS	HS	HS	HS	HS	HS
	2006	HS	HS	HS	HS	HS	HS	HS	HS	HS
Other	**Wen-M**	HS	HS	*	HS	HS	*	*	-	HS
	Leaven	HS	HS	*	HS	HS	HS	HS	HS	HS
	Entiat	HS	HS	*	HS	HS	HS	HS	HS	HS

Table 3 (con't)

		Chiwawa – Natural Origin								
		1989	**1993**	**1996**	**1998**	**2000**	**2001**	**2004**	**2005**	**2006**
Chiwawa – Natural Origin	**1989**		-	-	-	-	*	*	*	*
	1993	-		-	*	*	*	HS	*	HS
	1996	-	-		-	-	-	-	-	-
	1998	-	*	-		*	*	HS	*	*
	2000	-	*	-	*		HS	-	HS	HS
	2001	*	*	-	*	HS		HS	*	HS
	2004	*	HS	-	HS	-	HS		HS	HS
	2005	*	*	-	*	HS	*	HS		*
	2006	*	HS	-	*	HS	HS	HS	*	
Nason	**1996**	*	*	-	*	*	HS	HS	HS	HS
	2000	HS	HS	HS	HS	HS	HS	HS	HS	HS
	2001	HS	*	-	*	HS	HS	HS	HS	HS
	2004	HS	HS	-	HS	HS	HS	HS	HS	HS
	2005	*	*	-	*	HS	HS	HS	HS	HS
	2006	HS	HS	-	-	HS	HS	HS	HS	HS
White	**1989**	HS	HS	*	HS	HS	HS	HS	HS	HS
	1991	HS	HS	*	-	HS	HS	HS	HS	HS
	1992	HS	HS	-	*	HS	HS	HS	HS	HS
	1993	HS	*	-	*	HS	HS	HS	HS	HS
	2005	HS	*	*	*	HS	HS	HS	*	HS
	2006	HS	HS	*	HS	HS	HS	HS	HS	HS
Other	**Wen-M**	*	-	-	-	*	*	HS	*	*
	Leaven	HS	HS	*	*	HS	HS	HS	HS	HS
	Entiat	HS	HS	*	HS	HS	HS	HS	HS	HS

Table 3 (con't)

		Nason					
		1996	**2000**	**2001**	**2004**	**2005**	**2006**
Nason	**1996**		HS	-	HS	-	*
	2000	HS		HS	HS	HS	HS
	2001	-	HS		*	-	*
	2004	HS	HS	*		*	HS
	2005	-	HS	-	*		-
	2006	*	HS	*	HS	-	
White	**1989**	HS	HS	HS	HS	HS	HS
	1991	*	HS	HS	HS	*	*
	1992	HS	HS	HS	HS	HS	HS
	1993	*	HS	HS	HS	HS	HS
	2005	*	HS	HS	HS	HS	HS
	2006	HS	HS	HS	HS	HS	HS
Other	**Wen-M**	HS	HS	HS	HS	*	HS
	Leaven	HS	HS	HS	HS	HS	HS
	Entiat	HS	HS	HS	HS	HS	HS

Table 3 (con't)

		White						Other		
		1989	**1991**	**1992**	**1993**	**2005**	**2006**	**Wen-M 2001**	**Leaven 2000**	**Entiat 1997**
White	**1989**		-	*	-	HS	HS	HS	HS	HS
	1991	-		-	-	*	*	*	HS	HS
	1992	*	-		-	*	*	HS	HS	HS
	1993	-	-	-		*	*	HS	HS	HS
	2005	HS	*	*	*		*	HS	HS	HS
	2006	HS	*	*	*	*		HS	HS	HS
Other	**Wen-M**	HS	*	HS	HS	HS	HS		HS	HS
	Leaven	HS	HS	HS	HS	HS	HS	HS		HS
	Entiat	HS	HS	HS	HS	HS	HS	HS	HS	

Table 4 Probabilities (above diagonal) and levels of significance (below diagonal) for pairwise tests of genic differentiation among all Chiwawa hatchery broodstock and Chiwawa natural spawner collections used in this analysis. HS = highly significant (P < 0.000476; the Bonferroni corrected p-value for an alpha = 0.05); * = P < 0.05 (nominal critical value for most statistical test); - = P > 0.05 (considered not significant). A significant result between pairs of populations indicates that the allele frequencies between the pair are significantly different. Pairwise comparisons between the hatchery broodstock and natural spawner collections from 2001, 2004, 2005, and 2006, respectively, are highlighted.

		Smolt		Hatchery Broodstock								Natural Spawners				
		1993	1994	1993	1996	1998	2000	2001	2004	2005	2006	1989	2001	2004	2005	2006
Smolt	1993		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	1994	HS		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Hatchery Broodstock	1993	HS	HS		0.9155	0.0000	0.0073	0.3647	0.0003	0.0694	0.0000	0.2220	0.0039	0.0008	0.0095	0.0000
	1996	HS	HS	-		0.0151	0.8388	0.0452	0.4916	0.3189	0.0716	0.5591	0.0759	0.8101	0.2364	0.0786
	1998	HS	HS	HS	*		0.0000	0.0000	0.0000	0.0000	0.0043	0.0000	0.0000	0.0000	0.0000	0.0005
	2000	HS	HS	*	-	HS		0.0000	0.4720	0.0000	0.0000	0.0036	0.0000	0.0712	0.0000	0.0000
	2001	HS	HS	-	*	HS	HS		0.0000	0.0059	0.0000	0.0003	0.0000	0.0000	0.0126	0.0000
	2004	HS	HS	*	-	HS	-	HS		0.0000	0.0000	0.0001	0.0000	0.0012	0.0000	0.0000
	2005	HS	HS	-	-	HS	HS	*	HS		0.0005	0.0024	0.0137	0.0025	0.7782	0.0018
	2006	HS	HS	HS	-	*	HS	HS	HS	*		0.0000	0.0000	0.0000	0.0000	0.5770
Natural Spawners	1989	HS	HS	-	-	HS	*	*	HS	*	HS		0.0023	0.0317	0.0000	0.0003
	2001	HS	HS	*	-	HS	HS	HS	HS	*	HS	*		0.0000	0.2641	0.0000
	2004	HS	HS	*	-	HS	-	HS	*	*	HS	*	HS		0.0000	0.0000
	2005	HS	HS	*	-	HS	HS	*	HS	-	HS	HS	-	HS		0.0000
	2006	HS	HS	HS	-	*	HS	HS	HS	*	-	*	HS	HS	HS	

Table 5 Analysis of molecular variance (AMOVA) for the Chiwawa collections, showing the partition of molecular variance into (1) within collections, (2) among collections but within group, and (3) among group components. Each column in the table represents a separate analysis testing for differences under a different spatial or temporal hypothesis. The different analyses are grouped together in a single table for comparisons. The values within the table are percentages and the parenthetical values are P-values, or probabilities, associated with that percentage. P-values greater than 0.05 indicate that the percentage is not significantly different from zero. For example, when collections are organized by hatchery- versus natural-origin ("Origin" – fourth column), 0.11% of the molecular variance is attributed to among group (i.e., hatchery- versus natural-origin), which is not significantly different from zero. No collections (first column) indicates no organization or grouping among all collections, and the among-group percentage is equal to the F_{ST} for the entire data set.

	No Structure	Collection Year	Spawning Location	Origin	Origin-Spawning Location
Among Groups	0.26 (0.00)	0.20 (0.43)	0.05 (0.48)	0.11 (0.15)	0.11 (0.06)
Among collections - Within groups	-	0.08 (0.003)	0.24 (0.00)	0.21 (0.00)	0.18 (0.06)
Within collections	99.74 (0.00)	99.72 (0.00)	99.71 (0.00)	99.68 (0.00)	99.71 (0.00)

Table 6 F_{ST} values for all pairwise combinations of populations. Each F_{ST} is the median value for all pairwise combinations of collections within each population (the number of collections within each population is shown parenthetically next to each population name on each row). For example, the F_{ST} for the Chiwawa hatchery versus the White River (0.019) is the median value of 54 pairwise comparisons. The bold values along the center diagonal are the median F_{ST} values within each collection. For those populations with only one collection, the diagonal value was set at 0.000.

	Chiwawa-Hatchery	Chiwawa-Natural	Entiat	Leaven-worth	Nason	Wenatchee-main	White	Little Wenatchee
Chiwawa-Hatchery (9)	**0.013**	0.008	0.016	0.012	0.011	0.005	0.019	0.111
Chiwawa-Natural (9)		**0.003**	0.012	0.011	0.007	0.003	0.014	0.105
Entiat (1)			**0.000**	0.005	0.010	0.008	0.019	0.078
Leavenworth (1)				**0.000**	0.007	0.008	0.014	0.092
Nason (6)					**0.006**	0.008	0.015	0.099
Wenatchee-main (1)						**0.000**	0.012	0.098
White (6)							**0.005**	0.113
Little Wenatchee (1)								**0.000**

Table 7 As in Table 5, except data includes Chiwawa hatchery- and natural-origin, Nason Creek, and White River collections

	All Years	All Years	1989-1996	2005-2006	2005-2006
	No Structure	Origin	Origin	Origin	Collection Year
Among Groups	0.28 (0.00)	0.33 (0.00)	-0.07 (0.67)	0.43 (0.01)	-0.06 (0.57)
Among Collections - Within groups	-	0.04 (0.00)	0.22 (0.00)	0.25 (0.00)	0.64 (0.00)
Within Collections	99.72	99.63	99.85	99.32	99.41

Table 8 Individual assignment results reported are the numbers of individuals assigned to each population using the partial Bayesian criteria of Rannala and Mountain (1997) and a "jack-knife" procedure (see Methods). The population with the highest posterior probability is considered the stock of origin (i.e., no unassigned individuals). Individuals from each population are assigned to specific populations (along rows). Bold values indicate correct assignment back to population of origin. Individuals assigned to a population are read down columns. For example, of the 595 individuals from Chiwawa hatchery origin, 134 individuals were assigned to Chiwawa natural origin (reading across). Of the 511 individuals assigned to Chiwawa natural origin (reading down), 60 were from Nason Creek.

Population	**Total**	**Unassigned**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1) Chiwawa Hatchery	595	0	**371**	134	2	16	0	45	15	12
2) Chiwawa Natural	501	0	156	**269**	4	5	0	42	9	16
3) Entiat	37	0	4	5	**13**	8	0	6	1	0
4) Leavenworth	73	0	9	8	3	**33**	0	17	0	3
5) Little Wenatchee	19	0	0	0	0	0	**19**	0	0	0
6) Nason	268	0	49	60	5	11	0	**131**	1	11
7) Wenatchee Mainstem	32	0	12	9	0	1	0	2	**6**	2
8) White	179	0	22	26	0	2	0	13	1	**115**
TOTAL	1704	0	623	511	27	76	19	256	33	159

Table 9 As in Table 8, except the posterior probability from the partial Bayesian criteria of Rannala and Mountain (1997) must be 0.90 or greater, to be assigned to a population. Those individuals with posterior probabilities less than 0.90 are unassigned.

Aggregate	Total	Unassigned	1	2	3	4	5	6	7	8
1) Chiwawa Hatchery	595	332	**214**	31	1	4	0	10	3	0
2) Chiwawa Natural	501	375	30	**82**	0	1	0	5	2	6
3) Entiat	37	24	1	1	**5**	4	0	2	0	0
4) Leavenworth	73	51	0	1	1	**19**	0	1	0	0
5) Little Wenatchee	19	2	0	0	0	0	**17**	0	0	0
6) Nason	268	188	11	6	2	5	0	**53**	0	3
7) Wenatchee Mainstem	32	23	4	3	0	0	0	0	**2**	0
8) White	179	92	4	3	0	1	0	5	1	**73**
TOTAL	1704	1087	264	127	9	34	17	76	8	82

Table 10 Estimates of N_e based on bias correction method of Waples (2006) implemented in LDNe (Do and Waples unpublished). Collections are categorized by spawning location. Sample size is the harmonic mean of the sample size, 95% CI is the confidence interval calculated using Waples' (2006) equation 12, and Major Cohort assumes that each collection is 100% four-year-olds.

	Sample size	Estimated N_b	95% CI	Major Cohort	Census	N_e/N
1993 Chiwawa Broodstock	58.4	103.1	77.0 - 149.7	1989	1392	0.30
1996 Chiwawa Broodstock	15.5	30.4	19.6 - 58.1	1992	1099	0.11
1998 Chiwawa Broodstock	33.4	37.7	29.8 - 49.7	1994	280	0.54
2000 Chiwawa Broodstock	77.8	48.4	41.4 - 57.2	1996	182	1.06
2001 Chiwawa Broodstock	80.4	49.6	42.2 - 59.2	1997	389	0.51
2004 Chiwawa Broodstock	56.6	48.1	39.0 - 60.9	2000	688	0.28
2005 Chiwawa Broodstock	73	274.3	148.9 - 1131.8	2001	4130	0.27
2006 Chiwawa Broodstock	88.4	198.3	136.1 - 340.5	2002	1613	0.49
1989 Chiwawa River	26.6	5.2	3.9 - 6.3	1985		
2001 Chiwawa River	46.7	38.6	31.0 - 49.3	1997	389	0.40
2004 Chiwawa River	88.5	82.6	67.3 - 104.4	2000	688	0.48
2005 Chiwawa River	104.2	231.5	161.8 - 382.7	2001	4130	0.22
2006 Chiwawa River	101.1	107.3	87.2 - 136	2002	1613	0.27

Table 11 Summary of output from program SALMONNb and data for eight Chiwawa broodstock collections from Wenatchee River. For each pairwise comparison of samples *i* and *j*, $\widetilde{S}$ is the harmonic mean sample size, *n* is the number of independent alleles used in the comparison, $\hat{N}_{b(i,j)}$ are the pairwise estimates of N_b, and Var [$\hat{N}_{b(i,j)}$] is the variance of $\hat{N}_{b(i,j)}$. $\widetilde{N}_b$ is the harmonic mean of the $\hat{N}_{b(i,j)}$. Alleles with a frequency below 0.05 were excluded from the analysis to reduce potential bias.

Year	1993	1996	1998	2000	2001	2004	2005	2006
Pairwise $\widetilde{S}$ (above diagonal) and *n* (below diagonal):								
1993	-	24.5	42.5	66.4	67.2	57.2	64.6	70.3
1996	82	-	21.2	25.8	26.0	24.4	25.6	26.4
1998	80	81	-	46.7	47.2	42.0	45.8	48.4
2000	80	82	84	-	78.6	65.2	75.1	82.7
2001	73	77	81	76	-	66.0	76.2	84.2
2004	77	81	75	76	78	-	63.5	69.0
2005	71	75	82	73	73	69	-	80.0
2006	81	80	84	75	74	75	72	-
Pairwise $\hat{N}_{b(i,j)}$ (above diagonal) and Var [$\hat{N}_{b(i,j)}$] (below diagonal):								
1993	-	-742.7	406.9	1240.8	-5432.0	829.8	808.9	729.0
1996	22491.2	-	110.4	-1786.5	765.9	162.8	824.7	382.7
1998	10910.4	67299.1	-	101.8	237.1	69.6	307.0	140.0
2000	6910.0	742895.8	19122.7	-	490.6	1498.2	706.9	201.6
2001	49318.3	21402.8	9754.2	6126.6	-	307.8	82.0	362.5
2004	8338.4	257267.7	24283.0	145043.4	7095.7	-	269.7	140.1
2005	31511.8	22242.5	10015.8	6596.6	114931.1	8240.4	-	599.6
2006	6223.8	43935.2	73518.7	10152.5	5885.3	12827.0	6370.8	-

$\widetilde{N}_b = 269.4$

Table 12 Summary of output from program SALMONNb and data for five Chiwawa in-river spawner collections from Wenatchee River. For each pairwise comparison of samples *i* and *j*, $\tilde{S}$ is the harmonic mean sample size, *n* is the number of independent alleles used in the comparison, $\hat{N}_{b(i,j)}$ are the pairwise estimates of N_b, and Var [$\hat{N}_{b(i,j)}$] is the variance of $\hat{N}_{b(i,j)}$. $\tilde{N}_b$ is the harmonic mean of the $\hat{N}_{b(i,j)}$. Alleles with a frequency below 0.05 were excluded from the analysis to reduce potential bias.

Year	1989	2001	2004	2005	2006
Pairwise $\tilde{S}$ (above diagonal) and *n* (below diagonal):					
1989	-	33.3	40.2	41.7	42.2
2001	72	-	60.5	63.9	63.3
2004	72	77	-	95.3	94.0
2005	69	72	75	-	102.5
2006	76	76	77	78	-
Pairwise $\hat{N}_{b(i,j)}$ (above diagonal) and Var [$\hat{N}_{b(i,j)}$] (below diagonal):					
1989	-	118.4	299.0	143.3	165.3
2001	40378.8	-	181.7	-1537.3	153.5
2004	10455.2	7265.5	-	387.1	329.4
2005	20923.6	68660.6	5040.7	-	356.8
2006	16227.2	8886.9	3802.0	4522.8	-

$\tilde{N}_b = 224.2$

Table 13 Summary of output from program SALMONNb and data for three brood years that combined Chiwawa natural- and hatchery-origin samples from Wenatchee River. For each pairwise comparison of samples *i* and *j*, $\widetilde{S}$ is the harmonic mean sample size, *n* is the number of independent alleles used in the comparison, $\hat{N}_{b(i,j)}$ are the pairwise estimates of N_b, and Var [$\hat{N}_{b(i,j)}$] is the variance of $\hat{N}_{b(i,j)}$. $\widetilde{N}_b$ is the harmonic mean of the $\hat{N}_{b(i,j)}$. Alleles with a frequency below 0.05 were excluded from the analysis to reduce potential bias.

Year	2004	2005	2006
Pairwise $\widetilde{S}$ (above diagonal) and *n* (below diagonal):			
2004	-	162	164.3
2005	77	-	188.2
2006	76	75	-
Pairwise $\hat{N}_{b(i,j)}$ (above diagonal) and Var [$\hat{N}_{b(i,j)}$] (below diagonal):			
2004	-	611.3	210.8
2005	9351.5	-	727.5
2006	14965.5	8673.9	-

$\widetilde{N}_b = 386.8$

Appendix L

Fish Trapping at the Nason Creek Smolt Trap 2016

Population Estimates for Juvenile Salmonids in Nason Creek, WA

2016 Annual Report

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ABSTRACT

In 2016, Yakama Nation Fisheries Resource Management (YNFRM) monitored emigration of Endangered Species Act (ESA) listed Upper Columbia River (UCR) spring Chinook salmon and summer steelhead as well as naturally spawned juvenile coho salmon in Nason Creek. This report summarizes juvenile abundance and freshwater survival estimates for each of these species. Fish were captured using a 1.5m rotary smolt trap between March 1 and November 30, 2016. We collected 852 spring Chinook salmon, 672 summer steelhead, 1 bull trout, and 6 coho; all of natural origin and varying age classes. Daily fish abundances for spring Chinook, steelhead, and coho were expanded by stream discharge-to-trap efficiency regression or pooled estimates. All estimates were made with a 95% confidence interval (CI) with total emigration estimates for BY2014 spring Chinook juveniles and coho juveniles of 8,694 (± 5,207) and 223 (± 514), respectively. We estimated the total BY2013 summer steelhead emigration at the trap to be 13,417 (± 3,733). Egg-to-emigrant survival rates for BY2014 spring Chinook and BY2013 summer steelhead were both 1.7%. Productivity, as measured by emigrants-per-redd, for spring Chinook and summer steelhead, was 76 and 99, respectively.

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ACKNOWLEDGEMENTS

This project is part of a basin-wide monitoring program requiring close coordination between multiple agencies and contractors. We greatly appreciate the hard work of the Yakama Nation Fisheries Resource Management (YNFRM) crew members including Matthew Clubb, Jamie Hallman, Arlene Heemsah, Tim Jeffris, and Kevin Swager who maintained and operated the trap during all hours including nights/weekends and through challenging weather conditions. We would like to also thank the Wenatchee River Ranger District (U.S. Forest Service) and Mr. Duane Bolser for providing use of the trapping site and accommodating the needs of this project as well as to Peter Graf (Grant County PUD) for administering contracting and funding. Finally, thank you to Mike Hughes, Mclain Johnson, Andrew Murdoch, Ben Truscott, and Joshua Williams (Washington Department of Fish and Wildlife), and Tracy Hillman (Bio Analysts, Inc.) for shared data and smolt trap methodologies.

1.0 INTRODUCTION

Beginning in the fall of 2004, Yakama Nation Fisheries Resource Management (YNFRM) began operating a rotary smolt trap in Nason Creek for nine months per year. Prior to 2004, the smolt trap was operated on a limited basis solely for hatchery coho predation studies. This project is a cost share between the YNFRM's Mid-Columbia Coho Reintroduction Program (MCCRP) and Grant County PUD's Hatchery Monitoring Plan. Trap operations were conducted in compliance with ESA consultation specifically to address abundance and productivity of spring Chinook, steelhead trout, and coho salmon in Nason Creek.

Within this document we will report:

1) Juvenile abundance and productivity of spring Chinook salmon (tkwínat) *Oncorhynchus tshawytscha*, steelhead trout (shúshaynsh) *Oncorhynchus mykiss* and coho salmon (súnx) *Oncorhynchus kisutch* in Nason Creek.

2) Emigration timing of spring Chinook salmon, steelhead trout and coho salmon emigrating from Nason Creek.

The data presented will be directly used to address Objective 2 in the Monitoring and Evaluation Plan for PUD Hatchery Programs (Hillman et al. 2015) on a 5-year analytic cycle:

Objective 2: Determine if the proportion of hatchery fish on the spawning grounds affects the freshwater productivity of supplemented stocks (Hillman et al. 2013).

1.1 Watershed Description

The Nason Creek watershed drains 26,547 ha of alpine glaciated landscape where high precipitation and moderate rain on snow recurrence controls the hydrology and aquatic communities. Nason Creek originates near the Cascade crest at Stevens Pass and flows east for approximately 37 river kilometers (rkm) until joining the Wenatchee River at rkm 86.3 just below Lake Wenatchee. Both smolt trap locations employed in 2014 (see section 2.1 Trapping Equipment and Operations) were downstream from the majority of spring Chinook and steelhead spawning grounds (Figure 1). There are 26.4 rkm along the mainstem accessible to anadromous fish in Nason Creek. Private land ownership comprises 21,165 ha (79.7%) of the watershed while 5,180 ha (19.5%) are federal and 194 ha (0.1%) are state owned (USFS et al. 1996).

The channel morphology of the lower 25 rkm of Nason Creek has been impacted by development of highways, railroads, power lines, and residential development resulting in channel confinement and reduced side-channel habitat. The present condition is a low gradient (< 1.1%), low sinuosity (1:2 to 2:0 channel-to-valley length ratio) and depositional channel (USFS et al. 1996). Peak runoff typically occurs in May and June with occasional high water produced by rain on snow events in October and November.

In 2016, mean daily discharge for Nason Creek was 11.1 m^3/s (392 cfs; Figure 2). The onset of spring freshets was unseasonable early in 2016, with peak flows occurring approximately one

month earlier than the 12-year mean. Accordingly, this resulted in a prolonged summer base-flow period, as snowpack was deminished at a much faster rate than normal. Fall freshets began in mid-October with a significant spike in flow, followed by normal levels of discharge. Water temperature data for 2016 was not available through Washington State Deportment of Ecology (WDOE).

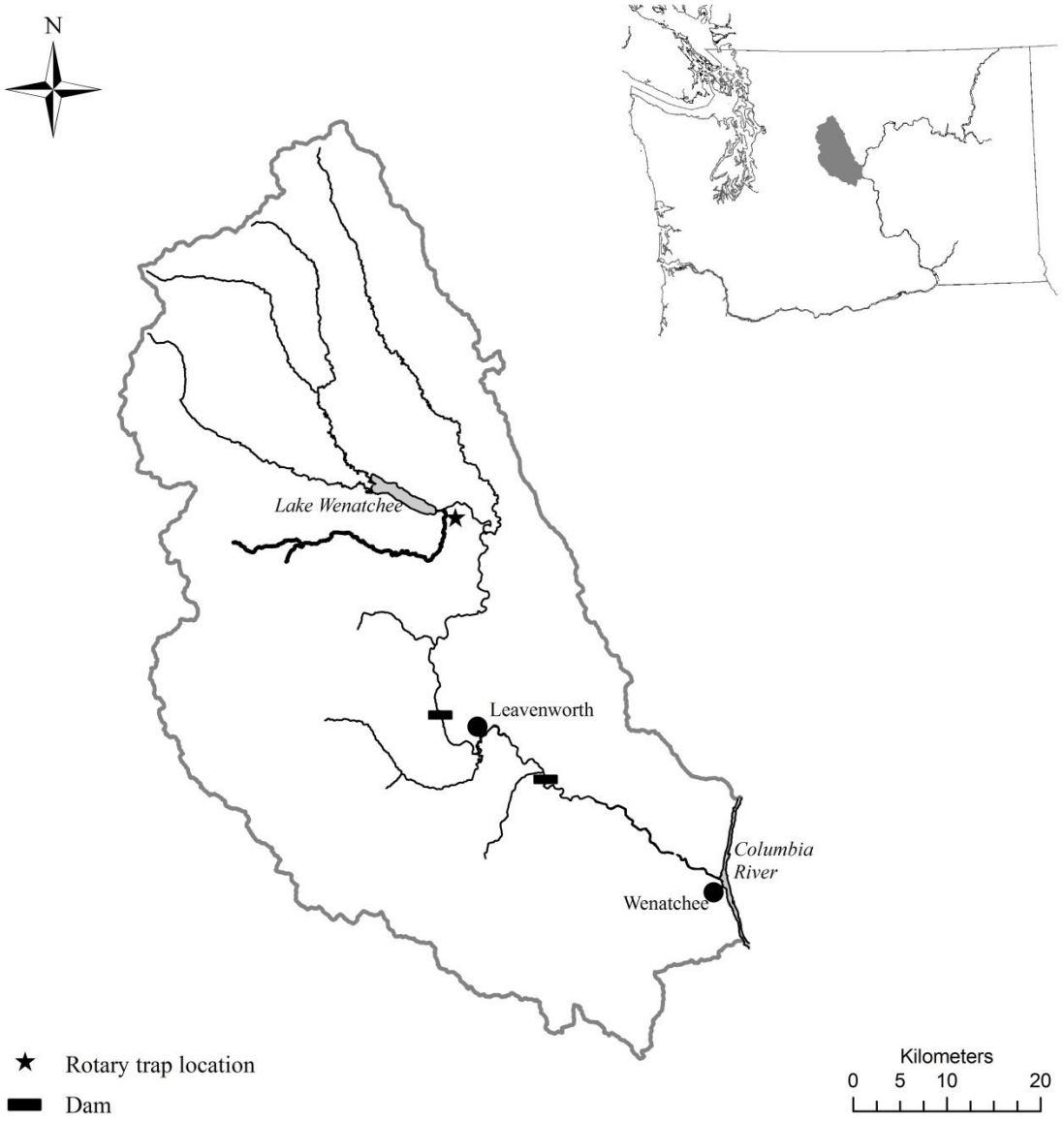


Figure 1. Map of Wenatchee River Subbasin with the Nason Creek rotary trap location.

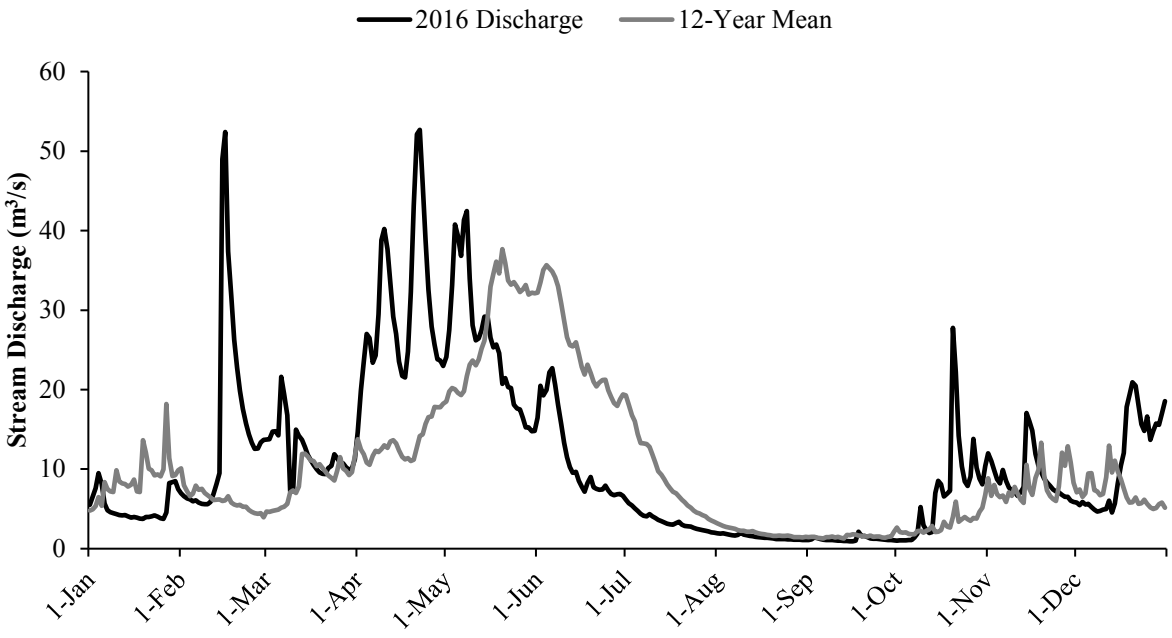


Figure 2. Mean daily stream discharge at the Nason Creek WDOE stream monitoring station in 2016.

2.0 METHODS

2.1 Trapping Equipment and Operation

The smolt trap was operated continually 24 hours per day, 7 days per week when conditions permitted. During spring snowmelt, operations occurred only during hours of darkness in order to minimize trap damage and capture mortality, while retaining the ability to sample during periods of peak fish movement. Without the threat of vandalism posed during periods of peak use at the previously-used campground location, summer operations at the Bolser location were not modified (daytime suspension).

On a daily basis, fish were removed from the primary collection box and retained in separate shore-anchored holding boxes until removed for efficiencies trials. A rotating drum-screen constantly removed small debris from the live box to avoid fish injury. All changes/modifications to the trap as well as periods of stoppage were noted.

2.2 Biological Sampling

Trap operating procedures and techniques followed a standardized basin-wide monitoring plan developed by the Upper Columbia Regional Technical Team (RTT) for the Upper Columbia

Salmon Recovery Board (UCSRB; Hillman 2004), which was adapted from Murdoch and Petersen (2000).

All fish were enumerated by species and size class. Fish to be sampled were anesthetized in a solution of MS-222, weighed with an electronic scale and measured in a wetted trough-type measuring board. Anesthetized fish received air through aquarium bubblers and were allowed to fully recover before being either released downstream of the trap or used in efficiency trials. Fork length (FL) and weight were recorded for all fish except when large numbers of fry or non-target species were collected; a sub-sample of 25 fish were measured and weighed while the remaining fish were tallied. Weight was measured to the nearest 0.1 gram and FL to the nearest millimeter. We used these data to calculate a Fulton-type condition factor (K-factor) using the formula:

$$K = (W/L^3) \times 100{,}000$$

where K = Fulton-type condition metric;
W = weight in grams;
L = fork length in millimeters;
And 100,000 is a scaling constant.

Scale samples were collected from steelhead measuring ≥ 60 mm FL so that age and brood year could be assigned. Samples were collected according to the needs and protocols set by Washington Department of Fish and Wildlife (WDFW), who conducted the analysis and provided YNFRM with results. Tissue samples were collected from spring Chinook and steelhead for DNA analysis. Samples from spring Chinook and steelhead were retained for reproductive success analyses conducted by WDFW and National Marine Fisheries Service (NMFS). All target salmonids were classified as either natural or hatchery origin by physical appearance, presence/absence of coded wire tags (CWTs), or post-orbital elastomer tags. Developmental stages were visually classified as fry, parr, transitional, or smolt. Fry were defined as newly emerged fish with or without a visible yolk sac and a FL measuring < 50 mm. Age-0 coho and spring Chinook salmon captured before July 1 were considered 'fry' and were excluded from subyearling population estimates because of the uncertainity that these fish were actively migrating (UCRTT, 2001).

2.3 PIT Tagging

All natural origin Chinook, steelhead and coho measuring ≥ 60 mm were PIT tagged. Once anesthetized, each fish was examined for external wounds or descaling, then scanned for the presence of a previously implanted PIT tag. If a tag was not detected, a pre-loaded 12mm Digital Angel 134.2 kHz type TX 1411ST PIT tag was inserted into the body cavity using a Biomark MK-25 Rapid Implant Gun. Each unique tag code was electronically recorded along with date of tag implantation, date of fish release, tagging personnel, FL, weight, and anesthetic bath temperature. Data were entered using P3 software and submitted to the PIT Tag Information System (PTAGIS). PIT tagging methods were consistent with methodologies

described in the PIT Tag Marking Procedures Manual (CBFWA 1999) as well as in 2008 ISEMP protocols (Tussing 2008).

After marking and sampling, fish were held for a minimum of 24-hours in holding boxes at the trap to; a) ensure complete recovery, b) assess tagging mortality, and c) determine a PIT tag shed rate. Mark groups were released by hand 0.8 rkm above the trap at nautical twilight. At each release, fish were distributed evenly along river-left, and river-right banks in pools and other protected areas. Fish that were not used in mark-recapture trials were released downstream from the trap.

2.4 Mark-Recapture Trials

Groups of marked juveniles were released during a range of stream discharges in order to determine the trapping efficiency. PIT tags were the only method of marking used in 2016. These releases followed the protocols described in Hillman (2004), in which the author suggests a minimum sample size of 100 fish for each mark-recapture trial. Although 100 fish/trial represented the ideal mark group, low abundance of fish often required mark-recapture trials be completed with smaller sample sizes. To achieve the largest marked group possible, we combined catch over a maximum of 72 hours. Fish being held for mark-recapture trials were kept in auxiliary live boxes attached to the end of each pontoon or floating holding boxed anchored to the stream bank. A pre-season, minimum mark group size for each species/life stage was initially determined based on past regression models. In light of high abundance, minimum trial sizes could be raised to a more robust mark group with the intention of strengthening existing regression models.

Each mark-recapture trial was conducted over a three-day (72 hour) period to allow time for passage or capture. Completed trials were only considered invalid if an interruption to trapping occurred or proper pre-release procedures were not followed. Trials resulting in zero recaptures were included in the efficiency regression (if determined valid once vetted through release/recapture protocols) as allowed by the new method of observed trap efficiency calculation. The model used (Bailey) employs use of recaptures +1 in the calculation of efficiency as a mode of bias correction. As a result, even trials yeilding no recaptures can be included in regression modeling (See equation 3 in **2.5.1 Estimate of Abundance**).

In the event that low juvenile abundaces could not provide any opportunities for efficiency trials, releases were performed to allow for a pooled estimate. These releases did not have a minimum size and were released at equal intervals across the migratory period. Pooled estimates at the Nason Creek trap were utilized as an alternative method of estimation prior to the development of a viable regression model.

2.5 Data Analysis

2.5.1 Estimate of Abundance During Smolt Trapping

Seasonal juvenile migration, N, was estimated as the sum of daily migrations, N_i, i.e., $N = \sum_i N_i$, and daily migration was calculated from catch and efficiency:

$$\hat{N}_i = \frac{C_i}{\hat{e}_i}, \quad \textbf{(1)}$$

where C_i = number of fish caught in period *I*;

$\hat{e}_i$ = trap efficiency estimated from the flow-efficiency relationship, $\sin^2(b_0 + b_1 flow_i)$,

where b_0 is estimated intercept and b_1 is the estimated slope of the regression.

The regression parameters b_0 and b_1 are estimated using linear regression for the model:

$$\arcsin\left(\sqrt{e_k^{obs}}\right) = \beta_0 + \beta_1 flow_k + \varepsilon, \quad \textbf{(2)}$$

where e_k^{obs} = observed trap efficiency of Eq. 2 for trapping period *k*;

β_0 = intercept of the regression model;

β_1 = slope parameter;

ε = error with mean 0 and variance σ^2.

In Equation 2, the observed trap efficiency, e_k^{obs}, is calculated as follows,

$$e_k^{obs} = \frac{r_k + 1}{m}. \quad \textbf{(3)}$$

The estimated variance of seasonal migration is calculated from daily estimates as:

$$Var\left(\sum_{i=1}^{n} \hat{N}_i\right) = \underbrace{\sum_i Var(N_i)}_{Part\ A} + \underbrace{\sum_i \sum_j Cov(N_i, N_j)}_{Part\ B},$$

or,

$$Var\left(\sum_{i=1}^{n} \hat{N}_i\right) = \underbrace{\sum_i Var\left(\frac{(C_i+1)}{\hat{e}_i}\right)}_{Part\ A} + \underbrace{\sum_i \sum_j Cov\left(\frac{(C_i+1)}{\hat{e}_i}, \frac{(C_j+1)}{\hat{e}_j}\right)}_{Part\ B}. \quad \textbf{(4)}$$

Part A of equation 4 is the variance of daily estimates. Part B is the between-day covariance. Note that the between-day covariance exists only for days that use the same trap efficiency

model. If, for example, day 1 is estimated with one trap efficiency model, and day 2 estimated from a different model, then there is no covariance between day 1 and day 2. The full expression for the estimated variance:

$$\hat{Var}\left(\sum_{i=1}^{n}\hat{N}_i\right)=\underbrace{\sum_i \hat{N}_i^2\left(\frac{N_i\hat{e}_i(1-\hat{e}_i)}{(C_i+1)^2}+\frac{4(1-\hat{e}_i)}{\hat{e}_i}\hat{Var}(b_0+b_1 flow_i)\right)}_{PartA}+$$
$$\underbrace{\sum_i\sum_j 4\left(\hat{N}_i(1-\hat{e}_i)\right)\left(\hat{N}_j(1-\hat{e}_j)\right)\cdot\left[\hat{Var}(b_0)+flow_i\,flow_j\hat{Var}(b_1)\right]}_{PartB}$$

where $\hat{Var}(b_0+b_1 flow_i)=\hat{MSE}\left(1+\frac{1}{n}+\frac{(flow_i-\overline{flow})^2}{(n-1)s_{flow}^2}\right)$, and $\hat{Var}(b_0)$ and $\hat{Var}(b_1)$ are

obtained from regression results. In Excel, the standard error (SE) of the coefficients is provided. The variance is calculated as the square of the standard error, SE^2.

In cases when there was no significant flow-efficiency relationship (i.e., low correlation), then a pooled, or average trap efficiency will suffice for the stratum. The estimator is calculated as follows:

$$\hat{\bar{e}}=\frac{\sum_{j=1}^{k} r_j}{\sum_{j=1}^{k} m_j}$$

where $\hat{\bar{e}}$ = the average or pooled trap efficiency for the stratum;
m_j = the number of smolts marked and released in efficiency trial j for the stratum;
r_j = the number of smolts recaptured out of m_j marked fish in efficiency trial j.

Abundance for a trapping period is estimated as:

$$\hat{N}_i^{pooled}=\frac{C_i}{\hat{\bar{e}}},$$

,and total stratum abundance is:

$$N^{pooled}=\sum_i \hat{N}_i^{pooled}.$$

The variance of seasonal abundance takes into account the variability in catch numbers that are a result of binomial sampling (Part A), the pooled variance of trap efficiency, $\hat{\bar{e}}$ (Part B), and the covariance in daily estimates that arises from using a common estimate of efficiency across all trapping days (Part C):

$$\hat{Var}\left(\sum_{i=1}^{n}\hat{N}_i^{pooled}\right)=\underbrace{\left(\sum_i\frac{\hat{N}_i\left(1-\hat{\hat{e}}\right)}{\hat{\hat{e}}}\right)}_{PartA}+\underbrace{\frac{Var\left(\hat{\hat{e}}\right)}{\hat{\hat{e}}^2}\sum_i\hat{N}_i^2}_{PartB}+\underbrace{\frac{Var\left(\hat{\hat{e}}\right)}{\hat{\hat{e}}^2}\sum_i\sum_j\hat{N}_i\hat{N}_j}_{PartC}.$$

The Part B and Part C terms are combined in the calculation as a new Part B:

$$\hat{Var}\left(\sum_{i=1}^{n}\hat{N}_i^{pooled}\right)=\underbrace{\left(\sum_i\frac{\hat{N}_i\left(1-\hat{\hat{e}}\right)}{\hat{\hat{e}}}\right)}_{PartA}+\underbrace{\frac{Var\left(\hat{\hat{e}}\right)}{\hat{\hat{e}}^2}\left[\sum_i\hat{N}_i^2+\sum_i\sum_j\hat{N}_i\hat{N}_j\right]}_{PartB}.$$

The variance of $\hat{\hat{e}}$ is calculated as:

$$\hat{Var}\left(\hat{\hat{e}}\right)=\hat{Var}\left(\frac{\sum_{k=1}^{n}r_k}{\sum_{k=1}^{n}m_k}\right)=\frac{\sum_{k=1}^{n}\left(r_k-\hat{\hat{e}}_k m_k\right)^2}{\overline{m}^2 n(n-1)}$$

where $\overline{m}$ is the average release size across all efficiency trial, $\frac{\sum_{k=1}^{n}m_k}{n}$.

Confidence intervals were calculated using the following formulas:

$$95\%\text{ confidence interval} = 1.96\times\sqrt{\sum\text{var}\left[\hat{N}_i\right]}$$

The single M-R estimator of abundance carries a set of well documented assumptions (Everhart and Youngs 1981; Seber 1982),

1. The population is closed to mortality.
2. The probability of capturing a marked or unmarked fish is equal.
3. Marked fish were randomly dispersed in the population prior to recapture.
4. Marking does not affect probabilities of capture.
5. Marks were not lost between the time of release and recapture.
6. All marks are reported upon recapture.
7. The number of fish in the trap, C, is fully enumerated and known without error.

2.5.2 Estimate of Abundace During Trap Stoppages and Suspended Operations

Daily catch during stoppages of seven days or less was estimated by averaging catch three days prior to, and after the discreet non-trapping event and then applying that value to the consecutive days without operation. This method had been used consistently in the past given the duration of the stoppage is limited, and is applied to all target species.

For periods of suspended trapping longer than seven days, a methodology developed and currently employed by local WDFW smolt trap operators was used (J. Williams, personal

communication, March 8, 2017). This method uses historic run-timing to determine the proportion of the entire emigrant estimate missed during the period of suspended trapping. Once determined, the estimated percentage can be used with in-year data to extrapolate how many fish were missed. This method is used exclusively during the fall migratory period, when low summer flows commonly result in extended stoppages. Because steelhead are considered non-migratory during this period, this type of estimate was only applied to spring Chinook subyearlings.

2.5.3 Estimate of Abundance During The Winter Non-Trapping Period

An estimate of spring Chinook emmigration during the non-trapping period (December 1 through February 28) was calculated using remote-tagged spring chinook parr and the lower Nason Creek PIT tag array (NAL). A flow-detection efficiency regression was developed using mark-groups previously released to test the efficiency of the smolt trap. Daily spring Chinook detections at the NAL array and the developed regression were then applied to the Bailey estimator, as was peformed with daily trap abundance data (See equation **2.5.1 Estimate of Abundance**). Tag rate determined at the Nason Creek smolt trap was used to account for unmarked emmigrants passing the NAL array.

Tag rate, t_i, was calculated as:

$$t_i = \frac{t}{p}$$

where t = total smolt trap recaptures subsequent to the tagging effort;
p = total catch at the smolt trap.

Daily abundace during the non-trapping period is calculated as:

$$\hat{N}_i = \left(\frac{C_i}{\hat{e}_i} \right) \Big/ t_i ,$$

where C_i = number of fish caught in period I;

$\hat{e}_i$ = trap efficiency estimated from the flow-efficiency relationship, $\sin^2(b_0 + b_1 flow_i)$;

t_i = tag rate.

2.5.4 Production and Survival

Production estimates by age class were summed to produce a total emigration estimate. For spring Chinook and coho, estimates of fall migrant parr were added to subsequent spring smolt estimates to generate a single brood year estimate. For steelhead, a single brood year may require up to three years for emigration from Nason Creek to occur. Pending scale analysis, steelhead captured in 2016 were aged via an age-length histogram built upon previously analyzed scale samples. For all three species, egg-to-emigrant estimates were calculated by

dividing estimated emigrants by approximated egg deposition during a spawning brood (average fecundity used to determine egg deposition derived from WDFW Chiwawa broodstock spawning). The number of emigrants-per-redd for each brood year was calculated by dividing the total emigrant estimate by the number of redds counted during spawning ground surveys.

3.0 RESULTS

3.1 Dates of Operation

The Nason Creek smolt trap was installed on February 25, and operated in its fixed position for the entirety of the trapping season (March 1 to November 30). Removal of the trap occurred on December 5. We attempted to run the trap continuously 24 hours a day, 7 days per week. Intentional suspension of trapping activities occurred for two periods in the summer-early fall due to base flows (July 31 – August 8 and August 10 - October 9; Table 1). Pulling of the trap also occurred on October 21 as a precautionary measure during a high-water event.

Table 1. Summary of Nason Creek rotary trap operation.

Date of Trap Operations	Trap Status	Description	Days
March 1 to June 30	Operating	Continuous data collection	120
	Interrupted	Interrupted by debris	2
	Pulled	Intentionally pulled due to high flow, low flow, or heavy debris load	0
July 1 to November 30	Operating	Continuous data collection	76
	Interrupted	Interrupted by debris	6
	Pulled	Intentionally pulled due to high flow, low flow, or heavy debris load	71

3.2 Daily Captures and Biological Sampling

3.2.1 Spring Chinook Yearlings (BY2014)

Between March 1 and June 30, a total of 61 wild Chinook yearlings were captured at the trap (Figure 3). A peak catch of 12 yearling smolts coincided with a secondary spike in discharge occurring in early April. Following this peak, catch dropped substantially with the last emigrating Chinook yearling captured on April 8. Mean FL and weight for Chinook yearlings was 96 mm (n = 61; SD = 5.5) and 9.0 g (n = 61; SD = 1.7; Table 2), respectively. Tissue samples were collected from 61 fish for an ongoing, parental-based DNA analysis by WDFW. There were no wild spring Chinook mortalities.

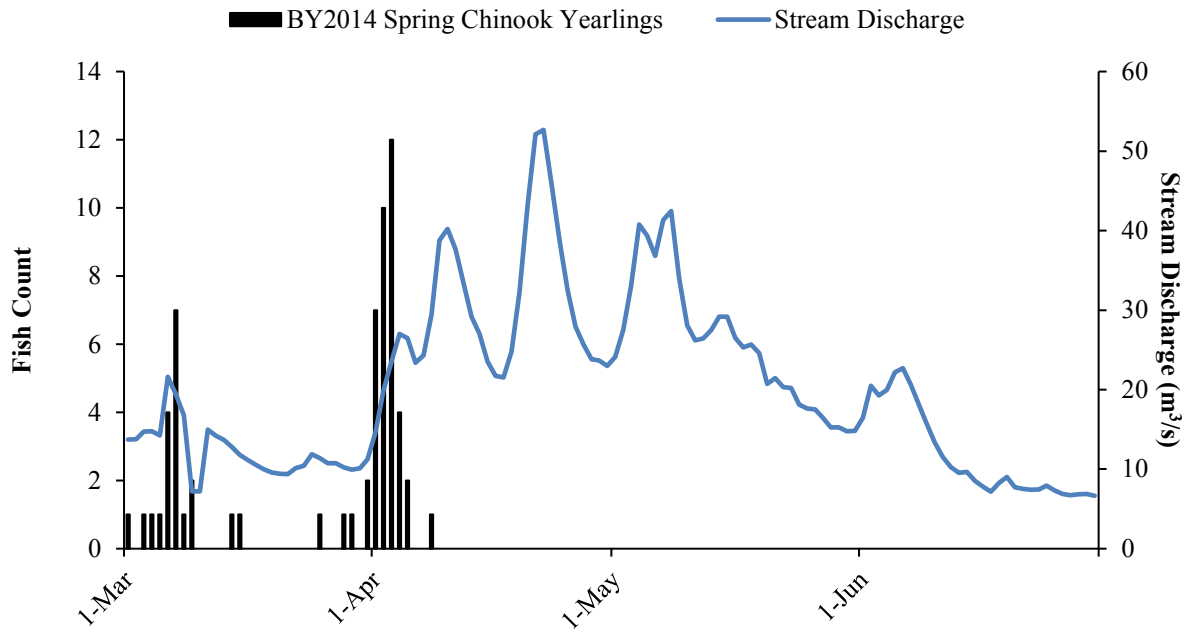


Figure 3. Daily catch of BY2014 spring Chinook yearlings with mean daily stream discharge at the Nason Creek rotary trap, March 1 to June 30, 2016.

Table 2. Summary of length and weight sampling of juvenile spring Chinook captured at the Nason Creek rotary trap in 2016.

Brood Year	Origin/Species/Stage	Fork Length (mm)			Weight (g)			K-Factor
		Mean	*n*	SD	Mean	*n*	SD	
2014	Wild Spring Chinook Yearling Smolt	96	61	5.5	9.0	61	1.7	1.01
2015	Wild Spring Chinook Subyearling Fry	38	285	3.0	0.5	285	0.2	0.78
2015	Wild Spring Chinook Subyearling Parr	85	491	12.7	6.9	490	2.5	1.07
2014	Hatchery Spring Chinook Yearling Smolt	119	87	13.5	19.6	87	7.6	1.09

3.2.2 Spring Chinook Subyearlings (BY2015)

A total of 491 wild spring Chinook subyearling parr (FL ≥ 50 mm) and 300 subyearling fry (FL < 50 mm) were captured in 2016 (Figure 4). The majority of parr movement was documented in late October following the first fall freshets. Mean FL and weight among subyearling parr was 85 mm (n = 491; SD = 12.7) and 6.9 g (n = 490; SD = 2.5), respectively. We estimate that an additional 20 Chinook subyearling parr would have been captured during short stoppages (≤7 days) had the trap run without interruption. Daily catch estimates were not made during the two periods of suspended trapping; total emmigrant estimates for these two periods will be included in section 3.4.2. Tissue samples were collected from 431 fish for an ongoing, parental-based DNA analysis by WDFW. Six subyearling Chinook (four fry and two parr) mortalities occurred in 2016. All deaths were attributed to trapping.

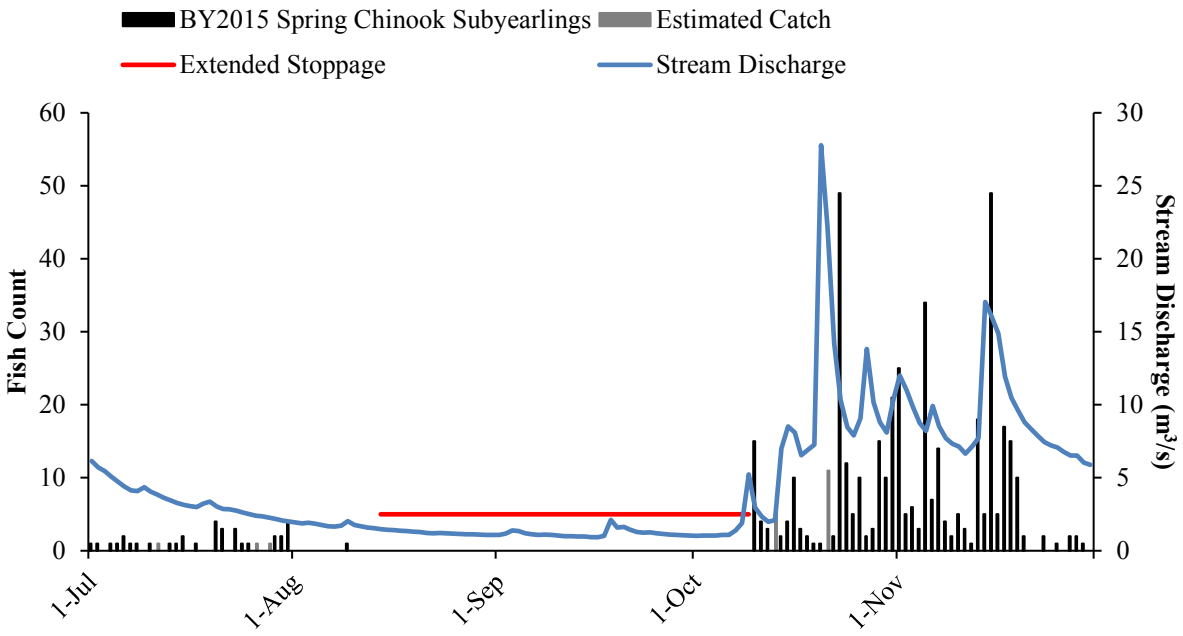


Figure 4. Daily catch of BY2015 spring Chinook subyearlings with mean daily stream discharge at the Nason Creek rotary trap, July 1 to November 30, 2016.

3.2.3 Hatchery Spring Chinook Smolts (BY2014)

In the spring of 2016, 31,651 hatchery spring Chinook smolts were released into Nason Creek. All hatchery spring Chinook were released directly from the Grant County Public Utility District (GCPUD) Nason Creek Acclimation Facility located at rkm17.3. Subsequently, a total of 124 smolts were captured with a mean FL and weight of 119 mm (n =87; SD = 13.5) and 19.6 g (n = 87; SD = 7.6), respectively (Figure 5). Hatchery spring Chinook were not captured at the smolt trap beyond June 3. There were no mortalities incurred.

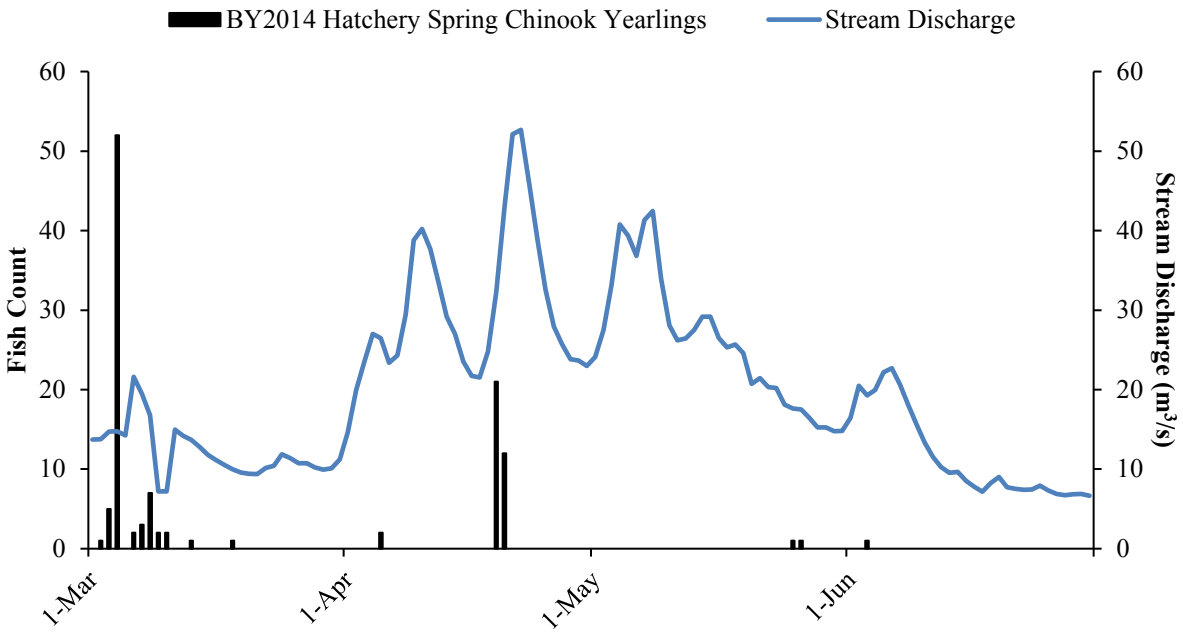


Figure 5. Daily catch of BY2014 hatchery spring Chinook smolts with mean daily stream discharge at the Nason Creek rotary trap, March 1 to June 30, 2016.

3.2.4 Summer Steelhead

A total of 1,007 wild summer steelhead juveniles were captured throughout the season from March 1 to November 30, with a peak catch of 79 juveniles on August 9 (Figs. 6&7). We estimated that an additional 6 age-1 juveniles would have been captured had there been no interruptions to trapping during the migratory period (Mar 1 to July 31). Histogram analysis of known steelhead ages sampled from 2005 to 2014 allowed us to estimate ages of fish captured in 2016 using FL. We estimate that of the total steelhead captured, 702 were young-of-the-year, 285 were age-1, 19 were age-2, and 1 was age-3. Subyearling steelhead had a mean FL of 56mm (n = 674; SD = 16.4), and a mean weight of 2.4 (n = 617; SD = 1.8). The majority of steelhead juveniles captured were age-1 parr emigrating past the trap in spring. Mean FL and weight of age-1 fish was 87 mm (n = 278; SD = 21.5; Table 3) and 8.3 g (n = 278; SD = 5.9), respectively. Age-2 steelhead were caught primarily in the spring, with only two fish being captured after July 31. Mean FL and weight of age-2 fish was 143 mm (n = 19; SD = 17.4) and 31.1 g (n = 19; SD = 9.6), respectively. A single age-3 fish with a FL of 202 mm and weight of 90.1 g was also captured. Scales were taken from a sub-sample (n = 141) to be used for future age analyses. One mortality was incurred (See **3.6 ESA Compliance**).

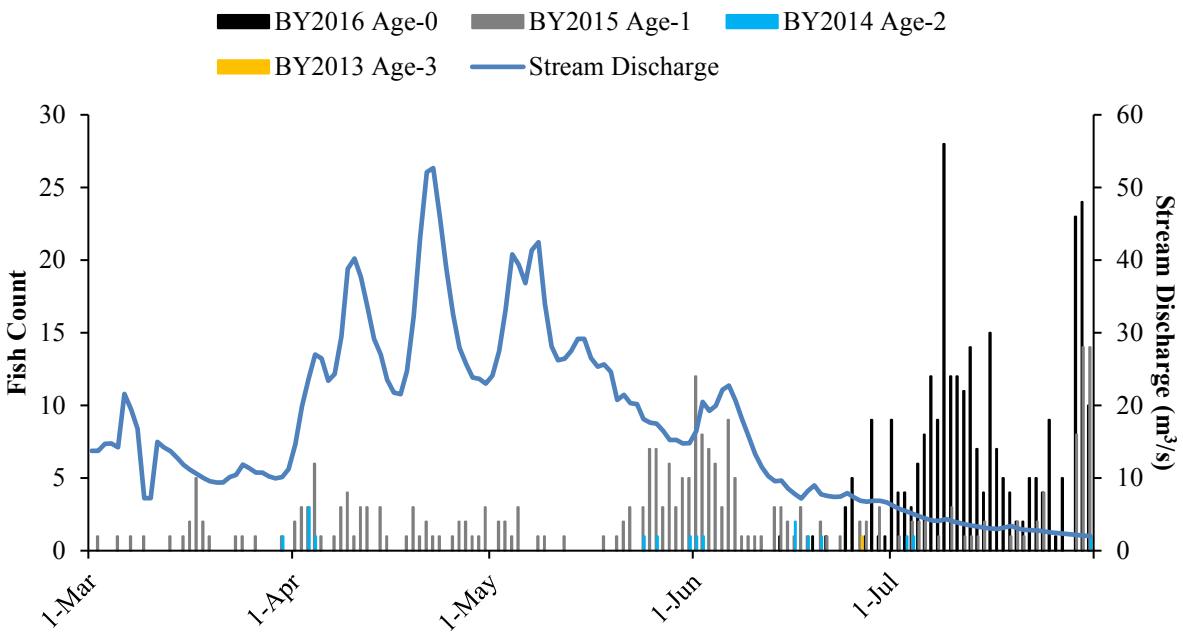


Figure 6. Daily catch of wild summer steelhead with mean daily stream discharge at the Nason Creek rotary trap, March 1 to July 31, 2016. Estimates of fish passage during trap interruptions are not depicted.

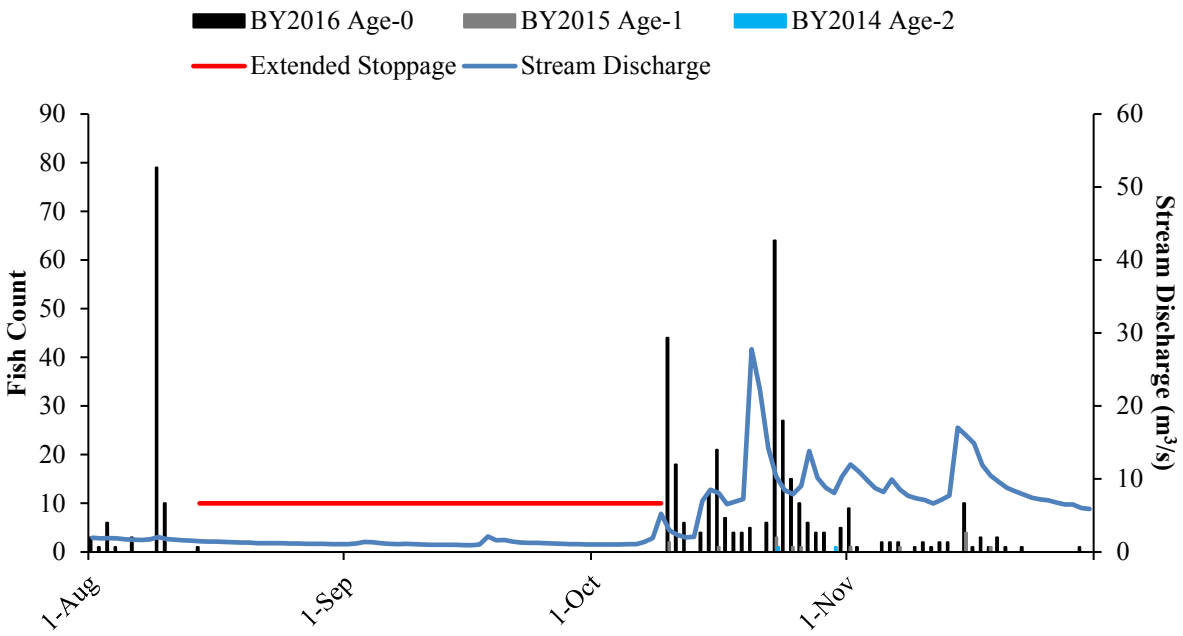


Figure 7. Daily catch of wild summer steelhead with mean daily stream discharge at the Nason Creek rotary trap, August 1 to November 30, 2016. Estimates of fish passage during trap interruptions are not depicted.

Table 3. Summary of length, weight and condition factor by age class of wild summer steelhead emigrants and hatchery steelhead captured at the Nason Creek rotary trap.

Brood Year	Origin/Species/Stage	Fork Length (mm)			Weight (g)			K-Factor
		Mean	*n*	SD	Mean	*n*	SD	
2016	Wild Summer Steelhead (Age-0)	56	674	16.4	2.4	617	1.8	1.02
2015	Wild Summer Steelhead (Age-1)	87	278	21.5	8.3	278	5.9	1.05
2014	Wild Summer Steelhead (Age-2)	143	19	17.4	31.1	19	9.6	1.04
2013	Wild Summer Steelhead (Age-3)	202	1	—	90.1	1	—	1.09
2015	Hatch. Summer Steelhead Smolt	175	95	15.5	55.1	95	16.2	0.99

3.2.5 Hatchery Steelhead Smolts (BY2015)

During April and May, WDFW directly planted a total of 55,105 hatchery summer steelhead smolts into Nason Creek above the smolt trap (M. Babiar, personal communication, February 8, 2017). Subsequently, a total of 98 hatchery steelhead were captured at the smolt trap with a mean FL and weight of 175 mm (*n* =95; *SD* = 15.5) and 55.1 g (*n* = 95; *SD* = 16.2), respectively (Figure 8). The last hatchery smolt was caught on June 14. Hatchery origin was determined by the presence of coded wire tags (CWT). There were no hatchery-origin steelhead smolt mortalities.

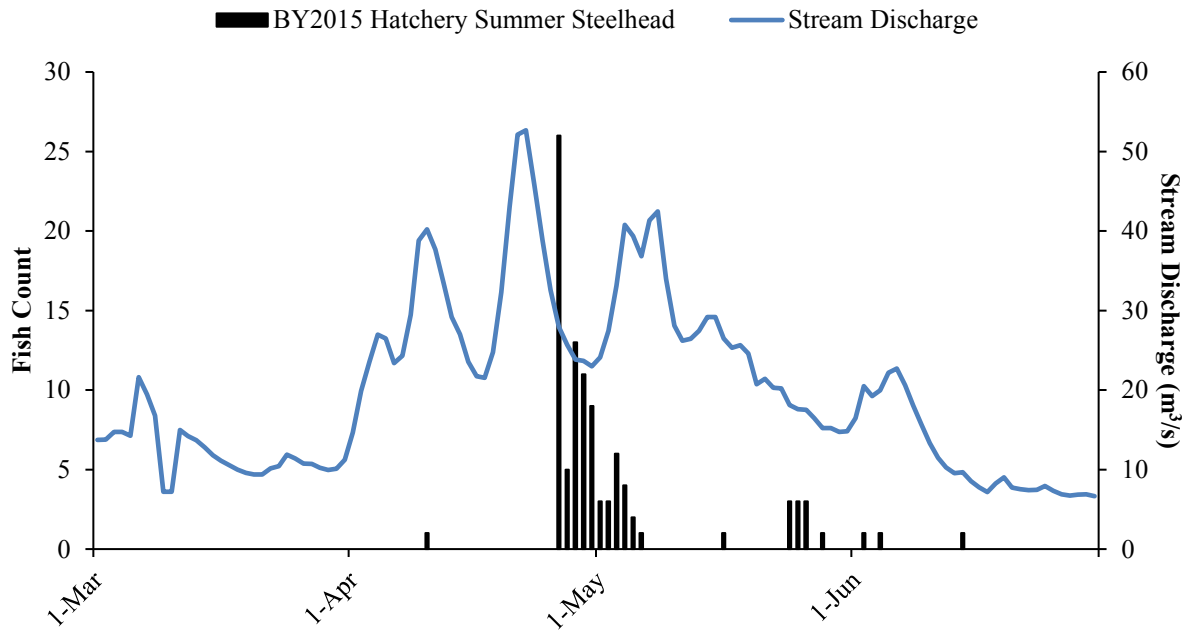


Figure 8. Daily catch of BY2015 hatchery steelhead smolt with mean daily stream discharge at the Nason Creek rotary trap, March 1 to June 30, 2016.

3.2.6 Bull Trout

Bull trout presence at the trap in 2016 was limited to a single fish with a FL of 199 mm and weight of 70.0 g. The bull trout was released immediately after morphometric measurements were taken. No other sampling/tagging activities were performed.

3.2.7 Coho Yearlings (BY2014)

Six naturally-produced coho yearlings were captured during spring emigration between March 1 and June 30 (Figure 9). Their mean FL and weight was 100 mm (n = 6; SD = 15.8) and 11.1 g (n = 6; SD = 5.5), respectively (Table 4). Scale and tissue samples were not taken from naturally-produced coho smolts in 2016. There were no coho yearling mortalities.

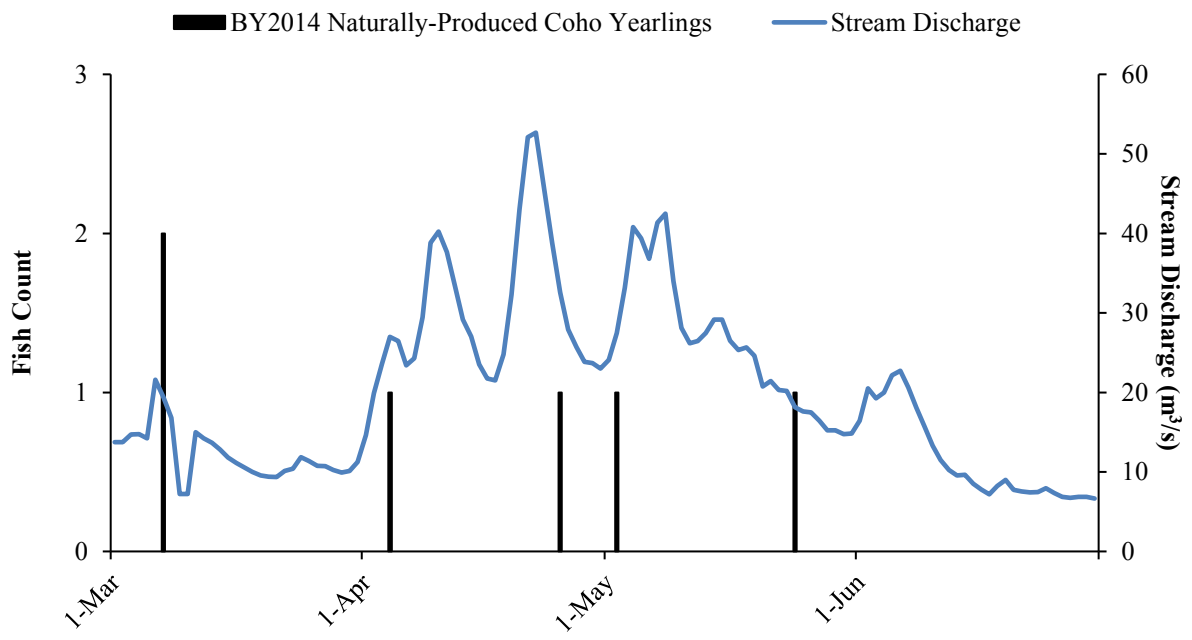


Figure 9. Daily catch of BY2014 naturally-produced coho yearlings with mean daily stream discharge at the Nason Creek rotary trap, March 1 to June 30, 2016.

Table 4. Summary of length and weight sampling of juvenile coho salmon captured at the Nason Creek rotary trap in 2016.

Brood Year	Origin/Species/Stage	Fork Length (mm)			Weight (g)			K-Factor
		Mean	*n*	SD	Mean	*n*	SD	
2013	Naturally Produced Coho Yearling Smolt	100	6	15.8	11.1	6	5.5	1.03
2013	Hatchery Coho Yearling Smolt	134	302	8.4	24.8	301	5.0	1.02

3.2.8 Coho Subyearlings (BY2015)

There were no BY2015 naturally-produced coho fry or parr captured at the Nason Creek smolt trap in 2016.

3.2.9 Hatchery Coho Smolts (BY2014)

A total of 276,063 hatchery coho were released into Nason Creek above the trap in spring of 2016. All hatchery coho released were acclimated in natural ponds adjacent to Nason Creek and

reared to smolt stage prior to volitional release. Between March 1 and June 30, a total of 343 hatchery coho were captured at the trap (Figure 10). Mean FL was 134 mm (n = 302; SD = 8.4) and mean weight was 24.8 g (n = 301; SD = 5.0; Table 2). A peak daily catch of 45 hatchery coho smolts occurred on April 29 following volitional release into Nason Creek. Two trapping mortalities were incurred. Hatchery coho emigration data at the Nason Creek trap assists the MCCRP by providing size-at-emigration, emigration timing and duration of residence in Nason Creek.

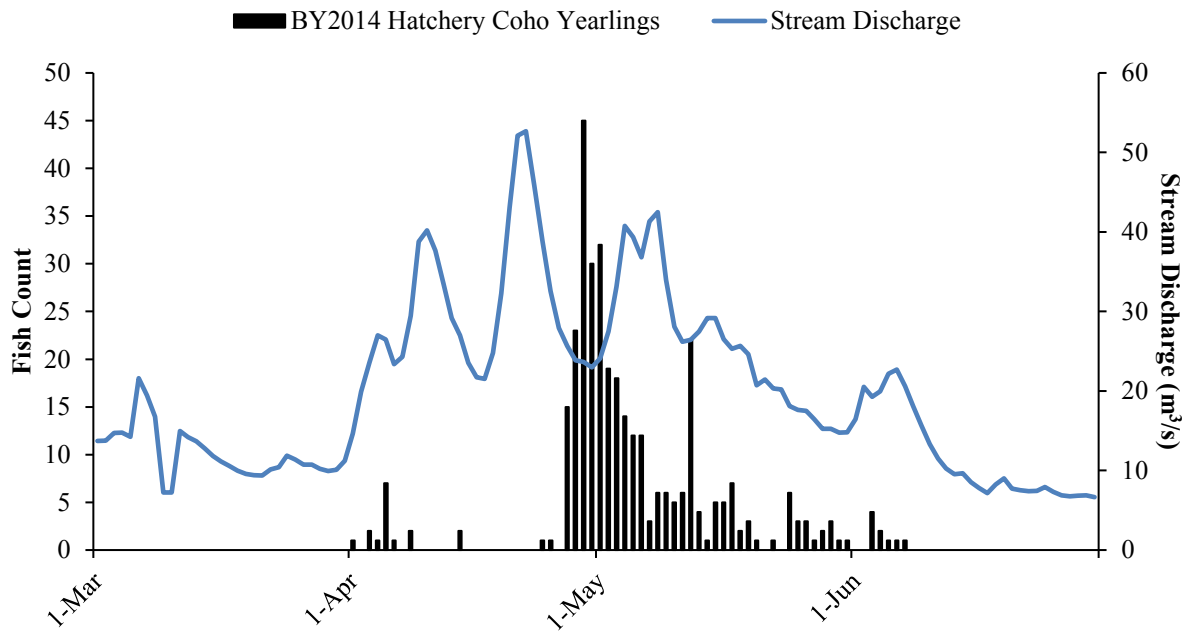


Figure 10. Daily catch of BY2014 hatchery coho smolt with mean daily stream discharge at the Nason Creek rotary trap, March 1 to June 30, 2016.

3.3 Remote Parr Tagging (BY2014 Spring Chinook)

YNFRM and WDFW personnel PIT tagged and released a total of 1,214 BY2014 spring Chinook parr between September 23 and October 15, 2015. The total surveyed area included Nason Creek from rkm 0.8 to 26.1. All collections were performed via backpack electrofisher. Equal capture effort (measured in electrofisher seconds used) was applied across all reaches.

Between October 1 and March 30, a total of 100 re-sights of the remote tagged spring Chinook were documented at the NAL array (Figure 11). Of these detections, only two were during the winter non-trapping period. High flows in November caused significant damages to the NAL array, resulting in antennas 1, 5, and 6 being inoperable throughout the non-trapping period (J. Deason Personal Communication, February 10, 2016).

Subsequent to the remote tagging effort, five remote-tagged BY2014 spring Chinook were recaptured at the Nason Creek smolt trap. Total spring Chinook catch at the smolt trap was 255 emigrants during the same period. The pooled tag rate for remote-tagged spring Chinook captured at the Nason smolt trap was 2.0%.

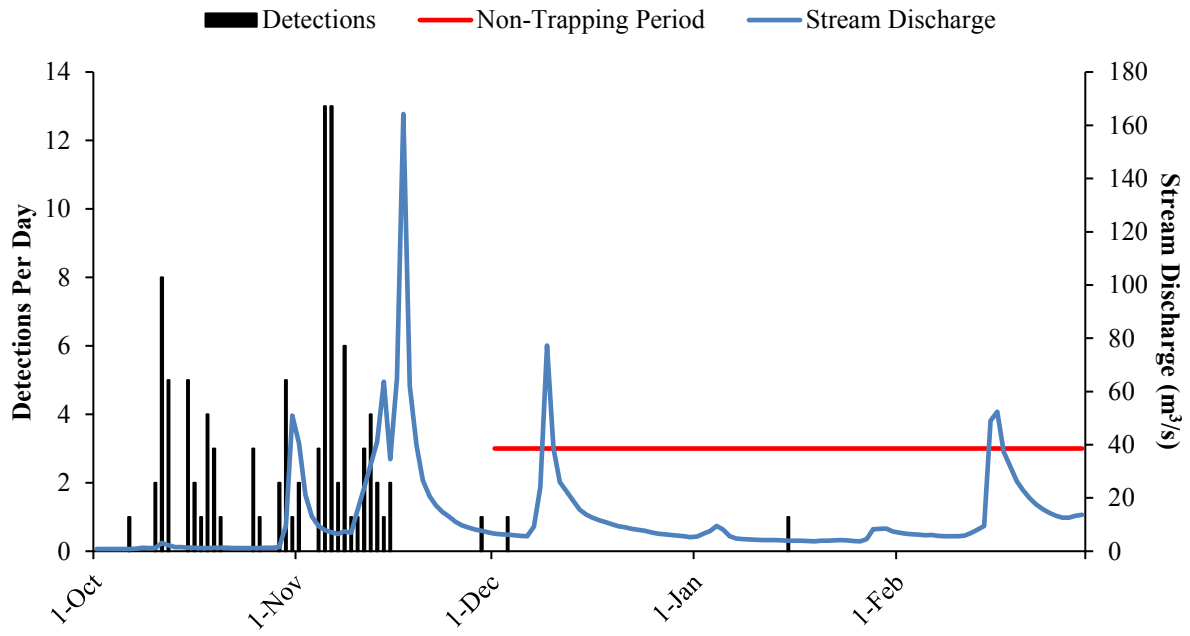


Figure 11. Daily detections of remote-tagged BY2014 spring Chinook at the lower Nason Creek PIT tag antenna array (NAL) between October 2015 and March 2016.

3.4 Trap Efficiency Calibration and Population Estimates

3.4.1 Spring Chinook Yearlings (BY2014)

Infrequent releases, low abundance, and a lack of recaptures did not allow a flow-efficiency model to be used on BY2014 yearling emigrants. In order to produce an estimate, a pooled efficiency (6.6%) composed of spring Chinook yearling releases in 2016 was used (Table 5). We recognize the sub-optimal nature of this estimation methodology, and will recalculate the estimates using linear regression analysis as soon as feasible. We estimated a total of 930 (± 5,083; 95% CI) BY2014 spring Chinook yearlings emigrated in spring of 2016 (Table 6). Parr emmigration during the non-trapping period was estimated using a flow-efficiency regression (r^2 = 0.38; p = 0.007) based on detections at the NAL pit tag array. This antenna efficiency is solely based on detections made on the three antennas that were functional during winter of 2016. We estimated that 1,442 (± 1,297; 95% CI) BY2013 spring Chinook emigrated out of Nason Creek during the non-trapping period. Combined with a recalculated BY2014 subyearling estimate of 8,694 (± 5,207; 95% CI), we estimated that a total of 7,280 (± 5,197; 95% CI) BY2014 spring Chinook juveniles emigrated from Nason Creek.

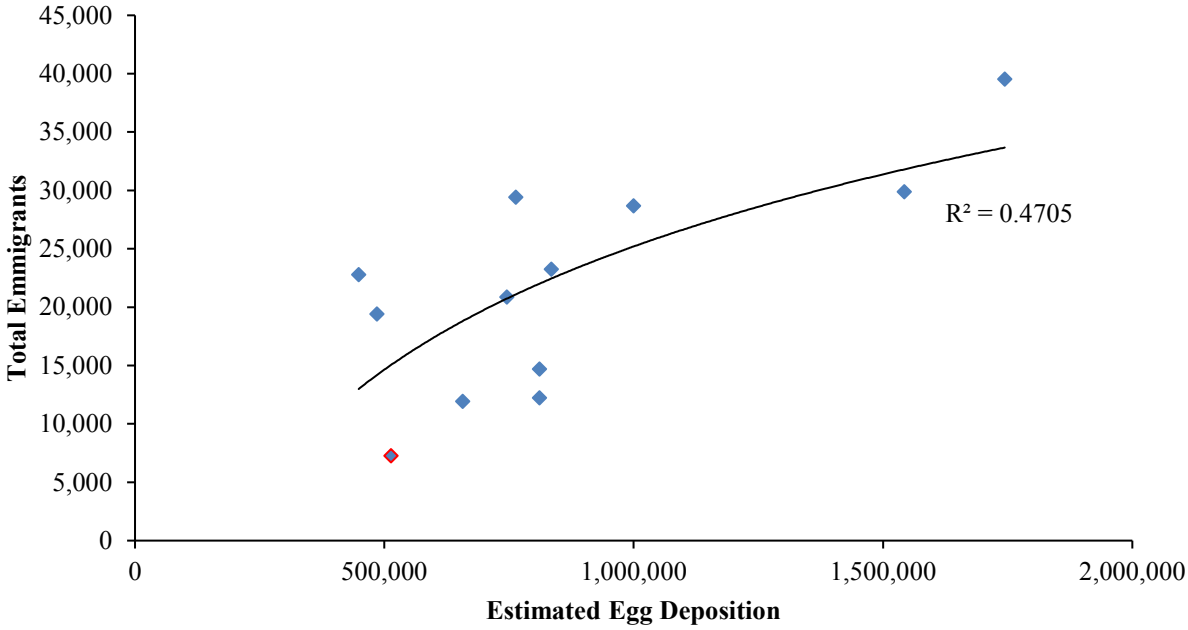
Table 5. Trap efficiency trials conducted with BY2014 wild spring Chinook yearlings and hatchery-origin coho yearling surrogates.

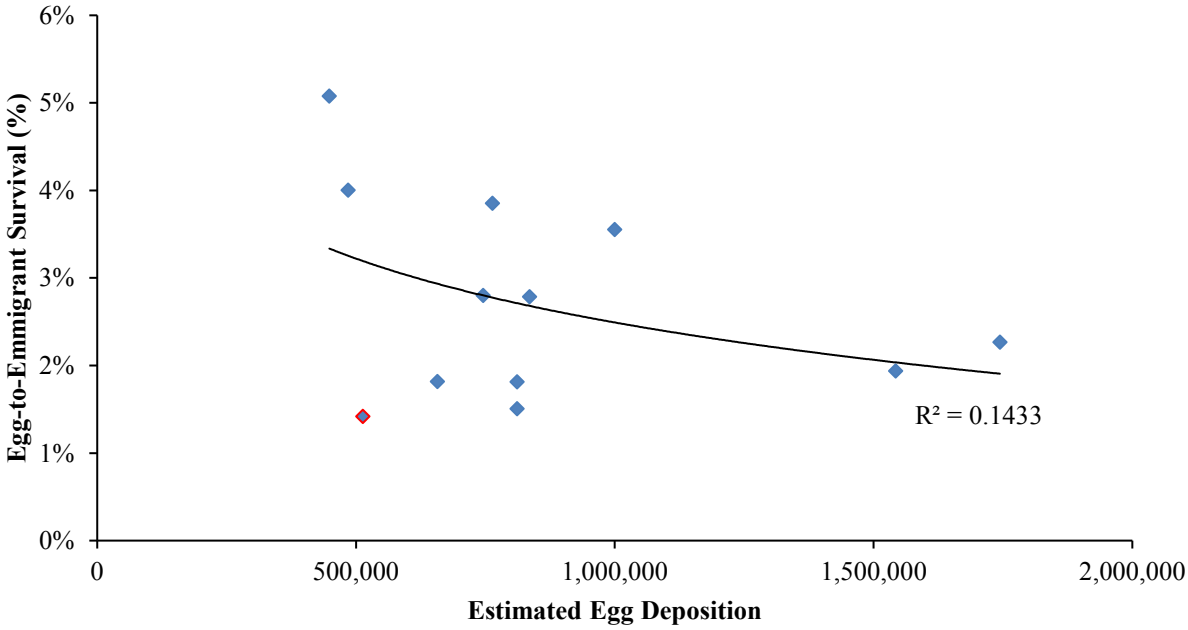
Origin/Species/Stage	Age	Date	Marked	Recaptured	Discharge (m^3/s)
Wild Chinook Yearlings	1+	3/4/2016	3	0	14.0
Wild Chinook Yearlings	1+	3/8/2016	12	4	15.9
Wild Chinook Yearlings	1+	3/12/2016	3	0	13.5
Wild Chinook Yearlings	1+	3/16/2016	2	0	10.5
Wild Chinook Yearlings	1+	3/28/2016	2	0	9.7
Wild Chinook Yearlings	1+	4/1/2016	10	0	13.9
Wild Chinook Yearlings	1+	4/5/2016	28	0	25.3
Wild Chinook Yearlings	1+	4/9/2016	1	0	37.7
Total			**61**	**4**	

Table 6. Estimated egg-to-emigrant survival and smolts-per-redd production for Nason Creek spring Chinook salmon.

Brood Year	No. Redds	Fecundity[a]	Est. Egg Deposition	No. of Emigrants				Egg-to-Emigrant	Emigrants per Redd
				Age-0[b]	Non Trap[d]	Age-1	Total ± 95% CI		
2002	294	4,654	1,368,276	—	—	4,683	—	—	—
2003	83	5,844	485,052	13,067	—	6,358	19,425 ± 1,993	4.0%	234
2004	169	4,799	811,031	12,111	—	2,597	14,708 ± 2,938	1.8%	87
2005	193	4,327	835,111	14,565	—	8,696	23,261 ± 5,440	2.8%	121
2006	152	4,324	657,248	4,144	—	7,798	11,942 ± 1,744	1.8%	79
2007	101	4,441	448,541	17,097	—	5,679	22,776 ± 2,983	5.1%	226
2008	336	4,592	1,542,912	26,284	—	3,611	29,895 ± 7,244	1.9%	89
2009	167	4,573	763,691	27,720	—	1,705	29,425 ± 12,777	3.9%	176
2010	188	4,314	811,032	8,685	—	3,535	12,220 ± 1,972	1.5%	65
2011	170	4,385	745,450	18,457	—	2,422	20,879 ± 3,887	2.8%	123
2012	413	4,223	1,744,099	34,961	—	4,561	39,522 ± 6,395	2.3%	96
2013	212	4,716	999,792	21,697	6,822	6,992[e]	35,511 ± 34,195	3.6%	168
2014	115	4,467	513,705	6,321	1,442	930[e]	8,694 ± 5,207	1.7%	76
2015	85	5,132	436,220	6,813	—	—	—	—	—
Avg.[c]	192	4,584	863,139	17,092	—	4,574	21,799	2.76%	128

[a] Data provided by Hillman et al. 2015.
[b] Does not include subyearling fry prior to July 1.
[c] 12-year average of complete brood data, BY2003-2014.
[d] Estimated emigration during the winter non-trapping period (December 1 – February 28).
[e] Pooled estimate


45,000
40,000
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
Total Emmigrants
0
500,000
1,000,000
1,500,000
2,000,000
Estimated Egg Deposition
R² = 0.4705


6%
5%
4%
3%
2%
1%
0%
Egg-to-Emmigrant Survival (%)
0
500,000
1,000,000
1,500,000
2,000,000
Estimated Egg Deposition
R² = 0.1433

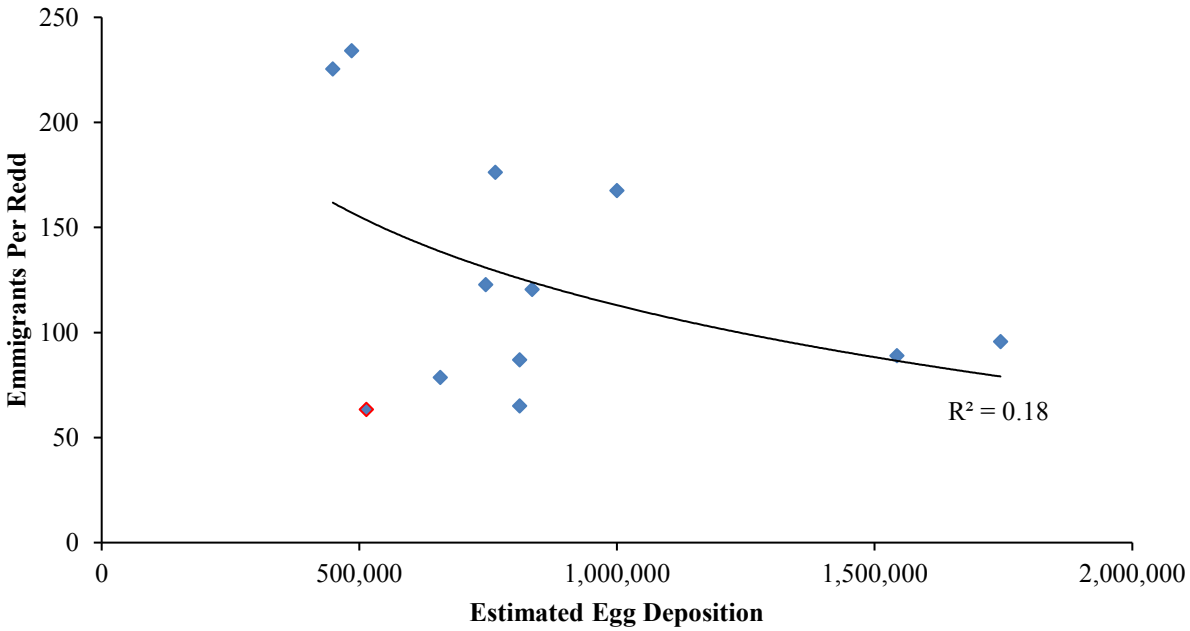


Figure 12. Relationships between estimated egg deposition and total emigrants produced, egg-to-emigrant survival, and emigrants per redd for Nason Creek spring Chinook, BY 2003 to 2014. *2014 brood (denoted by red border) does not include non-trapping estimate.

3.4.2 Spring Chinook Subyearlings (BY2015)

A linear regression model was developed using subyearling mark groups released in the fall of 2014 and 2016. The resulting regression ($r^2 = 0.60$; $p = 0.005$) was based on individual mark groups of ≥ 50 Chinook subyearlings only. Using this model we estimated that a total of 3,813 (± 1,116; 95% CI) BY2015 spring Chinook emigrated past the trap in the fall of 2016 (Table 6).

Table 7. Trap efficiency trials conducted with BY2015 wild spring Chinook subyearlings.

Origin/Species/Stage	Age	Date	Marked	Recaptured	Discharge (m^3/s)
Wild Chinook Subyearlings	0	7/2/2016	2	0	5.2
Wild Chinook Subyearlings	0	7/6/2016	4	0	3.9
Wild Chinook Subyearlings	0	7/14/2016	1	0	2.9
Wild Chinook Subyearlings	0	7/18/2016	2	0	2.9
Wild Chinook Subyearlings	0	7/22/2016	3	0	2.5
Wild Chinook Subyearlings	0	8/3/2016	1	0	1.7
Wild Chinook Subyearlings	0	10/24/2016	59	6	8.0
Wild Chinook Subyearlings	0	11/1/2016	68	8	10.6
Wild Chinook Subyearlings	0	11/6/2016	49	6	9.6
Wild Chinook Subyearlings	0	11/15/2016	69	11	15.3
Wild Chinook Subyearlings	0	11/20/2016	32	3	8.2
Total			**290**	**34**	

3.4.3 Summer Steelhead

Low abundance of summer steelhead emigrants in the spring of 2016 required a pooled estimate be used in light of the inability to meet minimum mark-group sizes (n = 50) for regression analysis. Releases of PIT-tagged steelhead were subsequently released every four days upstream at the established release location (Table 8). In a total of 31 separate trials, 216 wild summer steelhead were released upstream with 3 recaptures (1.4%). Estimates of age-0 fry and parr were not made due to insufficient evidence that active migration is occurring at this young age. Previous attempts at the old location to build a model based on young-of-the-year steelhead parr in the fall have yielded weak flow-efficiency relationships; further suggesting that age-0 parr catch is the result of displacement rather than active migration. We estimated that 19,872 (± 69,909; 95% CI) BY2015 age-1, 1,124 (± 4,437; 95% CI) BY2014 age-2, and 72 (± 294; 95% CI) BY2013 age-3 steelhead emigrated past the trap in 2016 (Table 9). We estimate that total (age 1-3) BY2013 emigration to be 13,417(± 9,133; 95% CI). All pooled estimates will be recalculated upon development of a species-specific flow-efficiency model.

Table 8. Efficiency trials conducted with wild summer steelhead juveniles.

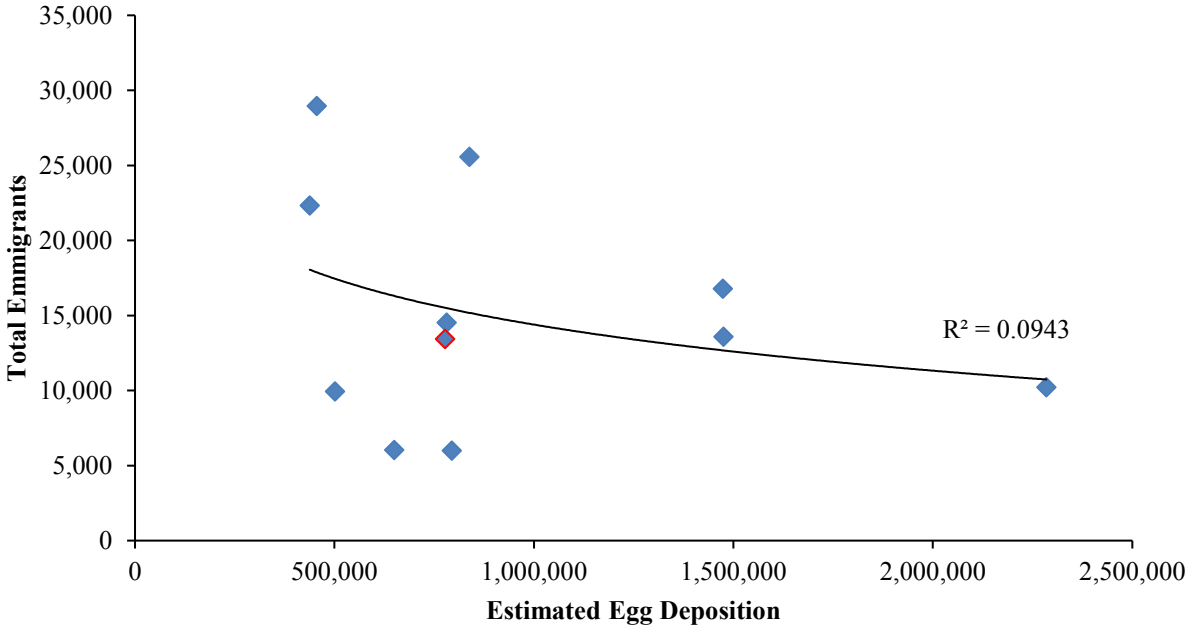
Origin/Species/Stage	Date	Marked	Recaptured	Discharge (m^3/s)
Wild Steelhead Parr/Smolt	3/4/2016	1	0	14.8
Wild Steelhead Parr/Smolt	3/8/2016	2	0	15.9
Wild Steelhead Parr/Smolt	3/12/2016	1	0	13.5
Wild Steelhead Parr/Smolt	3/16/2016	4	0	10.5
Wild Steelhead Parr/Smolt	3/20/2016	8	0	8.9
Wild Steelhead Parr/Smolt	3/24/2016	2	0	11.2
Wild Steelhead Parr/Smolt	4/1/2016	4	0	13.9
Wild Steelhead Parr/Smolt	4/5/2016	16	0	25.3
Wild Steelhead Parr/Smolt	4/9/2016	4	0	37.7
Wild Steelhead Parr/Smolt	4/13/2016	7	0	28.2
Wild Steelhead Parr/Smolt	4/17/2016	3	0	20.7
Wild Steelhead Parr/Smolt	4/21/2016	7	0	52.4
Wild Steelhead Parr/Smolt	4/25/2016	3	0	32.0
Wild Steelhead Parr/Smolt	4/29/2016	6	0	23.0
Wild Steelhead Parr/Smolt	5/3/2016	7	0	32.6
Wild Steelhead Parr/Smolt	5/7/2016	3	0	41.3
Wild Steelhead Parr/Smolt	5/11/2016	2	0	25.6
Wild Steelhead Parr/Smolt	5/23/2016	6	0	19.6
Wild Steelhead Parr/Smolt	5/27/2016	20	2	16.3
Wild Steelhead Parr/Smolt	5/31/2016	16	0	13.9
Wild Steelhead Parr/Smolt	6/4/2016	35	0	17.4
Wild Steelhead Parr/Smolt	6/8/2016	17	0	17.0
Wild Steelhead Parr/Smolt	6/12/2016	3	0	9.5
Wild Steelhead Parr/Smolt	6/16/2016	10	1	7.2
Wild Steelhead Parr/Smolt	6/20/2016	7	0	7.0

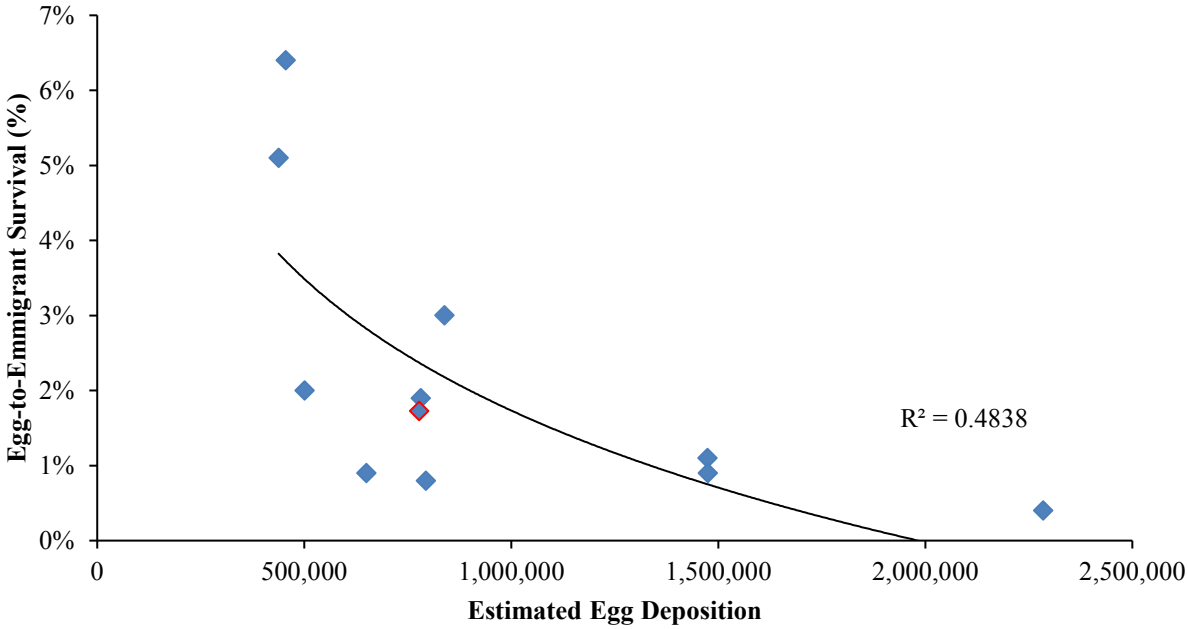
Origin/Species/Stage	Date	Marked	Recaptured	Discharge (m^3/s)
Wild Steelhead Parr/Smolt	6/24/2016	2	0	7.2
Wild Steelhead Parr/Smolt	6/28/2016	5	0	6.2
Wild Steelhead Parr/Smolt	7/2/2016	4	0	5.2
Wild Steelhead Parr/Smolt	7/6/2016	8	0	3.9
Wild Steelhead Parr/Smolt	7/10/2016	2	0	3.6
Wild Steelhead Parr/Smolt	7/14/2016	1	0	2.9
Total		**216**	**3**	

Table 9. Estimated egg-to-emigrant survival and emigrants-per-redd production for Nason Creek summer steelhead.

Brood Year	No. of Redds	Fecundity[a]	Est. Egg Deposition	No. of Emigrants				Egg-to-Emigrant	Emigrants per Redd
				1+	2+	3+	Total ± 95%CI		
2001	27	5,951	160,677	DNOT	DNOT	846	—	—	—
2002	80	5,776	462,080	DNOT	2,475	0	—	—	—
2003	121	6,561	793,881	4,906	1,054	27	5,987 ± 1,193	0.80%	49
2004	127	5,118	649,986	5,107	906	22	6,035 ± 885	0.90%	48
2005	412	5,545	2,284,540	7,416	2,502	298	10,216 ± 2,147	0.40%	25
2006	77	5,688	437,976	19,609	2,673	37	22,319 ± 5,722	5.10%	290
2007	78	5,840	455,520	26,518	2,325	117	28,960 ± 7,739	6.40%	371
2008	88	5,693	500,984	8,782	1,164	0	9,946 ± 2,382	2.00%	113
2009	126	6,199	781,074	13,606	608	312	14,526 ± 2,868	1.90%	115
2010	270	5,458	1,473,660	12,767	3,999	0	16,776 ± 3,885	1.10%	62
2011	235	6,276	1,474,860	13,109	482	0	13,591 ± 3,525	0.90%	58
2012	158	5,309	838,822	24,637	813	116[c]	25,566 ± 6,020	3.00%	162
2013	135	5,749	777,735	11,837	1,508[c]	72[c]	13,417 ± 9,133	1.73%	99
2014	198	5,831	1,154,538	22,504[c]	1,224[c]	—	—	—	—
2015	171	6,220	1,063,620	19,872[c]	—	—	—	—	—
Avg[b]	166	5,767	951,731	13,481	1,639	91	15,213	2.20%	127

[a] Data provided by Hillman et al. 2015
[b] 11-year average of complete brood estimates, BY2003-2013
[c] Pooled estimate


35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
Total Emmigrants
0
500,000
1,000,000
1,500,000
2,000,000
2,500,000
Estimated Egg Deposition
R² = 0.0943


7%
6%
5%
4%
3%
2%
1%
0%
Egg-to-Emmigrant Survival (%)
0
500,000
1,000,000
1,500,000
2,000,000
2,500,000
Estimated Egg Deposition
R² = 0.4838

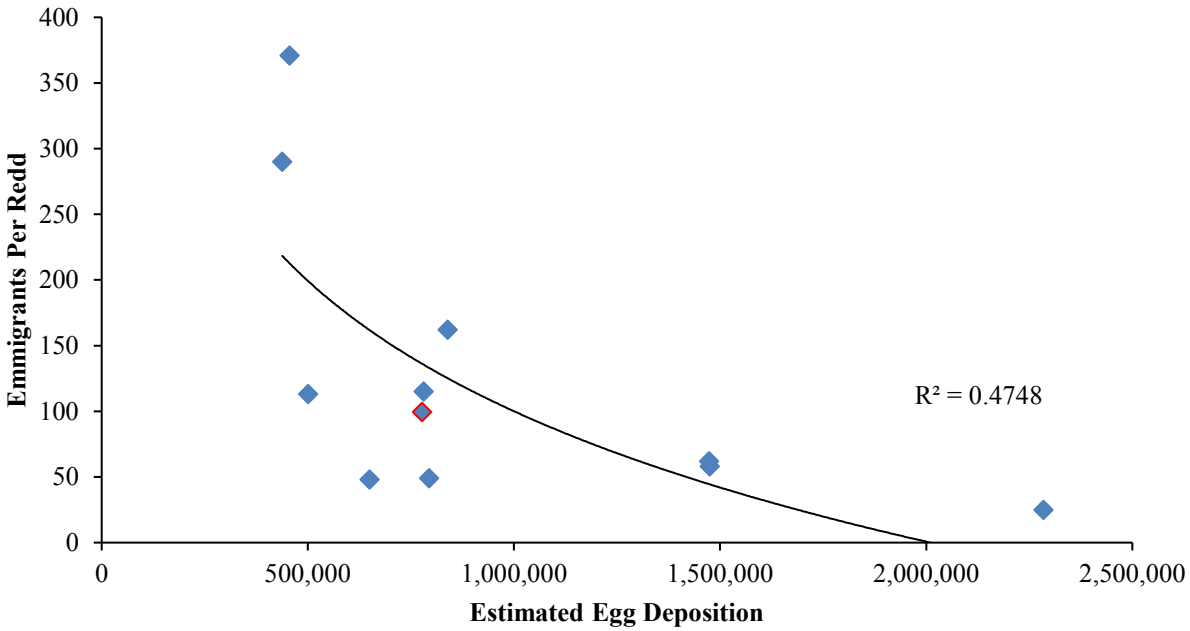


Figure 13. Relationships between estimated egg deposition and total emigrants produced, egg-to-emigrant survival, and emigrants per redd for Nason Creek summer Steelhead, BY 2003 to 2013. *2013 brood denoted by red border.

3.4.4 Coho Yearlings (BY2014)

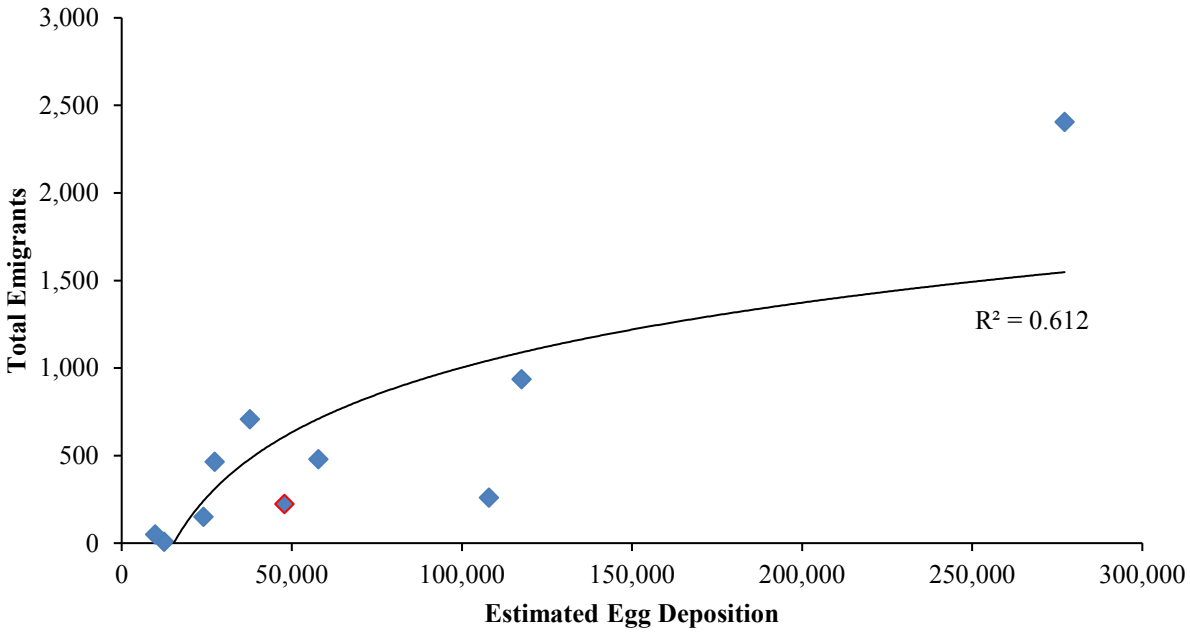
Limited abundance of BY2014 coho yearlings did not provide any opportunities to perform any efficiency trials in the spring of 2016. In lieu of a species-specific model, a pooled efficiency based on yearling spring Chinook releases was applied to wild coho smolts. In the spring of 2016, we estimated that 92 (± 504; 95% CI) emigrated past the trap (Table 10). Combined with a subyearling estimate of 131 (± 96; 95% CI),this gave us a total BY2014 emigrant estimate of 223 (± 514; 95% CI).

Table 10. Estimated egg-to-emigrant survival and smolts-per-redd production for Nason Creek coho salmon.

Brood Year	No. of Redds	Fecundity	Est. Egg Deposition	No. of Emigrants			Egg-to-Emigrant	Emigrants per Redd
				Age-0[a]	Age-1	Total ± 95% CI		
2003	6	2,458	14,748	DNOT	394	—	—	—
2004	35	3,084	107,940	204	56	260 ± 155	0.20%	7
2005	41	2,866	117,506	27	910	937 ± 347	0.80%	23
2006	4	3,126	12,504	7	0	7 ± 10	0.10%	2
2007	10	2,406	24,060	14	136	150 ± 104	0.60%	15
2008	3	3,275	9,825	50	0	50 ± 57	0.50%	17
2009	14	2,691	37,674	471	237	708 ± 478	1.90%	51
2010	8	3,411	27,288	27	437	464 ± 231	1.70%	58
2011	89	3,114	277,146	1,018	1,387	2,405 ± 612	0.90%	27
2012	21	2,752	57,792	46	434	480 ± 237	0.80%	23

Brood Year	No. of Redds	Fecundity	Est. Egg Deposition	No. of Emigrants			Egg-to-Emigrant	Emigrants per Redd
				Age-0[a]	Age-1	Total ± 95% CI		
2013	0	—	—	91	91[c]	182 ± 714	—	—
2014	16	2,992	47,872	131[c]	92[c]	223 ± 514	0.47%	14
2015	0	—	—	0	—	—	—	—
Avg.[b]	24	2,972	71,961	190	344	533	0.80%	24

[a] Does not include subyearling fry prior to July 1.
[b] 10-year average of complete brood data, BY2004-2014.
[c] Pooled estimate



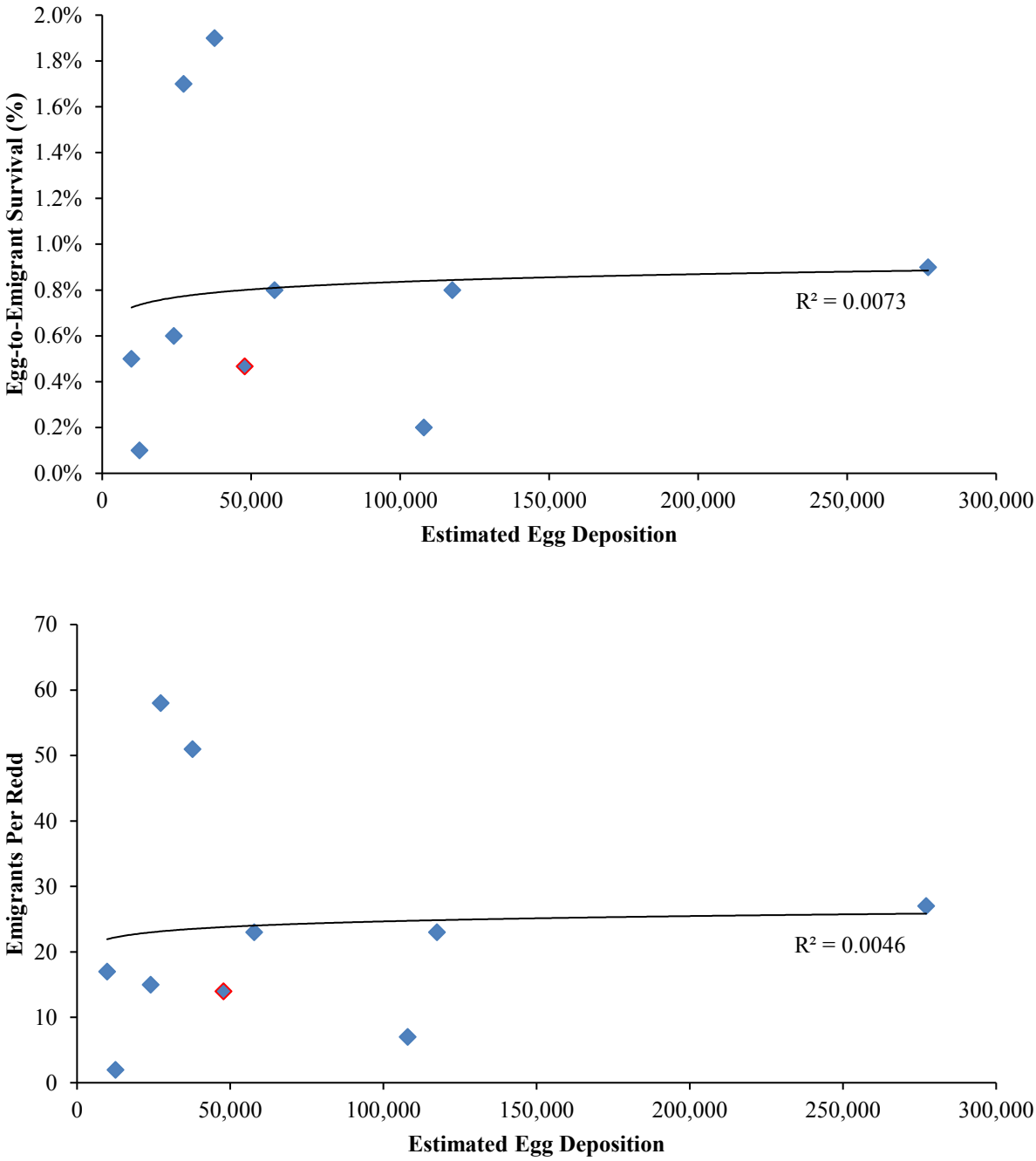


Figure 14. Relationships between estimated egg deposition and total emigrants produced, egg-to-emigrant survival, and emigrants per redd for Nason Creek natural-produced coho, BY 2003 to 2014. *2014 brood (denoted by red border).

3.4.5 Coho Subyearlings (BY2015)

Due to lack of BY2015 naturally-produced coho catch, we concluded that there were no emigrants from Nason in 2016.

3.5 PIT Tagging

During the 2016 trapping season, we PIT tagged 495 wild spring Chinook, 531 steelhead, and 6 naturally produced coho (Table 11). All tagging files were submitted to the PTAGIS database. One shed PIT tag (implanted in steelhead parr) was recovered in a holding box where fish had been held for 24-72 hours after tagging. During remote tagging efforts in the fall of 2015, 1,214 spring Chinook were PIT tagged by YNFRM and WDFW personnel.

Table 11. Number of PIT tagged coho, Chinook, and steelhead with shed rates at the Nason Creek rotary trap in 2016.

Species/Stage	Year-to-date Catch	Year-to-date PIT Tagged	No. of Shed Tags	Percent Shed Tags
Chinook Yearling Smolt	61	61	0	0.0%
Chinook Subyearling Parr (Mar 1 to June 30)	44	21	0	0.0%
Chinook Subyearling Parr (July 1 to Nov 30)	447	413	0	0.0%
Steelhead Parr	663	522	1	0.2%
Steelhead Smolt	9	9	0	0.0%
Coho Yearling Smolt	6	6	0	0.0%
Coho Subyearling Parr	0	0	—	—

* Counts do not include fish with FL<50mm (fry).

3.6 Incidental Species

Along with wild spring Chinook, wild steelhead/rainbow trout, and naturally produced coho, other resident fish species captured at the Nason Creek rotary trap and included in Table 12 are: bull trout *Salvelinus* confluentus, cutthroat trout *Oncorhynchus clarki*, flathead minnow *Pimephales promelas*, longnose dace *Rhinichthys cataractae*, northern pikeminnow *Ptychocheilus oregonensis*, redside shiner *Richardsonius balteatus,* sculpin *Cottus sp.,* sucker *Catostomus sp.,* and mountain whitefish *Prosopium williamsoni*.

Table 12. Summary of length and weight sampling of incidental species captured at the Nason Creek rotary trap in 2016.

Species	Total Count	Length (mm)			Weight (g)		
		Mean	N	SD	Mean	N	SD
Bull Trout	1	199	1	—	70.0	1	—
Cutthroat Trout	1	140	1	—	25.2	1	—
Fathead Minnow	4	52	4	3.7	1.7	4	0.3
Longnose Dace	230	52	230	19.2	2.5	228	4.1
Northern Pikeminnow	18	91	18	23.1	9.6	18	6.1
Redside Shiner	99	41	99	17.6	1.5	84	2.2
Sculpin	84	64	83	35.5	7.9	76	11.7
Sucker	319	58	319	23.4	3.8	317	18.7
Whitefish	81	58	81	39.8	4.8	79	25.8

3.7 ESA Compliance

The Nason Creek smolt trap was operated under consultation with NMFS and USFWS. Total numbers of UCR spring Chinook and UCR summer steelhead that were captured or handled (indirect take) at the trap were less than the maximum permitted (20%) for each species. Lethal take was well below the allowable level of 2% for all ESA-listed species (Table 13). Stream temperatures did not exceed 18˚C at any time in which fish were being handled.

Table 13. Summary of ESA species and coho salmon mortality at the Nason Creek rotary trap.

Species/Stage/Brood Year	Total Collected	Total Mortality	% Mortality
Spring Chinook Yearling (BY2014)	61	0	0.0%
Spring Chinook Subyearling (BY 2015)	791	6	0.8%
Total Wild Spring Chinook	**852**	**6**	**0.7%**
Total Hatchery Spring Chinook	**124**	**0**	**0.0%**
Steelhead Age-0 (BY2016)	702	1	0.1%
Steelhead Age-1 (BY2015)	285	0	0.0%
Steelhead Age-2 (BY2014)	19	0	0.0%
Steelhead Age-3 (BY2013)	1	0	0.0%
Total Wild Summer Steelhead	**1,007**	**1**	**0.1%**
Total Hatchery Summer Steelhead	**98**	**0**	**0.0%**
Total Bull Trout	**1**	**0**	**0.0%**
Coho Yearling (BY2014)	6	0	0.0%
Coho Subyearling (BY2015)	0	0	—
Total Naturally-Produced Coho	**6**	**0**	**0.0%**

4.0 DISCUSSION

Operation of the Nason Creek smolt trap in 2016 was, as in 2015, affected by an unseasonably early and warm spring that caused a quickly diminished snowpack. The resulting prolonged base-flow period meant that the trap could not be operated for much (70 d) of the mid to late summer due to insufficient water velocity. Aside from issues associated with the summer low flow period, inactivity due to other environmental conditions and mechanical issues was minimized. The critical assumptions noted in section 2.5.1, upon which the mark-recapture methodology was predicated, were not violated insofar as we could determine from measuring tag retention/tagging mortality, examining the health of all fish in mark groups prior to release, and ensuring that all fish encountered were thoroughly scanned for PIT tags post-release. All prudent measures were taken to ensure that fish used in mark groups avoided predation between point of release and the trap e.g., release into shallow water refugia.

Since establishment in the summer of 2014, smolt trap operations at the Bolser site have occurred largely under a prolonged period of El Niño spanning from approximately October 2014 through June 2016 (NOAA 2016). Oceanic Niño Index (ONI) levels for this period were especially high (≥ 2.0), with similar conditions not experienced since warming events in 1982/1983 and 1997/1998. Inland manifestations of this most recent El Niño included variable flow and temperature regimes, often deviating greatly from normal trends in both timing and magnitude (Figure 15). Comparison to the 12-year mean discharge and observed flows shows that high water events occurred early, and in periods in which cold temperature normally limit discharge. Quickly diminished snowpack caused by the high, early winter flows subsequently lead to early spring runoff and prolonged base-flow periods in the summer months.

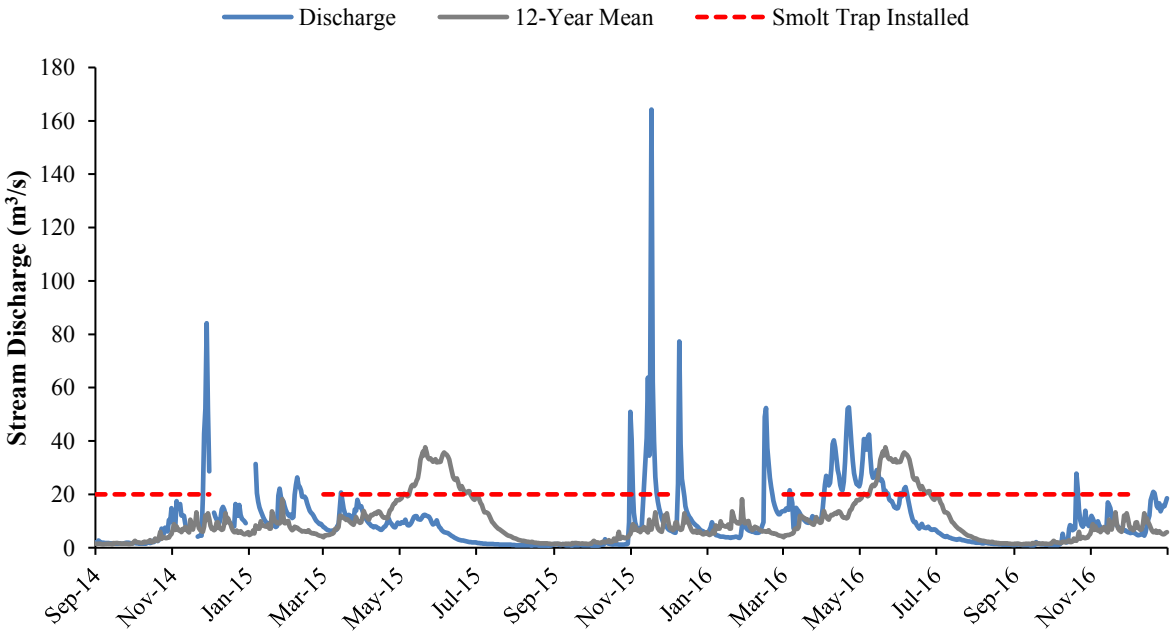


Figure 15. Nason Creek daily discharge from September 2014 through December 2016, with corresponding 12-year mean Nason Creek discharge.

Spring Chinook

The 2014 wild spring Chinook brood at Nason Creek yielded the smallest total emigrant estimate on record at the trap. Egg-to-emigrant survival in comparison to the nearby White River and Chiwawa River showed that Nason Creek was the only monitored tributary in the Wenatchee basin to demonstrate a decrease in in-stream survival between the 2013 and 2014 broods despite similar trends in redd deposition (Figure 17). Comparison of egg-to-emigrant survival and estimated egg deposition suggested that between the three tributaries, Nason Creek produced the most marked outlier (Figure 18). The degree to which Nason Creek deviated from the trends seen in the other tributaries may be due to the comparative effect that the El Niño event had on the individual watershed. The smallest, lowest elevation, and warmest of the three tributaries compared, Nason Creek saw the greatest physical impact of the warming phenomenon.

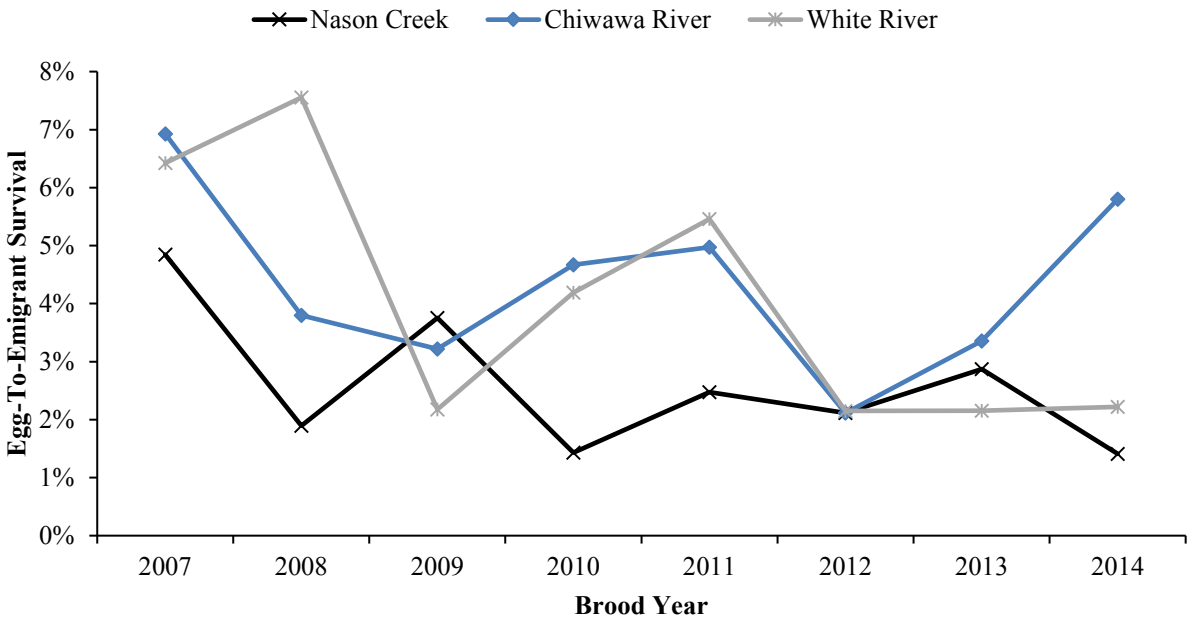


Figure 16. Comparison of wild spring Chinook abundance estimates (BY2007-2014) made at the White River, Nason Creek, and Chiwawa River smolt traps. *Non-trapping estimates not included.

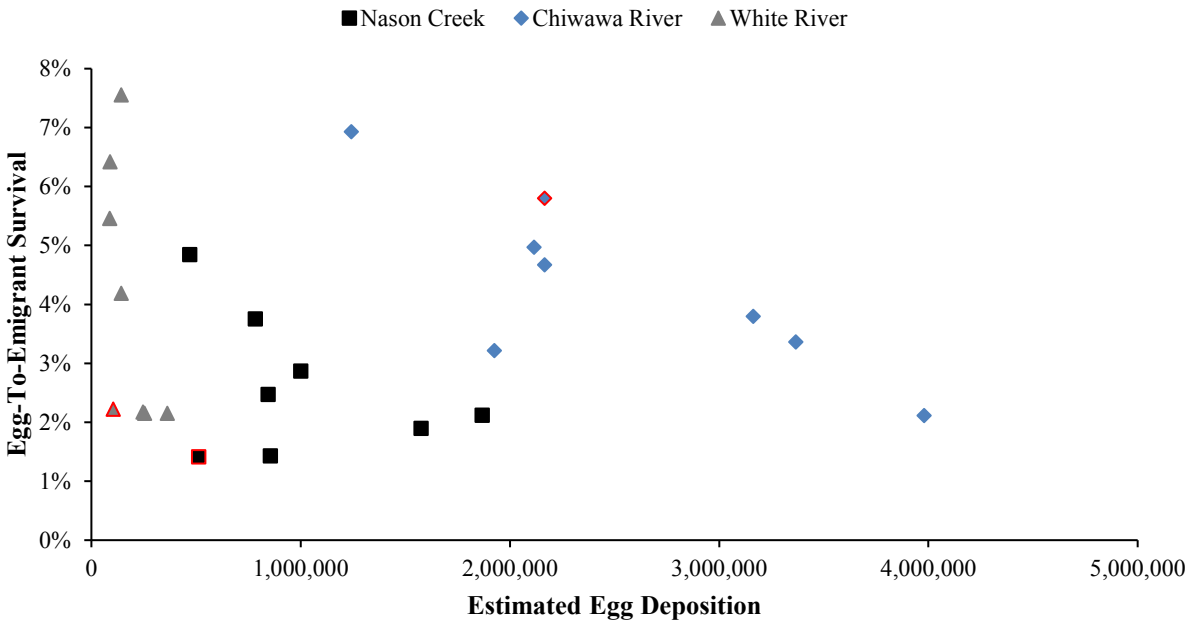


Figure 17. Comparison of egg-to-emigrant survival (BY 2007-2014) and egg deposition for Nason Creek, Chiwawa River, and White River spring Chinook. *Non-trapping estimates not included.

The low comparative survival of BY2014 Chinook was likely due in-apart to decreased survival associated with the anomalous flow and temperature regimes caused by El Niño. Redd scour and sedimentation brought on by irregularly high flows has been shown to increase in-gravel mortality (Montgomery et al. 1996 & Lotspeich and Everest 1981). Although difficult to quantify the exact influence of scour and sedimentation on our estimates, we assume that the strong negative correlation between juvenile survival and peak flow during incubation demonstrated in other tributaries had some negative influence in Nason Creek (Seiler et al. 2002). Some elevated level of increased mortality was also likely incurred as a result of warm water temperatures, decreased habitat available, and elevated competition for resources during the prolonged base flow period in the summer of 2015. Identified in normal years as an impaired watershed due to regular exceedance of 303(d) criteria, Nason Creek saw three consecutive months in 2015 (June-August) in which maximum temperatures exceeded 22ºC (Cristea and Pelletier 2005). Marine and Cech (2004) showed that between three laboratory-based rearing temperature regimes (13-16ºC, 17-20ºC, and 21-24ºC), higher water temperatures significantly decreased growth rates, smoltification indices, and predator avoidance capability. Though Marine and Cech did not see any increased mortality associated with higher rearing temperatures, we assume that effects noted in the study would have an impact on survival in-situ.

BY2014 spring Chinook parr that survived the summer months in Nason Creek were then met with extremely high discharges in the month of November 2015. Flows during this high-water event were large enough to cause a major reconfiguration of log jams and channel morphology in sections. During this period in which we presume a large proportion of the remaining Chinook in Nason Creek were involuntarily pushed out of the system, the trap was unable to run due to water high velocity and debris load. During this event, remote-tagged Chinook were also pushed

from the system when the PIT tag arrays were the least effective. We suspect that along with a higher incidence of in-stream mortality, much of the BY2014 brood left Nason Creek when estimation methodologies were unavailable or ineffective.

A total of only 85 redds in Nason Creek in 2015 was the lowest on record since 2003. The extent to which high winter flows of 2015/2016 affected the BY2015 emigration estimate will potentially be determined upon completion of the outmigration in the summer of 2017. Impact on this brood may be great in that much of the winter flooding occurred pre-emergence; a period of high vulnerability to both scour and sedimentation. The estimated survival of this brood will hopefully indicate the ability of Nason Creek spring Chinook to endure such in-gravel conditions.

Summer Steelhead

The 2013 Nason summer steelhead brood estimate did not reflect the low survival seen in BY2014 Chinook concluding their outmigration at the same time. Although BY2013 steelhead abundance and survival both fell below their 11-year averages, they were not outliers. This is presumably due to the fact that the overwhelming majority (88%) of BY2013 steelhead emigrants left during the spring of 2014; a period not characterized by irregularly high flows or preceded by adverse rearing conditions. The BY2013 age-2 and age-3 emigrant estimates are based on pooled efficiencies, and will be recalculated upon establishment of a viable multi-year regression. Recalculation of these estimates based on a flow-efficiency regression will most likely result in a slightly lower total estimate due to the pooled estimates use of low fixed efficiencies (0.86% and 1.34%). However, because age-2 & 3 steelhead emigrants comprise a relatively small proportion of the total outmigration, recalculation may not change in-stream survival to a great extent.

Potential effects of the El Niño period on developing (BY2014 and BY2015) estimates are still unclear due to the use of pooled estimates employing the aforementioned low fixed efficiencies. BY2014 and BY2015 estimates thus far have produced age-1 estimates that are markedly higher than the 11-year mean. Completion of both emigrant estimates as well as recalculation with a viable flow-efficiency regression will determine if this high abundance is accurate, and in stark contrast to the poor survival calculated in cohabitating spring Chinook.

Coho

Despite a relatively large Wenatchee basin spawner escapement in 2014, only 16 redds were documented in Nason Creek; below the 11-year mean of 24 redds. The resulting total emigrant estimate was also below the 11-year mean, and in the absence of a flow-efficiency regression, calculated with a pooled estimate. As with similar methodologies used to calculate other species abundances in the absence of a flow-efficiency relationship, we suspect that these pooled estimated are likely overestimated due to low efficiencies used. BY2014 coho were likely affected by the El Niño weather trend similarly to BY2014 spring Chinook, given similar in-stream residence times.

A poor adult coho return in 2015 required exhaustive broodstock retention at Tumwater dam to meet hatchery production goals. As a result, no coho were documented in Nason Creek. This is reflected in the complete lack of BY2015 subyearlings at the trap during the 2016 trap year. Given little coho passage above Tumwater dam, and a very small likelihood that any spawning activity occurred in Nason Creek in 2015, we suspect that yearling emigrants will be absent completely for this brood as well.

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APPENDIX A. Daily Stream Discharge

Date	Stream Discharge (m^3/s)
1/1/2016	5.5
1/2/2016	6.6
1/3/2016	7.6
1/4/2016	9.5
1/5/2016	8.1
1/6/2016	5.7
1/7/2016	4.8
1/8/2016	4.6
1/9/2016	4.5
1/10/2016	4.3
1/11/2016	4.2
1/12/2016	4.2
1/13/2016	4.2
1/14/2016	4.0
1/15/2016	3.9
1/16/2016	4.0
1/17/2016	3.9
1/18/2016	3.8
1/19/2016	3.7
1/20/2016	
1/21/2016	
1/22/2016	4.1
1/23/2016	4.2
1/24/2016	4.0
1/25/2016	3.8
1/26/2016	3.7
1/27/2016	4.5
1/28/2016	8.2
1/29/2016	
1/30/2016	8.5
1/31/2016	7.5
2/1/2016	7.0
2/2/2016	6.6
2/3/2016	6.3
2/4/2016	6.2
2/5/2016	6.0
2/6/2016	6.1
2/7/2016	5.8
2/8/2016	5.6
2/9/2016	5.6
2/10/2016	5.6
2/11/2016	5.9
2/12/2016	6.9
2/13/2016	
2/14/2016	9.5
2/15/2016	49.0
2/16/2016	52.4
2/17/2016	37.4
2/18/2016	31.7
2/19/2016	26.3
2/20/2016	22.8
2/21/2016	19.9
2/22/2016	17.6
2/23/2016	15.7
2/24/2016	14.4
2/25/2016	13.3
2/26/2016	12.6
2/27/2016	12.6
2/28/2016	13.4
2/29/2016	13.7
3/1/2016	
3/2/2016	13.8
3/3/2016	14.7
3/4/2016	14.8
3/5/2016	14.2
3/6/2016	21.6
3/7/2016	19.4
3/8/2016	16.8
3/9/2016	7.2
3/10/2016	7.2
3/11/2016	15.0
3/12/2016	14.2
3/13/2016	13.7
3/14/2016	12.8
3/15/2016	11.8
3/16/2016	11.1
3/17/2016	10.6
3/18/2016	10.0
3/19/2016	9.6
3/20/2016	9.4
3/21/2016	9.4
3/22/2016	10.1
3/23/2016	10.4
3/24/2016	11.9

3/25/2016	11.4
3/26/2016	10.8
3/27/2016	10.7
3/28/2016	10.2
3/29/2016	9.9
3/30/2016	10.1
3/31/2016	11.2
4/1/2016	14.6
4/2/2016	19.9
4/3/2016	23.6
4/4/2016	27.0
4/5/2016	26.5
4/6/2016	23.4
4/7/2016	24.3
4/8/2016	29.4
4/9/2016	38.8
4/10/2016	40.2
4/11/2016	37.7
4/12/2016	33.4
4/13/2016	29.2
4/14/2016	27.0
4/15/2016	23.5
4/16/2016	21.7
4/17/2016	21.5
4/18/2016	24.8
4/19/2016	32.3
4/20/2016	43.0
4/21/2016	52.1
4/22/2016	52.7
4/23/2016	45.9
4/24/2016	38.8
4/25/2016	32.6
4/26/2016	27.9
4/27/2016	25.7
4/28/2016	23.8
4/29/2016	23.7
4/30/2016	23.0
5/1/2016	24.1
5/2/2016	27.5
5/3/2016	33.1
5/4/2016	40.8
5/5/2016	39.4
5/6/2016	36.8
5/7/2016	41.3
5/8/2016	42.5
5/9/2016	34.0
5/10/2016	28.1
5/11/2016	26.2
5/12/2016	26.4
5/13/2016	27.5
5/14/2016	29.2
5/15/2016	29.2
5/16/2016	26.5
5/17/2016	25.3
5/18/2016	25.7
5/19/2016	24.6
5/20/2016	20.7
5/21/2016	21.4
5/22/2016	20.3
5/23/2016	20.2
5/24/2016	18.1
5/25/2016	17.6
5/26/2016	17.5
5/27/2016	16.5
5/28/2016	15.2
5/29/2016	15.2
5/30/2016	14.8
5/31/2016	14.8
6/1/2016	16.5
6/2/2016	20.5
6/3/2016	19.3
6/4/2016	20.0
6/5/2016	22.2
6/6/2016	22.7
6/7/2016	20.6
6/8/2016	18.1
6/9/2016	15.7
6/10/2016	13.3
6/11/2016	11.5
6/12/2016	10.3
6/13/2016	9.5
6/14/2016	9.7
6/15/2016	8.5
6/16/2016	7.8
6/17/2016	7.2
6/18/2016	8.2
6/19/2016	9.0
6/20/2016	7.7
6/21/2016	7.5
6/22/2016	7.4

6/23/2016	7.4
6/24/2016	7.9
6/25/2016	7.3
6/26/2016	6.9
6/27/2016	6.7
6/28/2016	6.9
6/29/2016	6.9
6/30/2016	6.7
7/1/2016	6.1
7/2/2016	5.7
7/3/2016	5.4
7/4/2016	5.1
7/5/2016	4.7
7/6/2016	4.4
7/7/2016	4.1
7/8/2016	4.1
7/9/2016	4.4
7/10/2016	4.0
7/11/2016	3.9
7/12/2016	3.6
7/13/2016	3.5
7/14/2016	3.3
7/15/2016	3.1
7/16/2016	3.1
7/17/2016	3.0
7/18/2016	3.2
7/19/2016	3.4
7/20/2016	3.0
7/21/2016	2.9
7/22/2016	2.8
7/23/2016	2.8
7/24/2016	2.6
7/25/2016	2.5
7/26/2016	2.4
7/27/2016	2.4
7/28/2016	2.3
7/29/2016	2.2
7/30/2016	2.1
7/31/2016	2.0
8/1/2016	1.9
8/2/2016	1.9
8/3/2016	1.9
8/4/2016	1.8
8/5/2016	1.8
8/6/2016	1.7
8/7/2016	1.6
8/8/2016	1.7
8/9/2016	2.0
8/10/2016	1.8
8/11/2016	1.7
8/12/2016	1.6
8/13/2016	1.5
8/14/2016	1.5
8/15/2016	1.4
8/16/2016	1.4
8/17/2016	1.4
8/18/2016	1.3
8/19/2016	1.3
8/20/2016	1.3
8/21/2016	1.2
8/22/2016	1.2
8/23/2016	1.2
8/24/2016	1.2
8/25/2016	1.2
8/26/2016	1.2
8/27/2016	1.1
8/28/2016	1.1
8/29/2016	1.1
8/30/2016	1.1
8/31/2016	1.1
9/1/2016	1.1
9/2/2016	1.2
9/3/2016	1.4
9/4/2016	1.3
9/5/2016	1.2
9/6/2016	1.1
9/7/2016	1.1
9/8/2016	1.1
9/9/2016	1.1
9/10/2016	1.0
9/11/2016	1.0
9/12/2016	1.0
9/13/2016	1.0
9/14/2016	1.0
9/15/2016	0.9
9/16/2016	0.9
9/17/2016	1.0
9/18/2016	2.1
9/19/2016	1.6
9/20/2016	1.6

9/21/2016	1.4
9/22/2016	1.3
9/23/2016	1.2
9/24/2016	1.3
9/25/2016	1.2
9/26/2016	1.2
9/27/2016	1.1
9/28/2016	1.1
9/29/2016	1.1
9/30/2016	1.0
10/1/2016	1.0
10/2/2016	1.0
10/3/2016	1.0
10/4/2016	1.0
10/5/2016	1.1
10/6/2016	1.1
10/7/2016	1.4
10/8/2016	1.9
10/9/2016	5.2
10/10/2016	2.9
10/11/2016	2.3
10/12/2016	2.0
10/13/2016	2.1
10/14/2016	7.0
10/15/2016	8.5
10/16/2016	8.1
10/17/2016	6.5
10/18/2016	
10/19/2016	7.3
10/20/2016	27.8
10/21/2016	22.2
10/22/2016	14.2
10/23/2016	10.3
10/24/2016	8.5
10/25/2016	7.9
10/26/2016	9.1
10/27/2016	13.8
10/28/2016	10.2
10/29/2016	8.8
10/30/2016	8.1
10/31/2016	10.4
11/1/2016	12.0
11/2/2016	11.0
11/3/2016	9.9
11/4/2016	8.7
11/5/2016	8.2
11/6/2016	9.9
11/7/2016	8.5
11/8/2016	7.7
11/9/2016	7.3
11/10/2016	7.1
11/11/2016	6.7
11/12/2016	7.1
11/13/2016	7.7
11/14/2016	17.0
11/15/2016	16.0
11/16/2016	14.9
11/17/2016	11.9
11/18/2016	10.5
11/19/2016	9.6
11/20/2016	8.8
11/21/2016	8.3
11/22/2016	7.9
11/23/2016	7.4
11/24/2016	7.2
11/25/2016	7.1
11/26/2016	6.8
11/27/2016	6.5
11/28/2016	6.5
11/29/2016	6.0
11/30/2016	5.9
12/1/2016	5.8
12/2/2016	5.5
12/3/2016	5.9
12/4/2016	
12/5/2016	5.6
12/6/2016	5.2
12/7/2016	4.9
12/8/2016	4.7
12/9/2016	4.7
12/10/2016	4.9
12/11/2016	5.0
12/12/2016	6.0
12/13/2016	4.6
12/14/2016	5.7
12/15/2016	8.2
12/16/2016	10.4

12/17/2016	12.0
12/18/2016	17.8
12/19/2016	19.4
12/20/2016	20.9
12/21/2016	20.5
12/22/2016	18.0
12/23/2016	15.7
12/24/2016	14.8
12/25/2016	16.6
12/26/2016	13.7
12/27/2016	14.8
12/28/2016	15.7
12/29/2016	15.6
12/30/2016	17.0
12/31/2016	18.5

APPENDIX B. Daily Trap Operation

Date	Trap Status	Comments
3/1/2016	Op.	
3/2/2016	Op.	
3/3/2016	Op.	
3/4/2016	Op.	
3/5/2016	Op.	
3/6/2016	Op.	
3/7/2016	Op.	
3/8/2016	Op.	
3/9/2016	Op.	
3/10/2016	Op.	
3/11/2016	Op.	
3/12/2016	Op.	
3/13/2016	Op.	
3/14/2016	Op.	
3/15/2016	Op.	
3/16/2016	Op.	
3/17/2016	Op.	
3/18/2016	Op.	
3/19/2016	Op.	
3/20/2016	Op.	
3/21/2016	Op.	
3/22/2016	Op.	
3/23/2016	Op.	
3/24/2016	Op.	
3/25/2016	Op.	
3/26/2016	Op.	
3/27/2016	Op.	
3/28/2016	Op.	
3/29/2016	Op.	
3/30/2016	Op.	
3/31/2016	Op.	
4/1/2016	Op.	
4/2/2016	Op.	
4/3/2016	Op.	
4/4/2016	Op.	
4/5/2016	Op.	
4/6/2016	Op.	
4/7/2016	Op.	
4/8/2016	Op.	
4/9/2016	Op.	
4/10/2016	Op.	
4/11/2016	Op.	
4/12/2016	Op.	
4/13/2016	Op.	
4/14/2016	Op.	
4/15/2016	Op.	
4/16/2016	Op.	
4/17/2016	Op.	
4/18/2016	Op.	
4/19/2016	Op.	
4/20/2016	Op.	
4/21/2016	Op.	
4/22/2016	Op.	
4/23/2016	Op.	
4/24/2016	Op.	
4/25/2016	Op.	
4/26/2016	Op.	
4/27/2016	Op.	
4/28/2016	Op.	
4/29/2016	Op.	
4/30/2016	Op.	
5/1/2016	Op.	
5/2/2016	Op.	
5/3/2016	Op.	
5/4/2016	Op.	
5/5/2016	Op.	
5/6/2016	Op.	
5/7/2016	Op.	
5/8/2016	Op.	
5/9/2016	Op.	
5/10/2016	Op.	
5/11/2016	Op.	
5/12/2016	Op.	
5/13/2016	Op.	
5/14/2016	Op.	
5/15/2016	Op.	
5/16/2016	Op.	
5/17/2016	Op.	
5/18/2016	Op.	

5/19/2016	Op.		7/1/2016	Op.	
5/20/2016	Op.		7/2/2016	Op.	
5/21/2016	Op.		7/3/2016	Op.	
5/22/2016	Op.		7/4/2016	Op.	
5/23/2016	Op.		7/5/2016	Op.	
5/24/2016	Op.		7/6/2016	Op.	
5/25/2016	Op.		7/7/2016	Op.	
5/26/2016	Op.		7/8/2016	Op.	
5/27/2016	Op.		7/9/2016	Op.	
5/28/2016	Op.		7/10/2016	Op.	
5/29/2016	Op.		7/11/2016	No Op.	Stopped - low flow
5/30/2016	Op.		7/12/2016	Op.	
5/31/2016	Op.		7/13/2016	Op.	
6/1/2016	Op.		7/14/2016	Op.	
6/2/2016	Op.		7/15/2016	Op.	
6/3/2016	Op.		7/16/2016	Op.	
6/4/2016	Op.		7/17/2016	Op.	
6/5/2016	No Op.	Stopped by debris	7/18/2016	Op.	
6/6/2016	Op.		7/19/2016	Op.	
6/7/2016	Op.		7/20/2016	Op.	
6/8/2016	Op.		7/21/2016	Op.	
6/9/2016	Op.		7/22/2016	Op.	
6/10/2016	Op.		7/23/2016	Op.	
6/11/2016	Op.		7/24/2016	Op.	
6/12/2016	Op.		7/25/2016	Op.	
6/13/2016	Op.		7/26/2016	No Op.	Stopped - low flow
6/14/2016	Op.		7/27/2016	Op.	
6/15/2016	Op.		7/28/2016	No Op.	Stopped - low flow
6/16/2016	Op.		7/29/2016	Op.	
6/17/2016	Op.		7/30/2016	Op.	
6/18/2016	Op.		7/31/2016	No Op.	Stopped - low flow
6/19/2016	Op.		8/1/2016	No Op.	Stopped - low flow
6/20/2016	Op.		8/2/2016	Op.	
6/21/2016	Op.		8/3/2016	No Op.	Stopped - low flow
6/22/2016	Op.		8/4/2016	No Op.	Stopped - low flow
6/23/2016	Op.		8/5/2016	No Op.	Stopped - low flow
6/24/2016	Op.		8/6/2016	No Op.	Stopped - low flow
6/25/2016	Op.		8/7/2016	No Op.	Stopped - low flow
6/26/2016	Op.		8/8/2016	No Op.	Pulled - low flow
6/27/2016	Op.		8/9/2016	Op.	
6/28/2016	Op.		8/10/2016	No Op.	Pulled - low flow
6/29/2016	Op.		8/11/2016	No Op.	Pulled - low flow
6/30/2016	No Op.	Stopped - debris	8/12/2016	No Op.	Pulled - low flow

8/13/2016	No Op.	Pulled - low flow
8/14/2016	No Op.	Pulled - low flow
8/15/2016	No Op.	Pulled - low flow
8/16/2016	No Op.	Pulled - low flow
8/17/2016	No Op.	Pulled - low flow
8/18/2016	No Op.	Pulled - low flow
8/19/2016	No Op.	Pulled - low flow
8/20/2016	No Op.	Pulled - low flow
8/21/2016	No Op.	Pulled - low flow
8/22/2016	No Op.	Pulled - low flow
8/23/2016	No Op.	Pulled - low flow
8/24/2016	No Op.	Pulled - low flow
8/25/2016	No Op.	Pulled - low flow
8/26/2016	No Op.	Pulled - low flow
8/27/2016	No Op.	Pulled - low flow
8/28/2016	No Op.	Pulled - low flow
8/29/2016	No Op.	Pulled - low flow
8/30/2016	No Op.	Pulled - low flow
8/31/2016	No Op.	Pulled - low flow
9/1/2016	No Op.	Pulled - low flow
9/2/2016	No Op.	Pulled - low flow
9/3/2016	No Op.	Pulled - low flow
9/4/2016	No Op.	Pulled - low flow
9/5/2016	No Op.	Pulled - low flow
9/6/2016	No Op.	Pulled - low flow
9/7/2016	No Op.	Pulled - low flow
9/8/2016	No Op.	Pulled - low flow
9/9/2016	No Op.	Pulled - low flow
9/10/2016	No Op.	Pulled - low flow
9/11/2016	No Op.	Pulled - low flow
9/12/2016	No Op.	Pulled - low flow
9/13/2016	No Op.	Stopped - low flow
9/14/2016	No Op.	Pulled - low flow
9/15/2016	No Op.	Pulled - low flow
9/16/2016	No Op.	Pulled - low flow
9/17/2016	No Op.	Pulled - low flow
9/18/2016	No Op.	Pulled - low flow
9/19/2016	No Op.	Pulled - low flow
9/20/2016	No Op.	Pulled - low flow
9/21/2016	No Op.	Pulled - low flow
9/22/2016	No Op.	Pulled - low flow
9/23/2016	No Op.	Pulled - low flow
9/24/2016	No Op.	Pulled - low flow
9/25/2016	No Op.	Pulled - low flow
9/26/2016	No Op.	Pulled - low flow
9/27/2016	No Op.	Pulled - low flow
9/28/2016	No Op.	Pulled - low flow
9/29/2016	No Op.	Pulled - low flow
9/30/2016	No Op.	Pulled - low flow
10/1/2016	No Op.	Pulled - low flow
10/2/2016	No Op.	Pulled - low flow
10/3/2016	No Op.	Pulled - low flow
10/4/2016	No Op.	Pulled - low flow
10/5/2016	No Op.	Pulled - low flow
10/6/2016	No Op.	Pulled - low flow
10/7/2016	No Op.	Pulled - low flow
10/8/2016	No Op.	Pulled - low flow
10/9/2016	No Op.	Pulled - low flow
10/10/2016	Op.	
10/11/2016	Op.	
10/12/2016	Op.	
10/13/2016	No Op.	Stopped - low flow
10/14/2016	Op.	
10/15/2016	No Op.	Stopped - debris
10/16/2016	Op.	
10/17/2016	Op.	
10/18/2016	Op.	
10/19/2016	Op.	
10/20/2016	Op.	
10/21/2016	No Op.	Pulled - high flow
10/22/2016	No Op.	Stopped - debris
10/23/2016	Op.	
10/24/2016	Op.	
10/25/2016	Op.	
10/26/2016	Op.	
10/27/2016	Op.	

10/28/2016	Op.		11/1/2016	Op.
10/29/2016	Op.		11/2/2016	Op.
10/30/2016	Op.		11/3/2016	Op.
10/31/2016	Op.		11/4/2016	Op.
			11/5/2016	Op.
			11/6/2016	Op.

11/7/2016	Op.	
11/8/2016	Op.	
11/9/2016	Op.	
11/10/2016	Op.	
11/11/2016	Op.	
11/12/2016	Op.	
11/13/2016	Op.	
11/14/2016	Op.	
11/15/2016	Op.	
11/16/2016	Op.	
11/17/2016	Op.	
11/18/2016	Op.	
11/19/2016	Op.	
11/20/2016	Op.	
11/21/2016	Op.	
11/22/2016	Op.	
11/23/2016	Op.	
11/24/2016	Op.	
11/25/2016	Op.	
11/26/2016	Op.	
11/27/2016	Op.	
11/28/2016	Op.	
11/29/2016	Op.	
11/30/2016	Op.	End Trapping

APPENDIX C. Regression Models

Model: Chinook Yearlings (Spring '06-'14) Back Position, ($r^2 = 0.15$; $p = 0.03$)

Origin/Species/Stage	Age	Date	Trap Position	Mark	Recap	Trap Efficiency (R+1) / M	ASIN Transform	Discharge (m^3/s)
Wild Chinook Smolt	1+	3/31/2007	Back	40	2	0.08	0.28	24.6
Wild Chinook Smolt	1+	4/6/2006	Back	42	9	0.24	0.51	7.5
Wild Chinook Smolt	1+	4/14/2010	Back	42	4	0.12	0.35	4.9
Wild Chinook Smolt	1+	3/31/2012	Back	43	5	0.14	0.38	7.1
Wild Chinook Smolt	1+	4/3/2007	Back	46	1	0.04	0.21	18.6
Wild Chinook Smolt	1+	4/19/2012	Back	48	7	0.17	0.42	12.3
Wild Chinook Smolt	1+	4/10/2007	Back	53	4	0.09	0.31	27.4
Wild Chinook Smolt	1+	4/21/2009	Back	53	0	0.02	0.14	20.7
Wild Chinook Smolt	1+	4/13/2012	Back	53	4	0.09	0.31	10.1
Wild Chinook Smolt	1+	4/16/2012	Back	53	7	0.15	0.40	12.5
Wild Chinook Smolt	1+	4/24/2008	Back	57	8	0.16	0.41	5.9
Wild Chinook Smolt	1+	4/23/2012	Back	58	1	0.03	0.19	39.1
Wild Chinook Smolt	1+	4/24/2006	Back	59	3	0.07	0.26	10.4
Wild Chinook Smolt	1+	3/23/2007	Back	59	7	0.14	0.38	24.8
Wild Chinook Smolt	1+	3/17/2007	Back	64	7	0.13	0.36	26.5
Wild Chinook Smolt	1+	4/18/2010	Back	67	2	0.05	0.21	9.3
Wild Chinook Smolt	1+	4/17/2008	Back	72	13	0.19	0.46	7.8
Wild Chinook Smolt	1+	4/3/2006	Back	81	10	0.14	0.38	5.3
Wild Chinook Smolt	1+	3/20/2007	Back	91	13	0.15	0.40	34.8
Wild Chinook Smolt	1+	5/1/2008	Back	102	16	0.17	0.42	8.9
Wild Chinook Smolt	1+	4/28/2008	Back	127	19	0.16	0.41	7.7
Wild Chinook Smolt	1+	4/14/2008	Back	195	40	0.21	0.48	9.3
Wild Chinook Smolt	1+	3/9/2014	Back	65	4	0.08	0.28	27.1
Wild Chinook Smolt	1+	3/13/2014	Back	67	9	0.15	0.40	16.0

Model: Chinook Subyearling (Fall '06-'13) Back Position, ($r^2 = 0.55$; $p = 0.001$)

Origin/Species/Stage	Age	Date	Trap Position	Mark	Recap	Trap Efficiency (R+1) / M	ASIN Transform	Discharge (m^3/s)
Wild Chinook Parr	0	10/26/2006	Back	183	50	0.28	0.56	1.4
Wild Chinook Parr	0	10/30/2006	Back	168	52	0.32	0.60	1.8
Wild Chinook Parr	0	11/1/2010	Back	254	42	0.17	0.42	5.6
Wild Chinook Parr	0	11/4/2010	Back	287	49	0.17	0.43	6.1
Wild Chinook Parr	0	11/7/2010	Back	168	32	0.20	0.46	6.8
Wild Chinook Parr	0	11/13/2010	Back	185	35	0.19	0.46	3.7
Wild Chinook Parr	0	11/3/2012	Back	201	25	0.13	0.37	11.4

Wild Chinook Parr	0	11/7/2012	Back	233	27	0.12	0.35	11.2
Wild Chinook Parr	0	11/11/2012	Back	328	87	0.27	0.54	6.1
Wild Chinook Parr	0	11/15/2012	Back	195	34	0.18	0.44	6.0
Wild Chinook Parr	0	9/30/2013	Back	171	12	0.08	0.28	15.3
Wild Chinook Parr	0	10/2/2013	Back	213	43	0.21	0.47	9.3
Wild Chinook Parr	0	10/3/2013	Back	181	41	0.23	0.50	8.4
Wild Chinook Parr	0	10/7/2013	Back	242	31	0.13	0.37	6.6
Wild Chinook Parr	0	10/9/2013	Back	203	40	0.20	0.47	8.6
Wild Chinook Parr	0	11/27/2013	Back	241	55	0.23	0.50	5.2

Model: Chinook Subyearling (Fall '06-'13) Forward Position, ($r^2 = 0.16$; $p = 0.02$)

Origin/Species/Stage	Age	Date	Trap Position	Mark	Recap	Trap Efficiency (R+1) / M	ASIN Transform	Discharge (m^3/s)
Wild Chinook Parr	0	7/13/2006	Back	52	8	0.17	0.43	4.8
Wild Chinook Parr	0	7/17/2006	Back	138	15	0.12	0.35	3.7
Wild Chinook Parr	0	7/20/2006	Back	74	5	0.08	0.29	3.2
Wild Chinook Parr	0	7/28/2006	Back	54	5	0.11	0.34	2.6
Wild Chinook Parr	0	7/31/2006	Back	99	7	0.08	0.29	2.2
Wild Chinook Parr	0	9/18/2006	Back	55	10	0.20	0.46	1.3
Wild Chinook Parr	0	7/31/2008	Back	60	15	0.27	0.54	3.4
Wild Chinook Parr	0	8/12/2008	Back	103	2	0.03	0.17	2.4
Wild Chinook Parr	0	8/22/2008	Back	75	11	0.16	0.41	2.7
Wild Chinook Parr	0	8/28/2008	Back	72	7	0.11	0.34	2.3
Wild Chinook Parr	0	10/9/2008	Back	110	22	0.21	0.48	1.8
Wild Chinook Parr	0	10/27/2008	Back	51	12	0.26	0.53	1.6
Wild Chinook Parr	0	10/30/2008	Back	84	15	0.19	0.45	1.5
Wild Chinook Parr	0	11/6/2008	Back	78	8	0.12	0.35	2.2
Wild Chinook Parr	0	11/10/2008	Back	88	0	0.01	0.11	8.7
Wild Chinook Parr	0	7/14/2009	Back	86	2	0.04	0.19	5.5
Wild Chinook Parr	0	7/15/2009	Back	105	4	0.05	0.22	5.1
Wild Chinook Parr	0	7/17/2009	Back	122	8	0.07	0.28	4.4
Wild Chinook Parr	0	7/20/2009	Back	89	2	0.03	0.19	3.8
Wild Chinook Parr	0	8/17/2009	Back	73	1	0.03	0.17	1.6
Wild Chinook Parr	0	9/10/2009	Back	56	7	0.14	0.39	1.7
Wild Chinook Parr	0	8/8/2010	Back	58	1	0.03	0.19	2.4
Wild Chinook Parr	0	8/11/2010	Back	114	8	0.08	0.29	2.2
Wild Chinook Parr	0	9/11/2010	Back	68	9	0.15	0.39	2.1
Wild Chinook Parr	0	10/12/2010	Back	216	42	0.20	0.46	3.6
Wild Chinook Parr	0	10/15/2010	Back	192	37	0.20	0.46	2.7
Wild Chinook Parr	0	10/18/2010	Back	193	36	0.19	0.45	2.3

Wild Chinook Parr	0	10/22/2010	Back	92	18	0.21	0.47	2.0
Wild Chinook Parr	0	10/25/2010	Back	60	7	0.13	0.37	2.2
Wild Chinook Parr	0	10/29/2010	Back	127	0	0.01	0.09	2.7
Wild Chinook Parr	0	8/19/2011	Back	106	5	0.06	0.24	3.5

Model: Chinook Subyearling (Fall '14-'16) Bolser Site ($r^2 = 0.60$; $p = 0.005$)

Origin/Species/Stage	Age	Date	Trap Position	Mark	Recap	Trap Efficiency (R+1)/M	ASIN Transform	Discharge (m^3/s)
Wild Chinook Parr	0	7/14/2014	1	89	7	0.09	0.30	6.8
Wild Chinook Parr	0	7/21/2014	1	74	4	0.07	0.26	4.3
Wild Chinook Parr	0	7/27/2014	1	72	4	0.07	0.27	3.3
Wild Chinook Parr	0	10/24/2014	1	53	4	0.09	0.31	5.0
Wild Chinook Parr	0	10/27/2014	1	71	3	0.06	0.24	5.4
Wild Chinook Parr	0	10/30/2014	1	70	5	0.09	0.30	8.4
Wild Chinook Parr	0	11/1/2014	1	96	6	0.07	0.27	9.6
Wild Chinook Parr	0	10/24/2016	1	59	6	0.12	0.35	8.0
Wild Chinook Parr	0	11/1/2016	1	68	8	0.13	0.37	10.6
Wild Chinook Parr	0	11/15/2016	1	69	11	0.17	0.43	15.3

Model: Summer Steelhead Back Position ('07-'14), ($r^2 = 0.35$; $p = 2.90E-05$)

Origin/Species/Stage	Age	Date	Trap Position	Mark	Recap	Trap Efficiency (R+1) / M	ASIN Transform	Discharge (m^3/s)
Wild Steelhead Parr/Smolt	1+	3/20/2007	Back	55	1	0.04	0.19	34.8
Wild Steelhead Parr/Smolt	1+	3/31/2007	Back	56	4	0.09	0.30	24.6
Wild Steelhead Parr/Smolt	1+	4/10/2007	Back	60	8	0.15	0.40	27.4
Wild Steelhead Parr/Smolt	1+	5/1/2007	Back	52	2	0.06	0.24	22.2
Wild Steelhead Parr/Smolt	1+	6/9/2007	Back	71	9	0.14	0.38	23.8
Wild Steelhead Parr/Smolt	1+	6/12/2007	Back	65	8	0.14	0.38	19.9
Wild Steelhead Parr/Smolt	1+	6/14/2007	Back	61	5	0.10	0.32	19.5
Wild Steelhead Parr/Smolt	1+	6/21/2007	Back	67	4	0.07	0.28	21.3
Wild Steelhead Parr/Smolt	1+	4/14/2008	Back	149	46	0.32	0.60	9.3
Wild Steelhead Parr/Smolt	1+	4/17/2008	Back	75	3	0.05	0.23	7.8
Wild Steelhead Parr/Smolt	1+	4/28/2008	Back	74	11	0.16	0.41	7.7
Wild Steelhead Parr/Smolt	1+	5/1/2008	Back	176	29	0.17	0.43	8.9
Wild Steelhead Parr/Smolt	1+	5/12/2008	Back	55	8	0.16	0.42	18.8
Wild Steelhead Parr/Smolt	1+	5/15/2008	Back	57	1	0.04	0.19	39.4
Wild Steelhead Parr/Smolt	1+	6/9/2008	Back	142	20	0.15	0.39	26.6
Wild Steelhead Parr/Smolt	1+	6/12/2008	Back	83	10	0.13	0.37	23.3

Wild Steelhead Parr/Smolt	1+	6/16/2008	Back	81	8	0.11	0.34	32.3
Wild Steelhead Parr/Smolt	1+	4/20/2010	Back	121	11	0.10	0.32	19.1
Wild Steelhead Parr/Smolt	1+	4/22/2010	Back	121	10	0.09	0.31	20.6
Wild Steelhead Parr/Smolt	1+	6/20/2010	Back	128	11	0.09	0.31	26.2
Wild Steelhead Parr/Smolt	1+	4/5/2011	Back	52	1	0.04	0.20	21.5
Wild Steelhead Parr/Smolt	1+	5/22/2011	Back	84	3	0.05	0.22	43.6
Wild Steelhead Parr/Smolt	1+	6/12/2012	Back	69	5	0.09	0.30	33.1
Wild Steelhead Parr/Smolt	1+	7/26/2012	Back	63	4	0.08	0.29	7.9
Wild Steelhead Parr/Smolt	1+	4/22/2013	Back	66	6	0.11	0.33	14.7
Wild Steelhead Parr/Smolt	1+	4/26/2013	Back	50	2	0.06	0.25	18.2
Wild Steelhead Parr/Smolt	1+	4/30/2013	Back	54	2	0.06	0.24	22.0
Wild Steelhead Parr/Smolt	1+	5/8/2013	Back	62	0	0.02	0.13	61.4
Wild Steelhead Parr/Smolt	1+	5/19/2013	Back	122	15	0.13	0.37	32.0
Wild Steelhead Parr/Smolt	1+	5/22/2013	Back	58	4	0.09	0.30	30.6
Wild Steelhead Parr/Smolt	1+	5/26/2013	Back	79	3	0.05	0.23	20.5
Wild Steelhead Parr/Smolt	1+	5/30/2013	Back	92	7	0.09	0.30	24.0
Wild Steelhead Parr/Smolt	1+	6/3/2013	Back	71	6	0.10	0.32	27.2
Wild Steelhead Parr/Smolt	1+	6/7/2013	Back	94	4	0.05	0.23	40.2
Wild Steelhead Parr/Smolt	1+	6/13/2013	Back	64	2	0.05	0.22	21.1
Wild Steelhead Parr/Smolt	1+	6/17/2013	Back	115	5	0.05	0.23	25.0
Wild Steelhead Parr/Smolt	1+	6/29/2013	Back	60	12	0.22	0.48	20.7
Wild Steelhead Parr/Smolt	1+	7/7/2013	Back	75	9	0.13	0.37	9.2
Wild Steelhead Parr/Smolt	1+	5/5/2014	Back	55	3	0.07	0.27	35.7
Wild Steelhead Parr/Smolt	1+	5/20/2014	Back	57	0	0.02	0.13	42.2
Wild Steelhead Parr/Smolt	1+	6/3/2014	Back	75	1	0.03	0.16	45.6

Model: 2013 Summer Steelhead Back Position (In-yr.), ($r^2 = 0.15$; $p = 0.05$)

Origin/Species/Stage	Age	Date	Trap Position	Mark	Recap	Trap Efficiency (R+1) / M	ASIN Transform	Discharge (m^3/s)
Wild Chinook Smolt	1+	3/31/2007	Back	40	2	0.08	0.28	24.6
Wild Chinook Smolt	1+	4/6/2006	Back	42	9	0.24	0.51	7.5
Wild Chinook Smolt	1+	4/14/2010	Back	42	4	0.12	0.35	4.9
Wild Chinook Smolt	1+	3/31/2012	Back	43	5	0.14	0.38	7.1
Wild Chinook Smolt	1+	4/3/2007	Back	46	1	0.04	0.21	18.6
Wild Chinook Smolt	1+	4/19/2012	Back	48	7	0.17	0.42	12.3
Wild Chinook Smolt	1+	4/10/2007	Back	53	4	0.09	0.31	27.4
Wild Chinook Smolt	1+	4/21/2009	Back	53	0	0.02	0.14	20.7
Wild Chinook Smolt	1+	4/13/2012	Back	53	4	0.09	0.31	10.1
Wild Chinook Smolt	1+	4/16/2012	Back	53	7	0.15	0.40	12.5
Wild Chinook Smolt	1+	4/24/2008	Back	57	8	0.16	0.41	5.9

Wild Chinook Smolt	1+	4/23/2012	Back	58	1	0.03	0.19	39.1
Wild Chinook Smolt	1+	4/24/2006	Back	59	3	0.07	0.26	10.4
Wild Chinook Smolt	1+	3/23/2007	Back	59	7	0.14	0.38	24.8
Wild Chinook Smolt	1+	3/17/2007	Back	64	7	0.13	0.36	26.5
Wild Chinook Smolt	1+	4/18/2010	Back	67	2	0.05	0.21	9.3
Wild Chinook Smolt	1+	4/17/2008	Back	72	13	0.19	0.46	7.8
Wild Chinook Smolt	1+	4/3/2006	Back	81	10	0.14	0.38	5.3
Wild Chinook Smolt	1+	3/20/2007	Back	91	13	0.15	0.40	34.8
Wild Chinook Smolt	1+	5/1/2008	Back	102	16	0.17	0.42	8.9
Wild Chinook Smolt	1+	4/28/2008	Back	127	19	0.16	0.41	7.7
Wild Chinook Smolt	1+	4/14/2008	Back	195	40	0.21	0.48	9.3
Wild Chinook Smolt	1+	3/9/2014	Back	65	4	0.08	0.28	27.1
Wild Chinook Smolt	1+	3/13/2014	Back	67	9	0.15	0.40	16.0

Model: Spring Chinook 2010-2014 Non-Trapping Period Array (NAL) – Full Antenna Function, ($r^2 = 0.61$; $p = 0.0002$)

Origin/Species/Stage	Age	Date	Mark	Detections	Trap Efficiency (R+1) / M	ASIN Transform	Discharge (m^3/s)
Wild Chinook Parr	0	11/4/2010	254	95	0.38	0.66	6.3
Wild Chinook Parr	0	11/7/2010	287	70	0.25	0.52	7.0
Wild Chinook Parr	0	11/10/2010	168	74	0.45	0.73	4.8
Wild Chinook Parr	0	11/13/2010	74	41	0.57	0.85	4.0
Wild Chinook Parr	0	11/18/2010	185	22	0.12	0.36	7.9
Wild Chinook Parr	0	11/3/2012	201	21	0.11	0.34	10.9
Wild Chinook Parr	0	11/7/2012	233	31	0.14	0.38	10.7
Wild Chinook Parr	0	11/11/2012	328	66	0.20	0.47	6.3
Wild Chinook Parr	0	11/15/2012	195	68	0.35	0.64	6.2
Wild Chinook Parr	0	11/4/2013	130	51	0.40	0.68	3.7
Wild Chinook Parr	0	11/8/2013	106	39	0.38	0.66	4.2
Wild Chinook Parr	0	3/9/2014	65	4	0.08	0.28	24.9
Wild Chinook Parr	0	3/13/2014	67	5	0.09	0.30	15.3
Wild Chinook Parr	0	11/4/2014	114	5	0.05	0.23	10.5
Wild Chinook Parr	0	11/1/2014	96	5	0.06	0.25	16.5
Wild Chinook Parr	0	11/10/2014	78	8	0.12	0.35	11.3

Model: Spring Chinook 2010-2014 Non-Trapping Period Array (NAL) – Partial Antenna Function, ($r^2 = 0.38$; $p = 0.007$)

Origin/Species/Stage	Age	Date	Mark	Detections	Discharge

					Trap Efficiency (R+1)/M	ASIN Transform	
Wild Chinook Parr	0	11/4/2010	254	39	0.16	0.41	6.3
Wild Chinook Parr	0	11/7/2010	287	16	0.06	0.25	7.0
Wild Chinook Parr	0	11/10/2010	168	34	0.21	0.47	4.8
Wild Chinook Parr	0	11/13/2010	74	17	0.24	0.52	4.0
Wild Chinook Parr	0	11/18/2010	185	8	0.05	0.22	7.9
Wild Chinook Parr	0	11/3/2012	201	7	0.04	0.20	10.9
Wild Chinook Parr	0	11/7/2012	233	8	0.04	0.20	10.7
Wild Chinook Parr	0	11/11/2012	328	24	0.08	0.28	6.3
Wild Chinook Parr	0	11/15/2012	195	30	0.16	0.41	6.2
Wild Chinook Parr	0	11/4/2013	130	40	0.32	0.60	3.7
Wild Chinook Parr	0	11/8/2013	106	30	0.29	0.57	4.2
Wild Chinook Parr	0	3/9/2014	65	1	0.03	0.18	24.9
Wild Chinook Parr	0	3/13/2014	67	5	0.09	0.30	15.3
Wild Chinook Parr	0	11/1/2014	96	1	0.02	0.15	10.5
Wild Chinook Parr	0	11/4/2014	114	4	0.04	0.21	16.5
Wild Chinook Parr	0	11/10/2014	78	3	0.05	0.23	11.3

APPENDIX D. Historical Morphometric Data

Spring Chinook (2004-2016)

Trap Year	Brood Year	Origin/Species/Stage	Fork Length (mm)			Weight (g)			K-factor
			Mean	n	SD	Mean	n	SD	
2004	2002	Wild Chinook Yearling Smolt	93.4	336	12.4	9	337	5	1.1
2004	2003	Wild Chinook Subyearling Fry	39.5	82	5.1	0.6	79	0.3	1
2004	2003	Wild Chinook Subyearling Parr	82.4	792	7.9	6.1	702	2.7	1.1
2005	2003	Wild Chinook Yearling Smolt	93.6	278	7.9	8.7	276	2.1	1.1
2005	2004	Wild Chinook Subyearling Fry	42.1	107	5.6	0.7	102	0.4	0.9
2005	2004	Wild Chinook Subyearling Parr	75.9	924	9.6	4.9	890	3.8	1.1
2006	2004	Wild Chinook Yearling Smolt	91.2	363	7.1	7.5	362	1.8	1
2006	2005	Wild Chinook Subyearling Fry	—	—	—	—	—	—	—
2006	2005	Wild Chinook Subyearling Parr	72.9	1,428	9.6	3.9	1,428	2.3	1
2007	2005	Wild Chinook Yearling Smolt	89	676	8.2	8	675	6.1	1.1
2007	2006	Wild Chinook Subyearling Fry	39	24	3.7	0.6	24	0.5	1
2007	2006	Wild Chinook Subyearling Parr	79.5	686	13.8	6.1	685	2.6	1.2
2008	2006	Wild Chinook Yearling Smolt	96.1	904	6.6	9.5	904	2.1	1.1
2008	2007	Wild Chinook Subyearling Fry	42.8	127	4.6	0.8	127	0.4	1
2008	2007	Wild Chinook Subyearling Parr	75.8	2,049	12.5	5.2	2,049	2.4	1.2
2009	2007	Wild Chinook Yearling Smolt	94.4	198	8.9	9.2	198	2.5	1.1
2009	2008	Wild Chinook Subyearling Fry	44.8	82	4.8	0.9	82	0.6	1
2009	2008	Wild Chinook Subyearling Parr	70.1	2,333	12	4.2	2,333	2	1.2
2010	2008	Wild Chinook Yearling Smolt	96.9	366	7.3	10.2	366	2.3	1.1
2010	2009	Wild Chinook Subyearling Fry	41.8	30	5	1.3	8	0.2	1.8
2010	2009	Wild Chinook Subyearling Parr	80.7	3,021	10.7	6.2	3,021	2.3	1.2
2011	2009	Wild Chinook Yearling Smolt	89.1	152	9.9	7.7	152	1.8	1.1
2011	2010	Wild Chinook Subyearling Fry	39.8	217	6.6	0.6	217	0.5	1
2011	2010	Wild Chinook Subyearling Parr	73.4	1,046	13.1	4.9	1,046	2.5	1.2
2012	2010	Wild Chinook Yearling Smolt	93.3	368	7	9.2	368	2.2	1.1
2012	2011	Wild Chinook Subyearling Fry	42.7	48	9.1	0.9	48	0.6	1.2
2012	2011	Wild Chinook Subyearling Parr	77.9	2,160	10.7	5.3	2,160	1.9	1.1
2013	2011	Wild Chinook Yearling Smolt	90.6	239	75	7.9	239	2.1	1.1
2013	2012	Wild Chinook Subyearling Fry	45.6	1,824	6.8	1	1,803	0.6	1.1
2013	2012	Wild Chinook Subyearling Parr	70	4,422	11.4	3.8	4,409	1.7	1.1
2014	2012	Wild Chinook Yearling Smolt	89.5	464	6.9	7.5	464	1.8	1
2014	2013	Wild Chinook Subyearling Fry	40.1	677	5.2	0.9	221	0.5	1.4
2014	2013	Wild Chinook Subyearling Parr	69.1	1,549	12.3	3.8	1,547	2.3	1.2
2015	2013	Wild Chinook Yearling Smolt	93	152	7	8.4	152	2.2	1
2015	2014	Wild Chinook Subyearling Fry	45	338	9.9	1	338	0.9	0.9

2015	2014	Wild Chinook Subyearling Parr	84	210	8	6.5	209	1.7	1.1
2015	2013	Hatchery Chinook Yearling Smolt	136	284	12.3	29.5	284	8.8	1.1
2016	2014	Wild Chinook Yearling Smolt	96	61	5.5	9.0	61	1.7	1.01
2016	2015	Wild Chinook Subyearling Fry	38	285	3.0	0.5	285	0.2	0.78
2016	2015	Wild Chinook Subyearling Parr	85	491	12.7	6.9	490	2.5	1.07
2016	2014	Hatchery Chinook Yearling Smolt	119	87	13.5	19.6	87	7.6	1.09

Summer Steelhead (2004-2016)

Trap Year	Brood Year	Age	Origin/Species	Fork Length (mm)			Weight (g)			K-factor
				Mean	n	SD	Mean	n	SD	
2004	2004	0	Wild Summer Steelhead	67	358	10	3.5	279	1.5	1.2
2004	2003	1	Wild Summer Steelhead	101.7	394	23.2	13.2	366	27.3	1.3
2004	2002	2	Wild Summer Steelhead	161.6	146	19.8	43.4	141	15.5	1
2004	2001	3	Wild Summer Steelhead	201.6	43	11.2	76	43	21.2	0.9
2004	2003	1	Hat. Summer Steelhead	182.8	523	22.4	62.1	497	21.2	1
2005	2005	0	Wild Summer Steelhead	54.1	649	15.7	2.2	616	3.2	1.4
2005	2004	1	Wild Summer Steelhead	93.6	585	25.6	10.8	575	10.1	1.3
2005	2003	2	Wild Summer Steelhead	153.5	103	21.2	38.1	102	16.4	1.1
2005	2002	3	Wild Summer Steelhead	144	1	—	43.2	1	—	1.4
2005	2004	1	Hat. Summer Steelhead	188.2	343	21.2	66	343	24	1
2006	2006	0	Wild Summer Steelhead	66.3	180	5.8	2.5	180	1	0.9
2006	2005	1	Wild Summer Steelhead	85.2	877	18.7	6.7	877	6.6	1.1
2006	2004	2	Wild Summer Steelhead	155.9	106	26.8	36.1	105	13.5	1
2006	2003	3	Wild Summer Steelhead	197	2	—	73.5	2	—	1
2006	2005	1	Hat. Summer Steelhead	—	—	—	—	—	—	—
2007	2007	0	Wild Summer Steelhead	54.2	329	11.7	2	328	1.4	1.3
2007	2006	1	Wild Summer Steelhead	82.7	1,330	16.8	7.2	1,329	6.3	1.3
2007	2005	2	Wild Summer Steelhead	143.8	102	20.6	31.4	102	11.9	1.1
2007	2004	3	Wild Summer Steelhead	143	1	—	26.8	1	—	0.9
2007	2006	1	Hat. Summer Steelhead	149.3	3	47	33.1	3	29.1	1
2008	2008	0	Wild Summer Steelhead	52.9	930	11.1	1.7	930	1.2	1.1
2008	2007	1	Wild Summer Steelhead	84.5	1,876	17.1	7.4	1,874	6.6	1.2
2008	2006	2	Wild Summer Steelhead	149.9	122	22.9	36	122	15.5	1.1
2008	2005	3	Wild Summer Steelhead	180.3	13	18.9	57.4	13	16.4	1
2008	2007	1	Hat. Summer Steelhead	179.4	389	16.5	55.9	388	14.8	1
2009	2009	0	Wild Summer Steelhead	55.6	843	10.5	2.2	688	1.1	1.3
2009	2008	1	Wild Summer Steelhead	82.6	452	18.6	7.1	447	5.5	1.3
2009	2007	2	Wild Summer Steelhead	156.9	72	22	40.9	72	15.5	1.1

2009	2006	3	Wild Summer Steelhead	195	3	5	73	3	6.7	1
2009	2008	1	Hat. Summer Steelhead	183.1	280	16.7	60.8	280	18.2	1
2010	2010	0	Wild Summer Steelhead	55	1,287	11.1	2.5	917	1.3	1.5
2010	2009	1	Wild Summer Steelhead	89.8	1,079	19.1	9	1,072	7.1	1.2
2010	2008	2	Wild Summer Steelhead	144.9	87	25.1	35	87	17.4	1.2
2010	2007	3	Wild Summer Steelhead	184	8	12.2	61.9	8	10.2	1
2010	2009	1	Hat. Summer Steelhead	183.5	531	19.5	61.3	526	19.6	1
2011	2011	0	Wild Summer Steelhead	43.5	1,093	10.1	1.1	783	0.9	1.3
2011	2010	1	Wild Summer Steelhead	75.7	818	18.5	5.5	811	5.7	1.3
2011	2009	2	Wild Summer Steelhead	144.8	27	41.3	42.1	27	62.1	1.4
2011	2008	3	Wild Summer Steelhead	—	—	—	—	—	—	—
2011	2010	1	Hat. Summer Steelhead	180.7	464	17	59.1	464	17.6	1
2012	2012	0	Wild Summer Steelhead	55.1	589	14.2	2.6	402	1.2	1.6
2012	2011	1	Wild Summer Steelhead	84.7	747	17.4	7.6	741	5.7	1.3
2012	2010	2	Wild Summer Steelhead	127.1	132	27	23.7	132	14.5	1.2
2012	2009	3	Wild Summer Steelhead	161	4	32	40.5	4	15.6	1
2012	2011	1	Hat. Summer Steelhead	154.8	318	20.9	37.7	318	14	1
2013	2013	0	Wild Summer Steelhead	56.1	878	11.3	2.1	777	1.1	1.2
2013	2012	1	Wild Summer Steelhead	44.5	1,777	14.7	5.4	1,772	4.2	1.2
2013	2011	2	Wild Summer Steelhead	144.7	21	15.7	36.1	21	10.2	1
2013	2010	3	Wild Summer Steelhead	—	—	—	—	—	—	—
2013	2012	1	Hat. Summer Steelhead	166.2	365	21.4	49.2	363	18.2	1.1
2014	2014	0	Wild Summer Steelhead	49.6	490	12.8	1.7	389	1.1	1.4
2014	2013	1	Wild Summer Steelhead	82.2	745	13.6	6.3	745	3.5	1.1
2014	2012	2	Wild Summer Steelhead	145.1	30	16.5	33	30	13.4	1.1
2014	2011	3	Wild Summer Steelhead	—	—	—	—	—	—	—
2014	2013	1	Hat. Summer Steelhead	173.4	632	18.7	52.6	633	15.9	1
2015	2015	0	Wild Summer Steelhead	70	182	15.5	4.3	176	2	1.1
2015	2014	1	Wild Summer Steelhead	88	233	20.2	8.3	233	6.7	1
2015	2013	2	Wild Summer Steelhead	149	14	13.5	33.7	14	8.2	1
2015	2012	3	Wild Summer Steelhead	191	1	—	73.8	1	—	1.1
2015	2014	1	Hat. Summer Steelhead	175	273	15.2	51.3	273	12.5	0.9
2016	2016	0	Wild Summer Steelhead	56	674	16.4	2.4	617	1.8	1.0
2016	2015	1	Wild Summer Steelhead	87	278	21.5	8.3	278	5.9	1.1
2016	2014	2	Wild Summer Steelhead	143	19	17.4	31.1	19	9.6	1.0
2016	2013	3	Wild Summer Steelhead	202	1	—	90.1	1	—	1.1
2016	2015	1	Hat. Summer Steelhead	175	95	15.5	55.1	95	16.2	1.0

Coho (2007-2016)

Trap Year	Brood Year	Origin/Species/Stage	Fork Length (mm)			Weight (g)			K-factor
			Mean	n	SD	Mean	n	SD	
2004	2002	Nat. Or. Coho Yearling Smolt	—	—	—	—	—	—	—
2004	2003	Nat. Or. Coho Subyearling Fry	—	—	—	—	—	—	—
2004	2003	Nat. Or. Coho Subyearling Parr	—	—	—	—	—	—	—
2004	2002	Hatchery Coho Yearling Smolt	136.6	847	12.8	27.4	820	7.5	1.1
2005	2003	Nat. Or. Coho Yearling Smolt	114.4	17	8.8	16.2	17	3.6	1.1
2005	2004	Nat. Or. Coho Subyearling Fry	49.1	9	10.4	1.3	9	0.8	1.1
2005	2004	Nat. Or. Coho Subyearling Parr	76.7	9	12.8	4.9	9	2.7	1.1
2005	2003	Hatchery Coho Yearling Smolt	137.3	689	11.3	28.6	690	7.2	1.1
2006	2004	Nat. Or. Coho Yearling Smolt	—	—	—	—	—	—	—
2006	2005	Nat. Or. Coho Subyearling Fry	—	—	—	—	—	—	—
2006	2005	Nat. Or. Coho Subyearling Parr	71	4	13.6	3.8	4	2.9	1.1
2006	2004	Hatchery Coho Yearling Smolt	—	—	—	—	—	—	—
2007	2005	Nat. Or. Coho Yearling Smolt	92.9	36	12.5	8.7	36	4	1.1
2007	2006	Nat. Or. Coho Subyearling Fry	—	—	—	—	—	—	—
2007	2006	Nat. Or. Coho Subyearling Parr	83	1	—	6.2	1	—	1.1
2007	2005	Hatchery Coho Yearling Smolt	116	2	—	16.8	2	—	1.1
2008	2006	Nat. Or. Coho Yearling Smolt	—	—	—	—	—	—	—
2008	2007	Nat. Or. Coho Subyearling Fry	—	—	—	—	—	—	—
2008	2007	Nat. Or. Coho Subyearling Parr	87	1	—	6.4	1	—	1
2008	2006	Hatchery Coho Yearling Smolt	130.2	843	10.4	23.6	843	6.2	1.1
2009	2007	Nat. Or. Coho Yearling Smolt	103	4	9.7	11.7	4	3.4	1.1
2009	2008	Nat. Or. Coho Subyearling Fry	—	—	—	—	—	—	—
2009	2008	Nat. Or. Coho Subyearling Parr	79.6	5	20.1	6.6	5	4.8	1.3
2009	2007	Hatchery Coho Yearling Smolt	135.3	625	8.9	26.2	579	5.2	1.1
2010	2008	Nat. Or. Coho Yearling Smolt	—	—	—	—	—	—	—
2010	2009	Nat. Or. Coho Subyearling Fry	48	2	—	1.3	2	—	1.2
2010	2009	Nat. Or. Coho Subyearling Parr	83.6	27	8.6	6.7	27	2.4	1.1
2010	2008	Hatchery Coho Yearling Smolt	130	1,051	10.1	23.8	1,049	5.3	1.1
2011	2009	Nat. Or. Coho Yearling Smolt	100.2	14	12.7	11.3	14	3.9	1.1
2011	2010	Nat. Or. Coho Subyearling Fry	—	—	—	—	—	—	—
2011	2010	Nat. Or. Coho Subyearling Parr	64.7	3	10.8	3	3	1.5	1.1
2011	2009	Hatchery Coho Yearling Smolt	124.6	969	8.6	21	969	4.8	1.1
2012	2010	Nat. Or. Coho Yearling Smolt	102.1	17	9.1	11.9	17	3	1.1
2012	2011	Nat. Or. Coho Subyearling Fry	36	1	—	—	—	—	—
2012	2011	Nat. Or. Coho Subyearling Parr	78.4	84	9.3	5	84	2.1	1
2012	2010	Hatchery Coho Yearling Smolt	126.2	1,684	7.6	21.5	1,684	5.5	1.1
2013	2011	Nat. Or. Coho Yearling Smolt	97	81	10	10	81	3.1	1.1
2013	2012	Nat. Or. Coho Subyearling Fry	47.3	3	1	1	3	1	0.9
2013	2012	Nat. Or. Coho Subyearling Parr	87.8	4	3.8	6.6	4	1	1

2013	2011	Hatchery Coho Yearling Smolt	130.1	982	8.5	23.3	977	4.9	1.1
2014	2012	Nat. Or. Coho Yearling Smolt	96.3	20	9.8	9.9	20	3	1.1
2014	2013	Nat. Or. Coho Subyearling Fry	36	1	—	—	—	—	—
2014	2013	Nat. Or. Coho Subyearling Parr	73	3	22.5	5.9	3	4.7	1.5
2014	2012	Hatchery Coho Yearling Smolt	127	1,203	9.7	21.7	1,207	5.0	1.1
2015	2013	Nat. Or. Coho Yearling Smolt	109	2	4.9	12.0	2	0.1	0.9
2015	2014	Nat. Or. Coho Subyearling Fry	47	7	13.7	1.4	7	1.5	0.9
2015	2014	Nat. Or. Coho Subyearling Parr	69	3	7	4.0	3	1.3	1.2
2015	2013	Hatchery Coho Yearling Smolt	131	952	9.9	23.3	952	4.8	1.0
2016	2014	Nat. Or. Coho Yearling Smolt	100	6	15.8	11.1	6	5.5	1.0
2016	2015	Nat. Or. Coho Subyearling Fry	—	—	—	—	—	—	—
2016	2015	Nat. Or. Coho Subyearling Parr	—	—	—	—	—	—	—
2016	2014	Hatchery Coho Yearling Smolt	134	302	8.4	24.8	301	5.0	1.0

Appendix M

Fish Trapping at the White River Smolt Trap during 2016

Population Estimates for Juvenile Spring Chinook Salmon in White River, WA

2016 Annual Report

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ABSTRACT

In 2007, Yakama Nation Fisheries Resource Management began monitoring emigration of Endangered Species Act (ESA) listed Upper Columbia River (UCR) spring Chinook salmon in the White River to provide abundance and freshwater survival estimates. This report summarizes data collected between March 1 and November 30, 2016. We used a 1.5 m rotary screw trap to collect 200 juvenile spring Chinook; 50 fry, 147 subyearling parr, and 3 yearling smolts. Daily counts at the trap were expanded via regression analysis derived from mark and recapture trials. We estimated that 386 (± 701; 95% CI) BY2014 wild spring Chinook smolts and 2,430 (± 723; 95% CI) BY2015 wild spring Chinook parr emigrated past the White River trap in 2016. Combined with data collected in 2015, this gives us a total estimate of 2,336 (± 807; 95% CI) BY2014 emigrants. Using spring Chinook spawning ground data collected by Washington Department of Fish and Wildlife (WDFW) in 2014, we estimated egg-to-emigrant survival of BY2014 spring Chinook to be 2.2% (90 smolts-per-redd).

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ACKNOWLEDGEMENTS

This project is part of a basin-wide monitoring program requiring close coordination between multiple agencies and contractors. We greatly appreciate the hard work of the Yakama Nation FRM crew members including Matthew Clubb, Jamie Hallman, Tim Jeffris and Kevin Swager who maintained and operated the trap during all hours including nights/weekends and inclement weather conditions. Also thank you to Peter Graf (Grant County PUD) for administering contracting and funding as well as Mike Hughes, Mclain Johnson, Andrew Murdoch, and Josh Williams (WDFW) for data sharing and collaboration on smolt trap methodologies.

1.0 INTRODUCTION

White River spring Chinook salmon (tkwínat) *Oncorhynchus tshawytscha* are part of the Upper Columbia River (UCR) spring Chinook salmon Evolutionarily Significant Unit (ESU), which was listed as endangered under the Endangered Species Act (ESA) in 1999. Due to critically low abundance, a captive broodstock program was operated in the White River between 1997 and 2015 as a risk aversion measure. Determining freshwater productivity of spring Chinook salmon in the White River is an essential component to overall population monitoring, and will help contribute to the body of knowledge needed to evaluate if further supplementation in the White River is warranted.

In the fall of 2005, Washington State Department of Fish and Wildlife (WDFW) began smolt trapping in the lower White River in order to provide an estimate of juvenile spring Chinook salmon production. No trapping was conducted in 2006 as there was a transition between trap operators. In 2007, Public Utility District No. 2 of Grant County (GCPUD) contracted with Yakama Nation Fisheries (YNF) to operate a rotary trap in the White River. This document reports data collected between March 1 and November 30, 2016, and provides emigration estimates for spring Chinook salmon yearlings (BY2014) and subyearlings (BY2015) during that time period. Fish trap operations were conducted in compliance with ESA consultation specifically to address abundance and productivity of spring Chinook salmon in the White River.

Within this document, we will report:

1) Juvenile abundance and productivity of spring Chinook salmon in the White River.

2) Emigration timing of spring Chinook salmon emigrating from the White River.

1.1 Watershed Description

The White River drainage encompasses 40,451 ha originating in alpine glaciers and perennial snow fields (Figure 1; USFS 2004). Elevation within the drainage varies from 569 m at the surface of Lake Wenatchee to 2,614 m at Clark Mountain (Andonaegui 2001). As one of two primary tributaries to Lake Wenatchee, the White River flows in a south-easterly direction for 42.9 rkm before emptying into the lake. Precipitation ranges from 79 cm at the mouth to more than 356 cm in the head waters (Andonaegui 2001). Due to its glacial origins, peak runoff for the White River typically occurs between April and July with occasional high

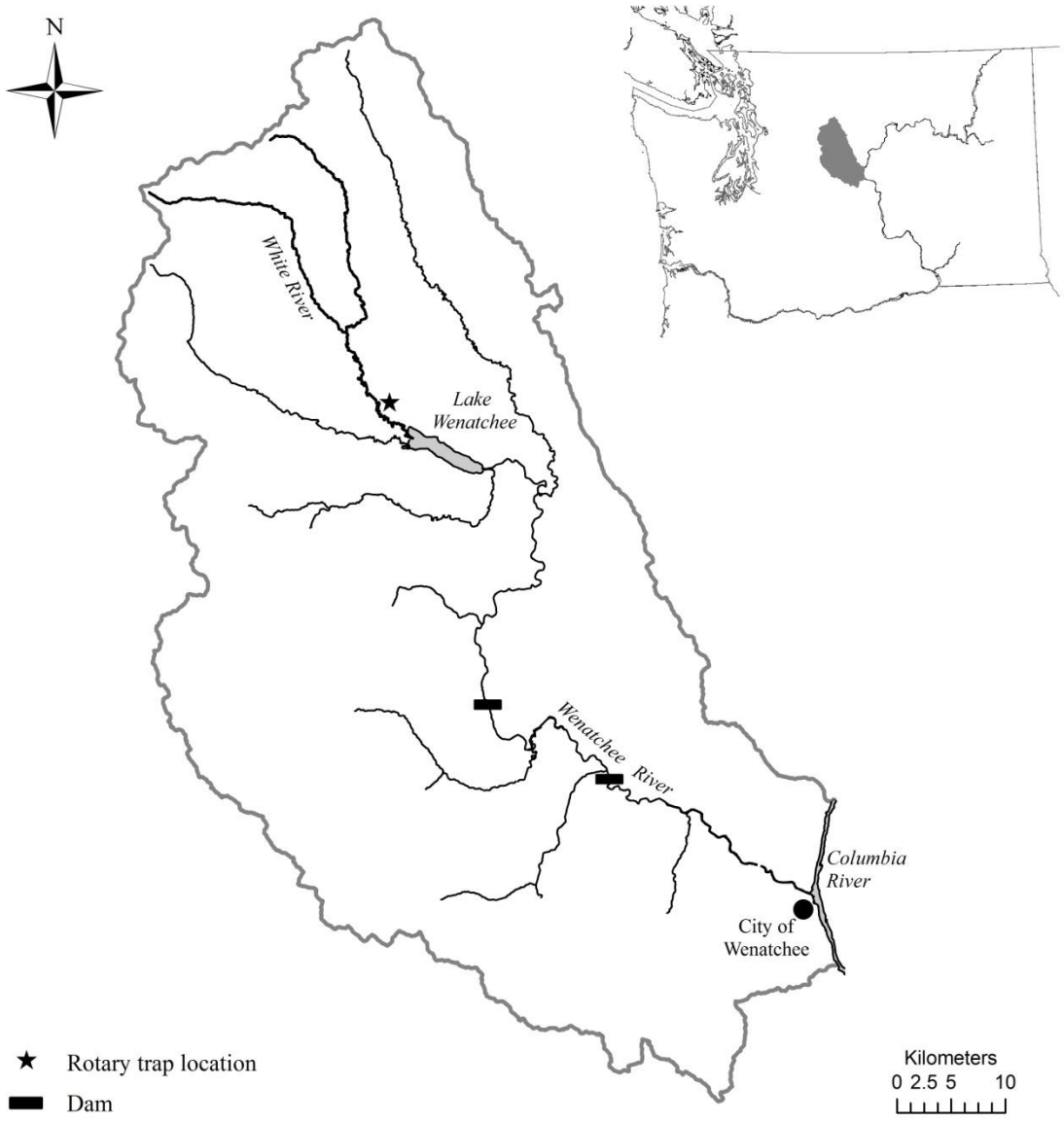


Figure 1. Map of the Wenatchee River subbasin with White River rotary trap location.

flows caused by rain-on-snow events in the fall and winter months. Water temperatures in this watershed tend to be cooler than other tributaries to the upper Wenatchee River subbasin. As of September 2002, Washington State Department of Ecology (WDOE) began operating a stream monitoring station at rkm 9.9. Operation of this station by WDOE is currently maintained with funding provided by GCPUD. In 2016, daily mean stream discharge ranged from 2.5 m^3/s (87 cfs) to 120 m^3/s (4,420 cfs) while mean daily stream temperatures ranged from 0.0°C to 14.6°C (Figs. 2 & 3). Discharge and temperature data provided by WDOE should be considered provisional and are presented in **Appendix A**.

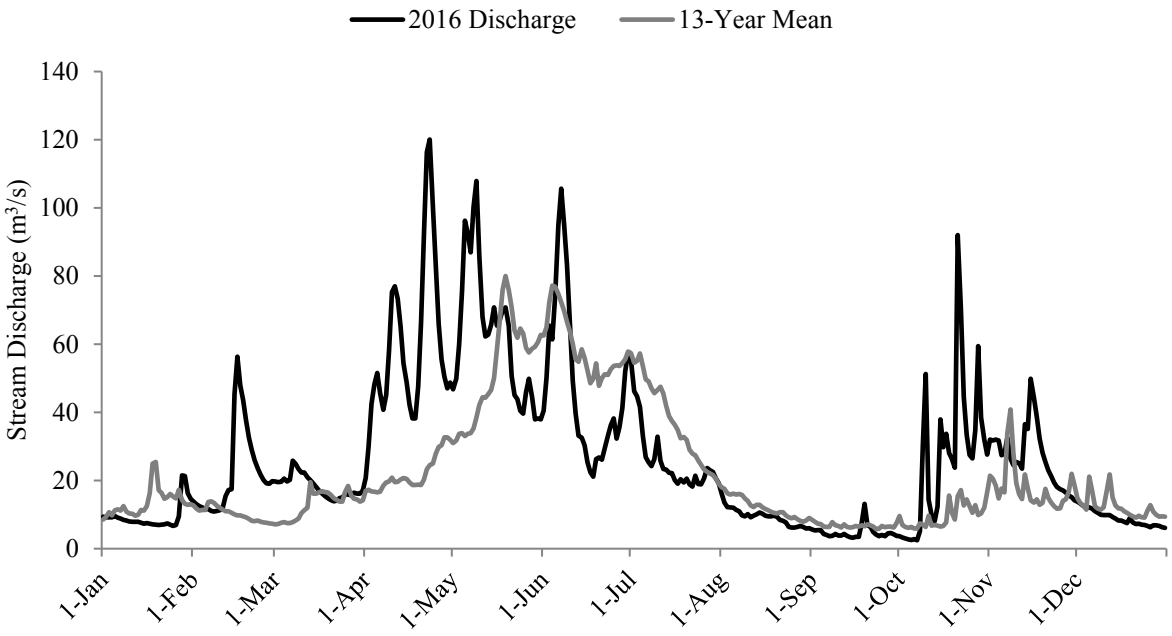


Figure 2. Mean daily stream discharge at the White River DOE stream monitoring station at Sears Creek Bridge, 2016.

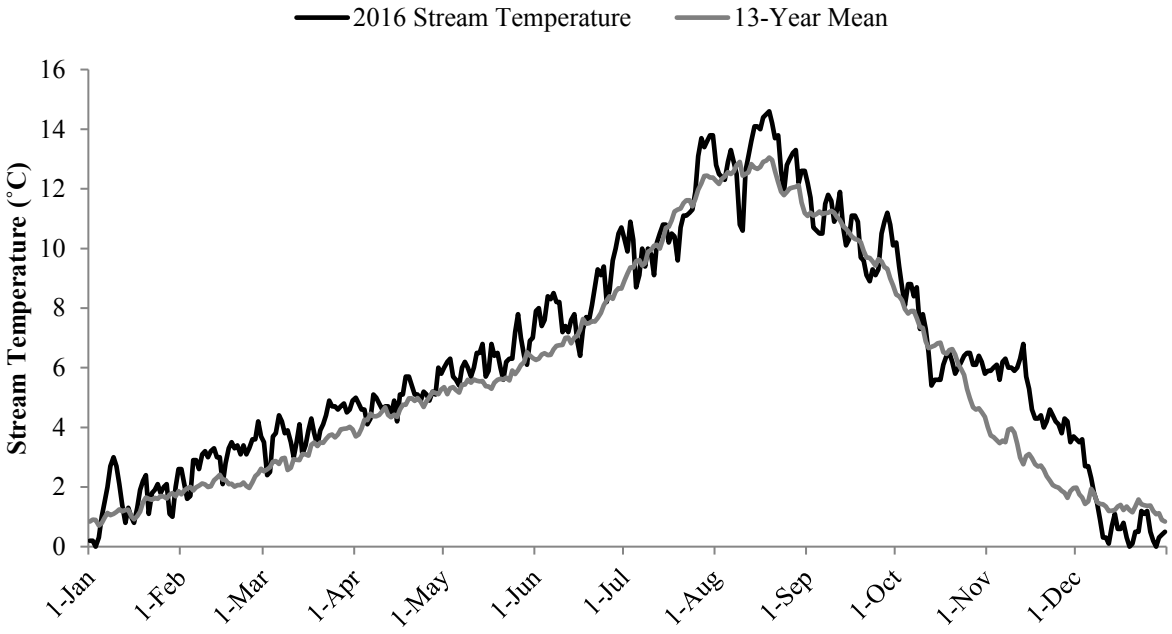


Figure 3. Mean daily water temperatures at the White River DOE stream monitoring station at Sears Creek Bridge, 2016.

The White River drainage has had minimal riparian harvest from the 1950's to the present on federally owned land. Turn of the century settlement and land clearing have impacted the

riparian reserve network up to the Napeequa confluence, yet, riparian areas in the mainstem below Panther Creek remain in fair condition (USFS 2004). In the remainder of the watershed, woody debris recruitment, shade, aquatic habitat connectivity, and riparian vegetation appear to be in good condition. Current habitat concerns pertaining to the development of homes and vacation retreats on private lands do exist. Rip-rapping, channel constriction, and stream degradation are considered minor in the watershed. Public ownership comprises 78% of the drainage area; more than half of public land is located within the Glacier Peak Wilderness. The remaining 22% of the drainage is in private ownership (USFS 2004).

Downstream of White River Falls are key spawning grounds for spring Chinook salmon (tkwínat) *Oncorhynchus tshawytscha*, sockeye salmon (kálux) *O. nerka*, and bull trout *Salvelinus confluentus*. Two large tributaries to the White River, Napeequa River and Panther Creek, are also known to support populations of anadromous salmonids (Mullen et al. 1992). For a complete list of known fish species encountered in the White River see (**3.4 Incidental Species**).

2.0 METHODS

2.1 Trapping Equipment and Operation

In 2016, a 1.5m diameter cone rotary trap was operated in a single position at all discharge levels. This revised trapping regime was implemented in 2013 to simplify data analysis by eliminating obsolete trap positions that generated very little data. Past attempts at developing a high flow position generated very few efficiency trials resulting in limited trap efficiency data. Operating season-long at a single position, the trap was suspended from a river-spanning cable from which its position could be adjusted perpendicular to stream flow by hand powered winches anchored on a tree on the river-right bank.

The trap was operated 24 hours per day, seven days per week for the majority of the season. During spring snowmelt, operations only occurred during hours of darkness to minimize trap damage and subsequent capture mortality; still enabling sampling during the hours of peak fish movement. When trap operations were suspended, the cone was raised to avoid damage by debris.

During all ranges of river discharge, fish were removed daily. Additional trap checks were necessary during periods of high discharge in the spring, and in the autumn due to increased leaf litter. Debris in the live-box was removed continually by a rotating drum screen located at the rear of the holding box and hydraulically powered by the cone. A record of daily trap operations is provided in **Appendix B**.

2.2 Biological Sampling

Trap operating procedures and techniques followed a standardized, basin-wide monitoring plan developed by the Upper Columbia Regional Technical Team (UCRTT) for the Upper Columbia Salmon Recovery Board (UCSRB; Hillman 2004), which was adapted from Murdoch & Petersen (2000).

Captured fish were transferred from the rotary trap's live box using covered five-gallon plastic buckets to a stream-side portable sampling station. Fish were anesthetized in a solution of tricaine methanesulfonate (MS-222) to facilitate sampling and reduce handling stress. Fork length (FL) and weight were recorded for all fish, except large numbers of sockeye fry. For these fish, a daily subsample of 25 individuals was measured while the remaining fish were enumerated and released. Weight was measured to the nearest 0.1g with a portable digital scale while FL was recorded to the nearest 1.0 mm using a trough-type measuring board. These data were used to calculate a Fulton-type condition factor (K-factor) for each target species using the formula:

$$K = (W/L^3) \times 100{,}000$$

where K = Fulton-type condition metric;
W = weight in grams;
L = fork length in millimeters;
And 100,000 is a scaling constant.

Portable aerators were used to oxygenate holding water during sampling. All fish were allowed to fully recover from anesthesia before being released. Spring Chinook salmon were classified as either natural or hatchery origin by the presence/absence of coded wire tags (CWT's). Developmental stages (fry, parr, transitional or smolt) were visually identified and assigned to each individual sampled. Transitional juveniles were identified as having both parr and smolt characteristics; visible parr marks, semi-transparent fin coloration along with silvery coloration throughout body. Smolts were identified by a strong silvery coloration over entire body and faint or absent parr marks. Fry were defined as newly emerged fish with or without a visible yolk sac and a FL measuring < 50 mm. Age-0 spring Chinook salmon captured before July 1 were considered 'fry' and excluded from population estimates due to the inconclusive nature of their movement (i.e. active emigration or local distribution in-stream). Age-0 spring Chinook salmon captured after 1 July were considered subyearling emigrants and included in the population estimate (UCRTT, 2001).

Tissue samples (caudal clip) were taken from spring Chinook salmon and applied to blotter sheets. Samples were provided to WDFW for reproductive success analysis. Scale samples were also collected from all steelhead captured. Scale samples were submitted to WDFW for age analysis. Bull trout tissue or scale samples were not collected in 2016.

During periods when the trap operations were suspended (e.g. - high discharge, high debris and/or mechanical problems), passage estimates were generated to account for emigrants during these time periods. This estimate was calculated using the average number of fish captured three days prior and three days after the break in operation (Hillman et al., 2013; Snow et al., 2013).

2.3 Mark-Recapture Trials

Groups of marked spring Chinook salmon were used for trap efficiency trials. Fish were marked by insertion of a passive integrated transponder (PIT) tag into the abdominal cavity. Ideally, marked groups of fish would be released over a broad range of stream discharges in order to determine a trap efficiency-discharge relationship. (See **2.4 Data Analysis**). However, due to low abundance and limited holding time of ESA-listed species (reducing the ability to meet trials size requirements on a more consistent basis), marked groups were released whenever the minimum sample size (≥ 20) was obtained. Mark-recapture (M-R) trials followed the protocol described in Hillman (2004). Although the protocol suggests a minimum sample size of 100 fish for each mark-group, the limited abundance of juvenile emigrants from the White River required that efficiency trials be completed with much smaller sample sizes. YN's continued goal is to increase individual mark-group sizes, when possible, to meet the standard described above.

Number of wild fish included in a marked group was maximized by combining catches from three days of trapping. Fish were held up to 72 hours prior to release in holding boxes located on the river-left bank. Fish to be used in efficiency trials were then transported in five gallon buckets ~1.0 rkm upstream to the release location at Sears Creek Bridge (rkm 10.3). All mark groups are released by hand at nautical twilight.

Each M-R trial was conducted over a three-day (72 hour) period to allow time for passage or capture. Completed trials were only considered invalid if an interruption to trapping occurred or proper pre-release procedures were not followed. Trials resulting in zero recaptures were included in the efficiency regression as allowed by the new method of observed trap efficiency calculation (See equation 3 in **2.5.1 Estimate of Abundance**).

2.3.1 Marking and PIT tagging

All spring Chinook and summer steelhead juveniles with FL ≥ 60mm were PIT tagged unless the health of a specimen was in question. Once anesthetized, each fish was examined for external wounds or descaling and scanned for the presence of a previously implanted PIT tag. If a tag was not detected, a pre-loaded 12mm Digital Angel 134.2 kHz type TX 1411ST PIT tag was inserted into the body cavity using a Biomark MK-25 Rapid Implant Gun. Each unique tag code was electronically recorded with an appropriate tagging date, release date, tagging personnel and biological data. These data were entered into P_3 and submitted to the PIT Tag Information System (PTAGIS) at the end of each month. Tagging methods were consistent with methodology described in the PIT Tag Marking Procedures Manual (CBFWA 1999) as well as with 2008 ISEMP protocols (Tussing 2008).

After marking and/or PIT tagging, fish were held for a minimum of 24-hours to a) ensure complete recovery, b) assess tagging mortality and c) determine tag-shed rate. Fish that were not to be used in an efficiency trial were released downstream of the smolt trap.

2.4 Data Analysis

2.4.1 Estimate of Abundance

Seasonal juvenile migration, N, was estimated as the sum of daily migrations, N_i, i.e., $N = \sum_i N_i$, and daily migration was calculated from catch and efficiency:

$$\hat{N}_i = \frac{C_i}{\hat{e}_i}, \qquad \textbf{(1)}$$

where C_i = number of fish caught in period *I*;

$\hat{e}_i$ = trap efficiency estimated from the flow-efficiency relationship, $\sin^2(b_0 + b_1 flow_i)$,

where b_0 is estimated intercept and b_1 is the estimated slope of the regression.

The regression parameters b_0 and b_1 are estimated using linear regression for the model:

$$\arcsin\left(\sqrt{e_k^{obs}}\right) = \beta_0 + \beta_1 flow_k + \varepsilon, \quad \textbf{(2)}$$

where e_k^{obs} = observed trap efficiency of Eq. 2 for trapping period k;

β_0 = intercept of the regression model;

β_1 = slope parameter;

ε = error with mean 0 and variance σ^2.

In Equation 2, the observed trap efficiency, e_k^{obs}, is calculated as follows,

$$e_k^{obs} = \frac{r_k + 1}{m}. \quad \textbf{(3)}$$

The estimated variance of seasonal migration is calculated from daily estimates as:

$$Var\left(\sum_{i=1}^{n} \hat{N}_i\right) = \underbrace{\sum_i Var\left(N_i\right)}_{Part\ A} + \underbrace{\sum_i \sum_j Cov\left(N_i, N_j\right)}_{Part\ B},$$

or,

$$Var\left(\sum_{i=1}^{n} \hat{N}_i\right) = \underbrace{\sum_i Var\left(\frac{\left(C_i + 1\right)}{\hat{e}_i}\right)}_{Part\ A} + \underbrace{\sum_i \sum_j Cov\left(\frac{\left(C_i + 1\right)}{\hat{e}_i}, \frac{\left(C_j + 1\right)}{\hat{e}_j}\right)}_{Part\ B}. \quad \textbf{(4)}$$

Part A of equation 4 is the variance of daily estimates. Part B is the between-day covariance. Note that the between-day covariance exists only for days that use the same trap efficiency model. If, for example, day 1 is estimated with one trap efficiency model, and day 2 estimated from a different model, then there is no covariance between day 1 and day 2. The full expression for the estimated variance:

$$\hat{Var}\left(\sum_{i=1}^{n}\hat{N}_i\right)=\underbrace{\sum_i \hat{N}_i^2\left(\frac{N_i\hat{e}_i(1-\hat{e}_i)}{(C_i+1)^2}+\frac{4(1-\hat{e}_i)}{\hat{e}_i}\hat{Var}(b_0+b_1 flow_i)\right)}_{PartA}+$$

$$\underbrace{\sum_i\sum_j 4\left(\hat{N}_i(1-\hat{e}_i)\right)\left(\hat{N}_j(1-\hat{e}_j)\right)\cdot\left[\hat{Var}(b_0)+flow_i\,flow_j\hat{Var}(b_1)\right]}_{PartB}$$

where $\hat{Var}(b_0+b_1 flow_i)=\hat{MSE}\left(1+\frac{1}{n}+\frac{(flow_i-\overline{flow})^2}{(n-1)s_{flow}^2}\right)$, and $\hat{Var}(b_0)$ and $\hat{Var}(b_1)$ are

obtained from regression results. In Excel, the standard error (SE) of the coefficients is provided. The variance is calculated as the square of the standard error, SE^2.

In cases when there was no significant flow-efficiency relationship (i.e., low correlation), then a pooled, or average trap efficiency will suffice for the stratum. The estimator is calculated as follows:

$$\hat{\bar{e}}=\frac{\sum_{j=1}^{k} r_j}{\sum_{j=1}^{k} m_j}$$

where $\hat{\bar{e}}$ = the average or pooled trap efficiency for the stratum;

m_j = the number of smolts marked and released in efficiency trial j for the stratum;

r_j = the number of smolts recaptured out of m_j marked fish in efficiency trial j.

Abundance for a trapping period is estimated as:

$$\hat{N}_i^{pooled}=\frac{C_i}{\hat{\bar{e}}},$$

,and total stratum abundance is:

$$N^{pooled}=\sum_i \hat{N}_i^{pooled}.$$

The variance of seasonal abundance takes into account the variability in catch numbers that are a result of binomial sampling (Part A), the pooled variance of trap efficiency, $\hat{\bar{e}}$ (Part B), and the covariance in daily estimates that arises from using a common estimate of efficiency across all trapping days (Part C):

$$\hat{Var}\left(\sum_{i=1}^{n}\hat{N}_i^{pooled}\right)=\underbrace{\left(\sum_i\frac{\hat{N}_i\left(1-\hat{\bar{e}}\right)}{\hat{\bar{e}}}\right)}_{PartA}+\underbrace{\frac{Var\left(\hat{\bar{e}}\right)}{\hat{\bar{e}}^2}\sum_i\hat{N}_i^2}_{PartB}+\underbrace{\frac{Var\left(\hat{\bar{e}}\right)}{\hat{\bar{e}}^2}\sum_i\sum_j\hat{N}_i\hat{N}_j}_{PartC}.$$

The Part B and Part C terms are combined in the calculation as a new Part B:

$$\hat{Var}\left(\sum_{i=1}^{n}\hat{N}_i^{pooled}\right)=\underbrace{\left(\sum_i\frac{\hat{N}_i\left(1-\hat{\bar{e}}\right)}{\hat{\bar{e}}}\right)}_{PartA}+\underbrace{\frac{Var\left(\hat{\bar{e}}\right)}{\hat{\bar{e}}^2}\left[\sum_i\hat{N}_i^2+\sum_i\sum_j\hat{N}_i\hat{N}_j\right]}_{PartB}.$$

The variance of $\hat{\bar{e}}$ is calculated as:

$$\hat{Var}\left(\hat{\bar{e}}\right)=\hat{Var}\left(\frac{\sum_{k=1}^{n}r_k}{\sum_{k=1}^{n}m_k}\right)=\frac{\sum_{k=1}^{n}\left(r_k-\hat{\bar{e}}_k m_k\right)^2}{\bar{m}^2 n(n-1)}$$

where $\bar{m}$ is the average release size across all efficiency trial, $\frac{\sum_{k=1}^{n}m_k}{n}$.

Confidence intervals were calculated using the following formulas:

$$95\%\text{ confidence interval} = 1.96\times\sqrt{\sum\text{var}\left[\hat{N}_i\right]}$$

The single M-R estimator of abundance carries a set of well documented assumptions (Everhart and Youngs 1981; Seber 1982),

1. The population is closed to mortality.
2. The probability of capturing a marked or unmarked fish is equal.
3. Marked fish were randomly dispersed in the population prior to recapture.

4. Marking does not affect probabilities of capture.
5. Marks were not lost between the time of release and recapture.
6. All marks are reported upon recapture.
7. The number of fish in the trap, C, is fully enumerated and known without error.

3.0 RESULTS

3.1 Dates of Operation

In 2016, YNF operated a 1.5m rotary trap between March 1 and November 30. During this period, the trap operated 24 hours per day, 7 days per week barring inoperable environmental conditions (i.e. heavy debris loads or high discharge). Trapping was interrupted a total of 29 days (Table 1).

Table 1. Summary of White River smolt trap operation, 2016.

Trap Status	Description	Days
Operating	Continuous data collection	246
Interrupted	Unexpected interruption by debris, etc.	29
Pulled	Intentionally pulled to protect the trap during high flows	0

3.2 Daily Captures and Biological Sampling

3.2.1 Wild Spring Chinook Yearlings (BY2014)

Three wild yearling Chinook smolts were collected between March 1 and June 30 (Figure 4). Mean fork-length (FL) was 106 mm (n = 3; SD = 1.5) and mean weight was 12.4 g (n = 3; SD = 0.3; Table 2). All spring Chinook smolts were implanted with PIT tags and sampled for genetics. There were no BY2014 spring Chinook mortalities incurred (See **3.4 ESA Compliance**).

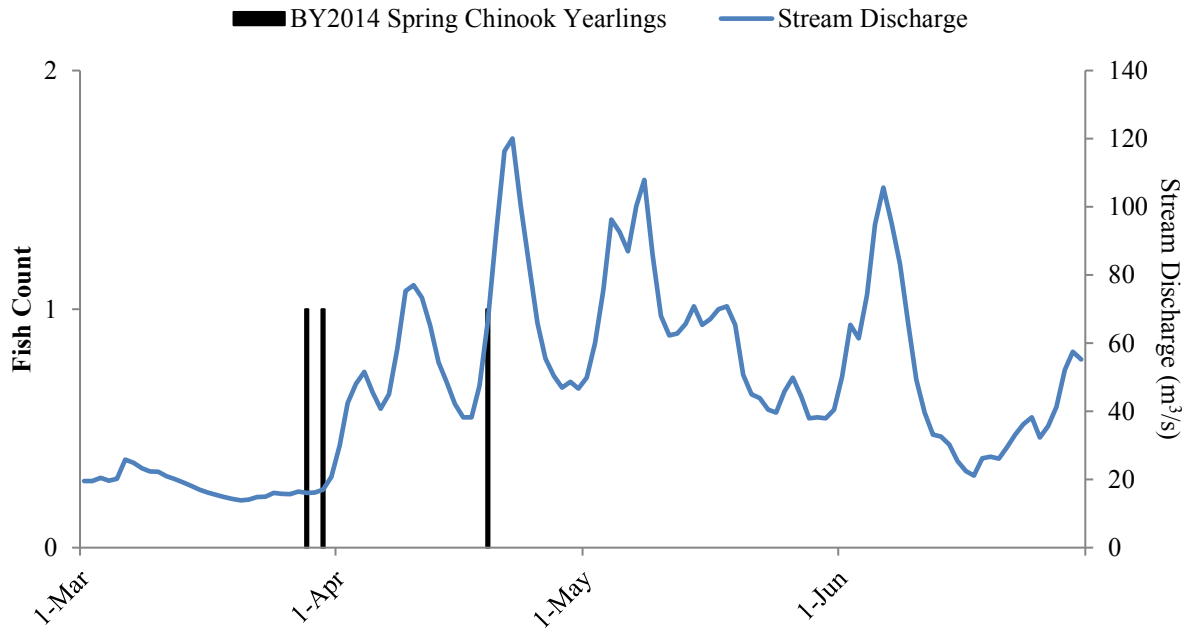


Figure 4. Daily catch of yearling spring Chinook smolt with mean daily stream discharge at the White River rotary trap, March 1 to June 30, 2016.

3.2.2 Wild Spring Chinook Subyearlings (BY2015)

Spring Chinook fry were captured at the trap between March 7 and June 22 (n = 49). During this period there were no fry trapping mortalities incurred. One additional subyearling Chinook with FL<50 mm was captured after June 30. Because this fish is considered a "fry" it was excluded from the parr estimate. A total of 147 wild subyearling Chinook parr were collected between May 25 and November 30, with peak catch occurring on August 25 (n = 14; Figure 5). The mean FL for subyearling parr was 89 mm (n = 147; SD = 10.7) and the mean weight was 8.3 g (n = 147; SD = 2.8); see Table 2. Four of the spring Chinook parr were captured prior to July 1. Because these were therefore considered "fry" they were excluded from the parr estimate. PIT tags were implanted into a total of 137 subyearling Chinook parr. One tag was shed during the 24hr holding period (Table 4). Genetic samples were taken from 137 parr. There were two BY2015 spring Chinook mortalities during the 2016 trapping season (See **3.4 ESA Compliance**).

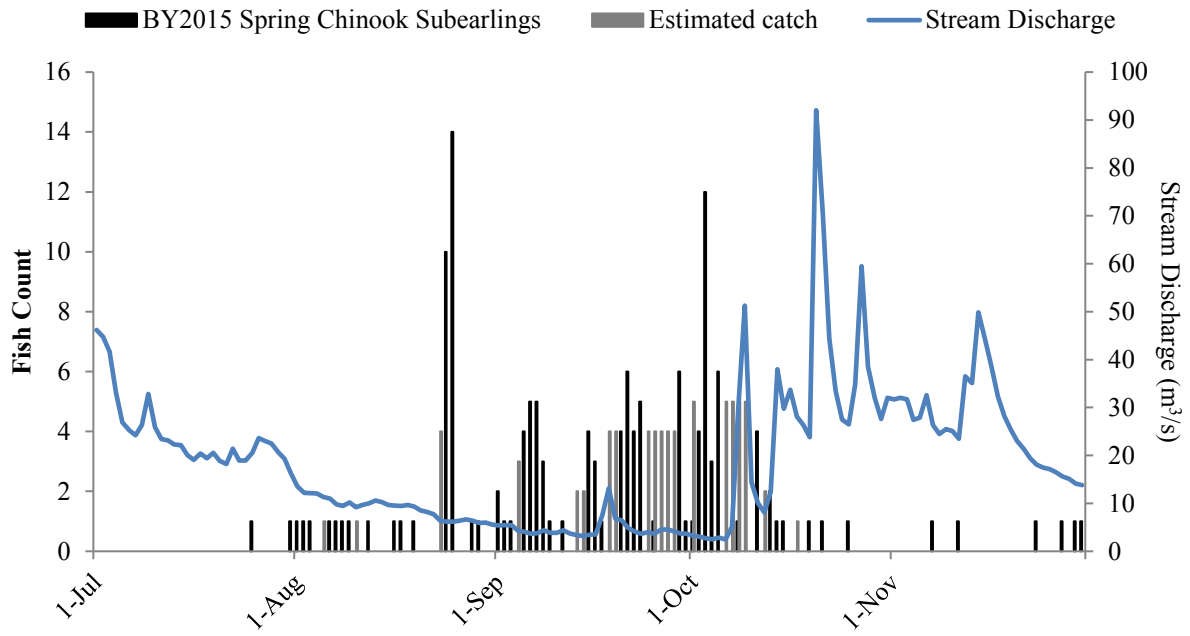


Figure 5. Daily catch of wild subyearling spring Chinook with mean daily stream discharge at the White River rotary trap, July 1 to November 30, 2016.

Table 2. Summary of length and weight sampling of juvenile spring Chinook captured at the White River rotary trap, 2016.

Brood Year	Origin/Species/Stage	Fork Length (mm)			Weight (g)			K-factor
		Mean	n	SD	Mean	n	SD	
2014	Wild Yearling Smolt	106	3	1.5	12.4	3	0.3	1.05
2015	Wild Subyearling Fry	38	50	3.0	0.5	49	0.3	0.82
2015	Wild Subyearling Parr	89	147	10.7	8.3	147	2.8	1.13

3.3 Trap Efficiency Calibration and Population Estimates

3.3.1 Wild Spring Chinook Yearlings (BY 2014)

Due to low abundance, no BY2014 wild yearling Chinook efficiency trials were performed in 2016. A composite regression model using previous year's (2008-2012) efficiency trials showed statistically significant ($r^2 = 0.57$; $p = 0.001$) flow-efficiency relationship, and was used to calculate yearling abundance. Use of a single spring trapping position allowed this regression to be applied to all yearling Chinook captured in 2016. Weighting of this regression via an R script (provided by WDFW) did not affect calculation parameters greatly and yielded the same r-square and *p*-values. In the fall of 2015, we estimated that 1,950 (± 400; 95% CI) BY2014 subyearlings emigrated past the trap. In the spring of 2016, we estimated that 386 (± 701; 95% CI) emigrated past the trap. Combining the two estimates, total BY2014 wild spring Chinook emigrants was 2,336 (± 807; 95% CI; Table 3).

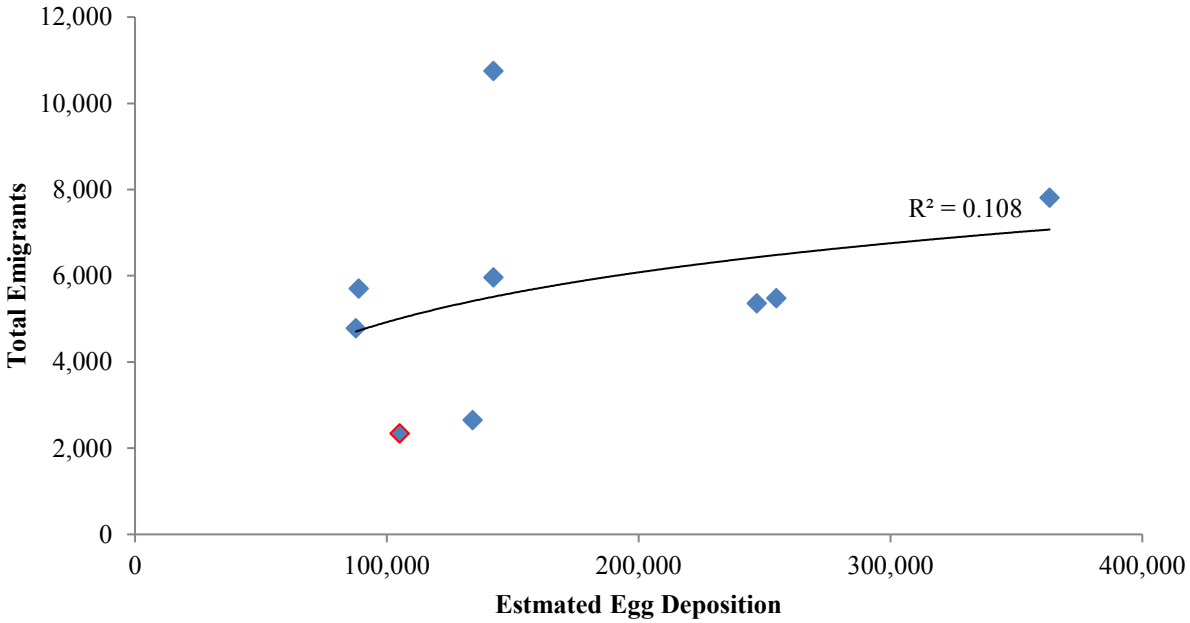
3.3.2 Wild Spring Chinook Subyearling (BY 2015)

Due to low abundance, no BY2015 wild yearling Chinook efficiency trials were performed in 2016. Instead, a composite regression based on previous year's data (2009-2015) was used to expand daily catch. This regression was comprised of all trails conducted fulfilling the minimum number marked ($n \geq 20$) including efforts in which zero recaptured were made (Appendix C). Mark-groups in which validity of the trial could be called into question (suspected trap stoppage or improper pre-release handling of the mark group) were removed. The weighted regression was not significant ($r^2 = 0.12$; $p = 0.086$) at our accepted limit ($\alpha = 0.05$). However, after comparison with a pooled method and considerations of the pooled estimate limitations, we decided to use the regression model despite its slightly higher *p*-value. This single regression was the only model required to estimate total subyearling migration due to the fact only one fall trapping position was used in 2015. We estimated that in 2016, 2,430 (± 723; 95% CI) spring Chinook subyearling parr moved past the trap (Table 3).

Table 3. Estimated egg-to-emigrant survival and emigrants per redd for White River spring Chinook

Brood Year	No. of Redds[a]	Fecundity[b]	No. of Eggs	No. of Emigrants			Egg-to Emigrant	Emigrants per Redd
				Age-0[c]	Age-1	Total ± 95% CI		
2005	86	4,327	372,122	DNOT[d]	4,856	—		—
2006	31	4,324	134,044	652	2,004	2,656 ± 1,597	2.0%	86
2007	20	4,441	88,820	2,309	3,395	5,704 ± 2,201	6.4%	285
2008	31	4,592	142,352	5,560	5,193	10,753 ± 3,783	7.6%	347
2009	54	4,573	246,942	2,428	2,939	5,367 ± 2,497	2.2%	99
2010	33	4,314	142,362	1,859	4,103	5,962 ± 3,448	4.2%	181
2011	20	4,385	87,700	3,128	1,659	4,787 ± 2,022	5.5%	239
2012	86	4,223	363,178	3,816	3,995	7,811 ± 3,847	2.2%	91
2013	54	4,716	254,664	2,461	3,023	5,484 ± 2,836	2.2%	102
2014	26	4,045	105,170	1,950	386	2,336 ± 807	2.2%	90
2015	70	4,847	339,290	2,430	—	—	—	—
Avg	39	4,401	173,915	2,685	2,966	5,651	3.8%	169

[a] Number of complete redds in White River (Hillman et al. 2015)
[b] Mean annual fecundity of spring Chinook broodstock at Chiwawa River Hatchery
[c] Estimate is based on capture of parr collected during summer/fall and does not include fry captured prior to July1
[d] Did not operate trap; no production estimates were made



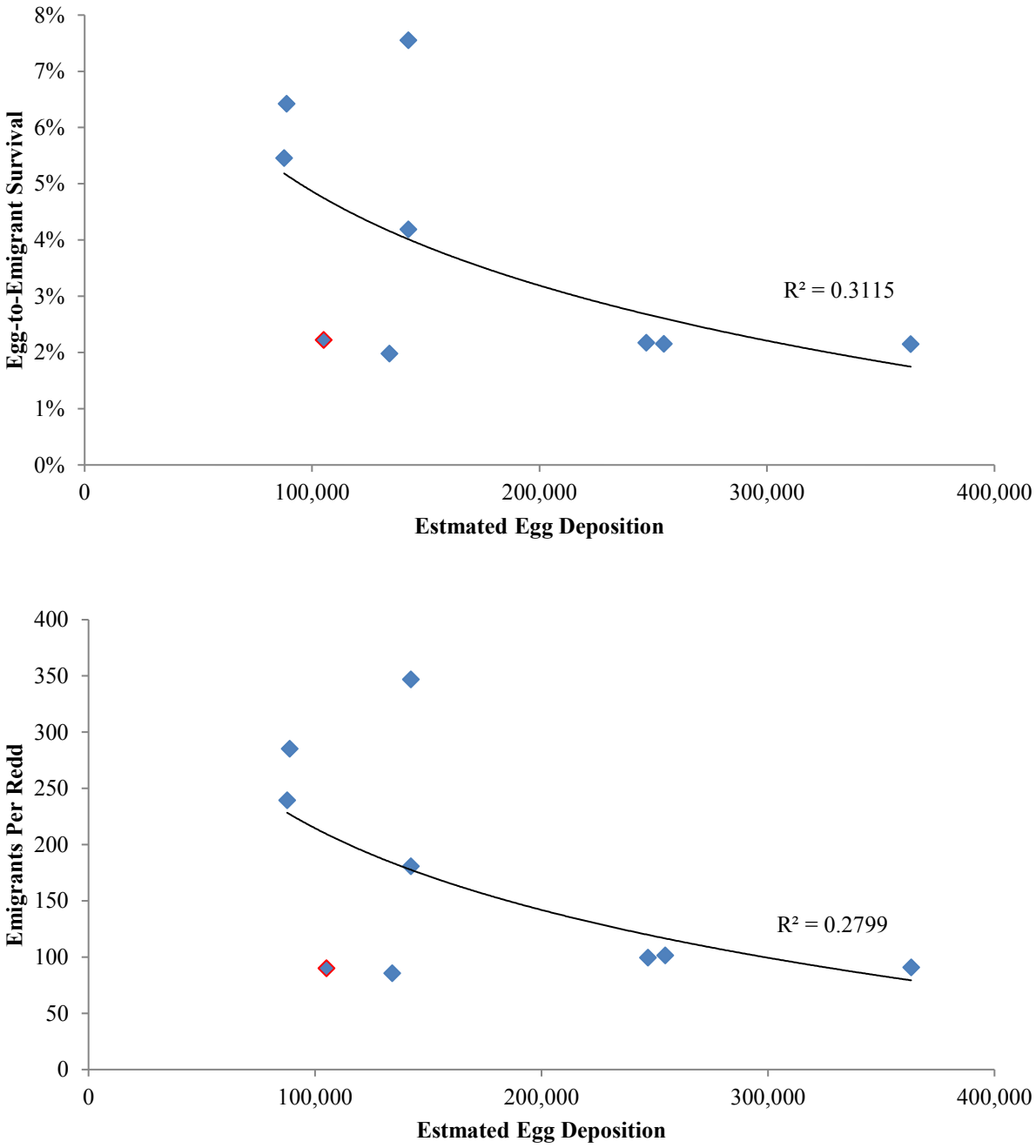


Figure 6. Relationships between estimated egg deposition and total emigrants produced, egg-to-emigrant survival, and emigrants per redd for White River spring Chinook, BY 2007 to 2014. *BY2014 values denoted by red border.

3.4 PIT Tagging

In 2016, a total of 140 spring Chinook and 5 steelhead were PIT tagged at the trap. PIT tag retention after 24 hours of observation yielded only one shed tag (wild spring Chinook parr; Table 4). There no tagging mortalities (Table 6).

Table 4. Number of PIT tagged spring Chinook and steelhead with shed rates at the White River rotary trap, 2016.

Brood Year	Species/Stage	Total Catch	Total PIT Tagged	Percent Tagged	Percent Tags Shed
2014	Yearling Chinook Smolt	3	3	100.0%	0.0%
2015	Subyearling Chinook Parr	147	137	93.2%	0.7%
*	Steelhead Parr	5	5	100.0%	0.0%

* Brood year unknown

3.5 Incidental Species

Incidental species were enumerated and sampled for length and weight (Table 5). Incidental species included: bull trout, longnose dace *Rhinichthys cataractae*, mountain whitefish *Prosopium williamsoni*, northern pikeminnow *Ptychocheilus oregonensis*, steelhead/rainbow trout (shúshaynsh) *Oncorhynchus mykiss*, redside shiner *Richardsonius balteatus*, sculpin *Cottus sp.*, sockeye salmon, sucker *Catostomus sp.*, and westslope cutthroat *Oncorhynchus clarkii lewisi*.

Table 5. Summary of length and weight sampling of incidental species captured at the White River rotary trap, 2016.

Species	Total Count	Fork Length (mm)			Weight (g)		
		Mean	*n*	SD	Mean	*n*	SD
Bull Trout Parr	5	341	5	220.5	98.9	3	89.5
Longnose Dace	4	73	4	24.5	5.9	4	4.7
Mountain Whitefish	93	64	93	29.7	6.2	83	19.6
Northern Pikeminnow	5	211	5	142.8	51.7	4	77.6
Rainbow Trout/Steelhead Parr	5	10	5	23.1	5.6	0	158.8
Redside Shiner	25	67	25	13.8	5.5	25	5.0
Sculpin	60	61	60	16.5	3.1	57	2.4
Sockeye Fry	1,784	27	864	1.1	—	—	—
Sockeye Parr	1	68	1	—	3.1	1	—
Sucker	20	213	20	76.9	159.0	20	109.3
Westslope Cutthroat	6	229	6	75.2	90.3	5	46.8

3.6 ESA Compliance

ESA-listed species mortalities incurred in 2016 included two subyearling Chinook parr (Table 6). At no point during the trapping season did the lethal take of wild spring Chinook exceed the maximum allowed 2%. All fish handled were inspected prior to tagging or further sampling with any sign of injury or stress warranting immediate release.

Table 6. Summary of White River ESA listed species catch and mortality, 2016.

Species/Stage	Total Catch	Total Mortality	Total % Mortality
Yearling Chinook Smolt	3	0	0.0%
Subyearling Chinook Parr	147	2	1.4%
Subyearling Chinook Fry	50	0	0.0%
Total Wild Spring Chinook	**200**	**2**	**1.0%**
Bull Trout	5	0	0.0%
Steelhead/Rainbow Trout	5	0	0.0%

4.0 DISCUSSION

Previously, below-average spring Chinook spawner escapements at the White River have resulted in elevated egg-to-emigrant survival estimates for their respective juveniles produced. Conversely, above-average spawner escapements have trended toward comparatively lowered rates of in-stream survival. Although replication at the highest escapement levels is limited, the trend thus far suggests that density-dependent constraints are influencing in-stream survival in the White River spring Chinook population. An estimated egg deposition in 2014 that fell well-below the White River average failed to produce the expected response of an elevated egg-to-emigrant survival. Instead, the BY2014 egg-to-emigrant survival rate of 2.2% showed no change over the two preceding broods, which had markedly higher estimated egg depositions. Potential explanations of this unexpected result are twofold: 1) the survival estimated is in fact a reflection of decreased survival, and contrary to the density-dependent trend previously noted, and/or 2) catch at the trap during the BY2014 migration did not effectively capture a representative sample of the outmigration. The likelihoods of both of these influences were exacerbated by the strong El Niño occurring during the majority of BY2014's in-stream rearing period (NOAA 2016).

Oceanic Niño Index (ONI) values were particularly high in 2015 and 2016, with levels not experienced since strong El Niño events in 1982/1983 and 1997/1998 (NOAA 2016). Inland manifestations of this oceanic phenomenon at the White River included high fall and winter discharges (Figure 7). High, irregular flows were likely to have produced some degree of increased mortality prior to gravel emergence as a result of redd scouring and sedimentation (Montgomery et al. 1996 & Lotspeich and Everest 1981). Flood events in November 2014 and 2015 were both great (>170 m^3/s [6,000cfs]), and included significant movement of bedload, suspended sediments, and large woody debris (LWD). Though difficult to quantify the impact of this flooding on incubating eggs, a strong negative correlation between egg-to-emigrant survival and peak flow during incubation has been shown in other tributaries (Seiler et al. 2002). Also, low snowpack and early snowmelt brought on by mild winter temperatures caused prolonged periods of summer base flows in 2015 and 2016. Though stream temperatures did not reach levels in which mass die-off was incurred (Max = 17.6ºC), prolonged low stream levels presumably resulted in a higher than average competition for critical resources.

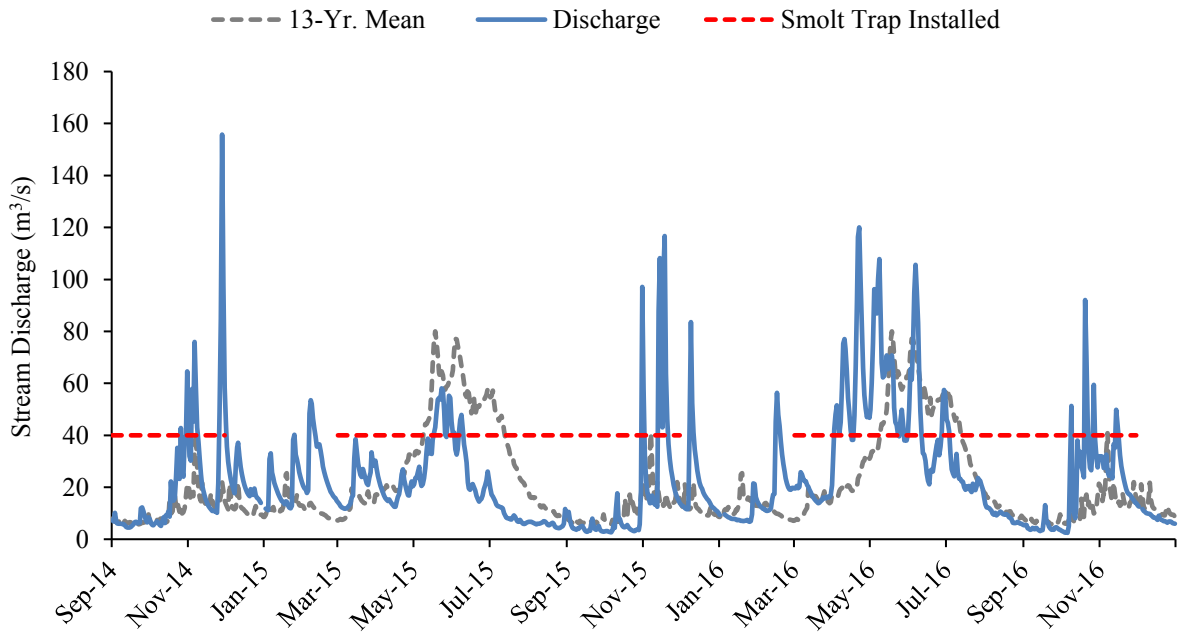


Figure 7. White River daily mean and 13-year mean discharge during strong El Niño, 2014-2016.

In addition to direct decreases to survival, we suspect that irregular weather patterns attributed to El Niño resulted in a potentially large portion of the BY2014 juvenile population being prematurely displaced during periods of low trap efficiency (high discharge), and/or early outmigration during the non-trapping period (December through February). While some displacement below the trap may be a simple function of pre-migratory fish being unable to maintain positioning during high-water events, Chinook populations elsewhere have displayed early migratory behavior in years with early snowmelt and warm water temperatures (Quinn 2005 & Achord et al. 2007). Early outmigration has also been associated with elevated growth, with larger fish tending to emigrate earlier (Achord et al. 2007). BY2014 subyearling parr had the highest average FL of any brood recorded. Given fulfillment of both conditions (warm water temperature and rapid-growth), BY2014 yearlings may have actively emigrated from the White River earlier than in previous years with typical temperature and flow regimes. If the bulk of movement was initiated prior to the start of trapping (March 1), spring operations may have captured a smaller than average proportion of the total outmigration i.e., only the tail-end of the downstream movement.

A comparison of egg-to-emigrant survival rates in the White River, Chiwawa River, and Nason Creek shows that BY2014 survivals deviated markedly from each other in comparison to the preceding two broods (Figure 8). We suspect that this may be explained in-part by differing felt effects of El Niño on each tributary, and capability of each trap to measure outmigration in light of high flows and early migratory behavior. Stronger influence of El Niño on a tributary would therefore cause a lowered estimated survival rate via the aforementioned effects on both survival and smolt trap efficacy. All three tributaries saw smaller spawner escapements in 2014. Based on previous data, all should have in-turn responded with elevated rates of egg-to-emigrant survival. We suspect that although the Chiwawa River did experience some adverse

environmental effects, influence of El Niño on the Chiwawa BY2014 emigrant estimate was the least affected of the three tributaries. Nason Creek showed potentially the greatest negative response to El Niño, with a decrease in survival. The smallest of the three tributaries, Nason Creek is listed as impaired due to water temperatures exceeding 303(d) criteria (Cristea and Pelletier 2005). Survival in Nason Creek may have been impacted by the prolonged, extremely warm temperatures to a higher degree than the Whiter River and Chiwawa River; two tributaries with much cooler summer water temperatures. Like Nason Creek, the White River failed to show an increase in survival in-light of a smaller adult return. However, given the assumption that a potentially significant proportion of the run was missed producing an underestimate of abundance, we assume that BY2014 survival did in fact increase over the previous brood, as did the Chiwawa River population.

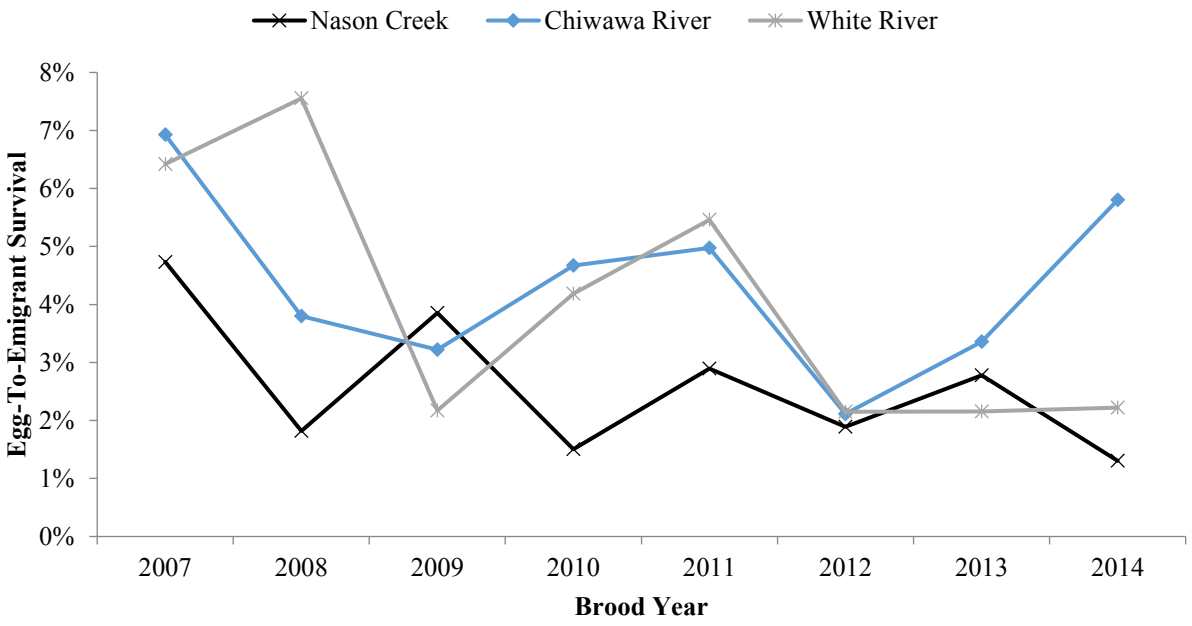


Figure 8. Comparison of wild spring Chinook abundance estimates (BY2007-2014) made at the White R., Nason Cr., and Chiwawa R. smolt traps. Chiwawa R. data provided by Hillman et al. (2015).

The 2015 White River spring Chinook brood in-stream rearing period also coincided partially with the El Niño event. The initial subyearling estimate is below the nine-year mean despite high estimated egg deposition; potentially the result of decreased survival and/or shifts in movement to low-efficiency or suspended periods of trapping. Completion of the migratory period in the spring of 2017 will help to determine the cumulative effect of the anomalous weather trends on the brood estimate. Given a change to cooler conditions associated with non-El Niño periods, we anticipate that the majority of BY2015 smolt emigration will occur after the smolt trap has been installed.

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APPENDIX A: White River Temperature and Discharge Data

Date	Stream Discharge (m^3/s)	Water Temperature (°C)
3/1/2016	20	2.4
3/2/2016	20	2.5
3/3/2016	21	3.7
3/4/2016	20	3.8
3/5/2016	20	4.4
3/6/2016	26	4.2
3/7/2016	25	3.8
3/8/2016	23	3.9
3/9/2016	22	3.5
3/10/2016	22	3.0
3/11/2016	21	3.5
3/12/2016	20	4.1
3/13/2016	19	3.1
3/14/2016	18	3.4
3/15/2016	17	3.9
3/16/2016	16	4.3
3/17/2016	16	3.8
3/18/2016	15	3.4
3/19/2016	14	3.9
3/20/2016	14	4.1
3/21/2016	14	4.4
3/22/2016	15	4.9
3/23/2016	15	4.7
3/24/2016	16	4.7
3/25/2016	16	4.6
3/26/2016	16	4.7
3/27/2016	16	4.8
3/28/2016	16	4.5
3/29/2016	16	4.6
3/30/2016	17	4.9
3/31/2016	21	5.0
4/1/2016	30	4.8
4/2/2016	42	4.6
4/3/2016	48	4.6
4/4/2016	52	4.1
4/5/2016	46	4.3
4/6/2016	41	5.1
4/7/2016	45	5.0
4/8/2016	58	4.8
4/9/2016	75	4.6
4/10/2016	77	4.7
4/11/2016	73	4.7
4/12/2016	65	4.4
4/13/2016	54	4.9
4/14/2016	49	4.2
4/15/2016	42	5.1
4/16/2016	38	5.1
4/17/2016	38	5.7
4/18/2016	48	5.7
4/19/2016	67	5.4
4/20/2016	92	5.1
4/21/2016	116	5.1
4/22/2016	120	4.9
4/23/2016	100	5.2
4/24/2016	83	5.1
4/25/2016	66	4.9
4/26/2016	56	5.2
4/27/2016	50	5.1
4/28/2016	47	6.0
4/29/2016	49	5.8
4/30/2016	47	6.0
5/1/2016	50	6.2
5/2/2016	60	6.3
5/3/2016	76	5.7
5/4/2016	96	5.6
5/5/2016	93	5.4
5/6/2016	87	6.0
5/7/2016	100	6.2
5/8/2016	108	6.0
5/9/2016	86	5.7
5/10/2016	68	6.0
5/11/2016	62	6.5
5/12/2016	63	6.5
5/13/2016	66	6.8
5/14/2016	71	5.7

5/15/2016	65	5.9	6/29/2016	57	10.7
5/16/2016	67	6.8	6/30/2016	55	10.3
5/17/2016	70	6.4	7/1/2016	46	9.9
5/18/2016	71	6.5	7/2/2016	45	10.9
5/19/2016	65	6.0	7/3/2016	42	10.3
5/20/2016	51	5.6	7/4/2016	33	8.7
5/21/2016	45	6.2	7/5/2016	27	9.1
5/22/2016	44	6.3	7/6/2016	25	10.0
5/23/2016	40	6.3	7/7/2016	24	9.4
5/24/2016	40	7.2	7/8/2016	26	10.0
5/25/2016	46	7.8	7/9/2016	33	9.8
5/26/2016	50	7.0	7/10/2016	26	9.1
5/27/2016	44	6.4	7/11/2016	23	10.2
5/28/2016	38	6.1	7/12/2016	23	10.5
5/29/2016	38	6.9	7/13/2016	22	10.8
5/30/2016	38	7.0	7/14/2016	22	10.8
5/31/2016	40	7.9	7/15/2016	20	10.2
6/1/2016	50	8.0	7/16/2016	19	10.5
6/2/2016	65	7.4	7/17/2016	20	10.4
6/3/2016	61	7.6	7/18/2016	19	9.6
6/4/2016	74	8.4	7/19/2016	21	10.7
6/5/2016	95	8.3	7/20/2016	19	11.1
6/6/2016	106	8.5	7/21/2016	18	11.1
6/7/2016	95	8.2	7/22/2016	21	11.2
6/8/2016	83	8.2	7/23/2016	19	11.3
6/9/2016	66	7.2	7/24/2016	19	11.9
6/10/2016	49	7.4	7/25/2016	21	13.1
6/11/2016	40	7.2	7/26/2016	24	13.7
6/12/2016	33	7.6	7/27/2016	23	13.4
6/13/2016	33	7.8	7/28/2016	23	13.6
6/14/2016	30	6.9	7/29/2016	21	13.8
6/15/2016	25	6.4	7/30/2016	19	13.8
6/16/2016	22	7.3	7/31/2016	16	12.8
6/17/2016	21	7.7	8/1/2016	14	12.5
6/18/2016	26	7.6	8/2/2016	12	12.4
6/19/2016	27	8.1	8/3/2016	12	12.3
6/20/2016	26	8.7	8/4/2016	12	12.9
6/21/2016	29	9.3	8/5/2016	11	13.3
6/22/2016	33	9.1	8/6/2016	11	12.9
6/23/2016	36	9.4	8/7/2016	10	12.5
6/24/2016	38	8.2	8/8/2016	9	10.8
6/25/2016	32	8.6	8/9/2016	10	10.6
6/26/2016	36	9.6	8/10/2016	9	12.6
6/27/2016	41	10.0	8/11/2016	10	13.1
6/28/2016	52	10.5	8/12/2016	10	13.6

8/13/2016	11	14.1	9/27/2016	5	11.2
8/14/2016	10	14.1	9/28/2016	4	10.8
8/15/2016	10	14.0	9/29/2016	4	10.1
8/16/2016	10	14.4	9/30/2016	4	10.2
8/17/2016	9	14.5	10/1/2016	3	9.4
8/18/2016	10	14.6	10/2/2016	3	8.7
8/19/2016	9	14.2	10/3/2016	3	8.1
8/20/2016	8	13.7	10/4/2016	3	8.8
8/21/2016	8	13.8	10/5/2016	3	8.8
8/22/2016	8	12.6	10/6/2016	2	8.4
8/23/2016	6	11.9	10/7/2016	5	8.7
8/24/2016	6	12.8	10/8/2016	30	7.3
8/25/2016	6	13.0	10/9/2016	51	7.8
8/26/2016	6	13.2	10/10/2016	14	7.3
8/27/2016	7	13.3	10/11/2016	10	6.5
8/28/2016	6	12.2	10/12/2016	8	5.4
8/29/2016	6	12.6	10/13/2016	12	5.6
8/30/2016	6	12.6	10/14/2016	38	5.6
8/31/2016	6	12.2	10/15/2016	30	5.6
9/1/2016	5	11.7	10/16/2016	34	6.1
9/2/2016	5	10.7	10/17/2016	28	6.4
9/3/2016	5	10.6	10/18/2016	26	6.5
9/4/2016	4	10.5	10/19/2016	24	6.2
9/5/2016	4	10.5	10/20/2016	92	5.8
9/6/2016	4	11.5	10/21/2016	70	6.0
9/7/2016	4	11.8	10/22/2016	44	6.2
9/8/2016	4	11.6	10/23/2016	33	6.4
9/9/2016	4	10.9	10/24/2016	27	6.5
9/10/2016	4	11.3	10/25/2016	27	6.5
9/11/2016	4	11.9	10/26/2016	35	6.1
9/12/2016	4	10.7	10/27/2016	59	6.1
9/13/2016	3	10.1	10/28/2016	39	6.4
9/14/2016	3	10.3	10/29/2016	32	6.2
9/15/2016	3	11.1	10/30/2016	28	5.8
9/16/2016	4	11.1	10/31/2016	32	5.9
9/17/2016	8	10.9	11/1/2016	32	5.9
9/18/2016	13	9.7	11/2/2016	32	6.0
9/19/2016	7	9.6	11/3/2016	32	6.1
9/20/2016	6	9.1	11/4/2016	27	5.6
9/21/2016	5	8.9	11/5/2016	28	6.2
9/22/2016	4	9.3	11/6/2016	33	6.3
9/23/2016	4	9.1	11/7/2016	26	6.0
9/24/2016	4	9.3	11/8/2016	24	6.0
9/25/2016	4	10.5	11/9/2016	25	5.9
9/26/2016	4	10.9	11/10/2016	25	6.0

11/11/2016	23	6.3
11/12/2016	37	6.8
11/13/2016	35	5.7
11/14/2016	50	5.3
11/15/2016	44	4.6
11/16/2016	39	4.3
11/17/2016	32	4.3
11/18/2016	28	4.4
11/19/2016	25	4.0
11/20/2016	23	4.2
11/21/2016	21	4.6
11/22/2016	19	4.4
11/23/2016	18	4.2
11/24/2016	17	4.1
11/25/2016	17	3.8
11/26/2016	16	4.3
11/27/2016	16	4.2
11/28/2016	15	3.5
11/29/2016	14	3.7
11/30/2016	14	3.6

APPENDIX B: Daily Trap Operation Status

Date	Trap Status	Comments
3/1/2016	Op.	
3/2/2016	Op.	
3/3/2016	Op.	
3/4/2016	Op.	
3/5/2016	Op.	
3/6/2016	Op.	
3/7/2016	Op.	
3/8/2016	Op.	
3/9/2016	Op.	
3/10/2016	Op.	
3/11/2016	Op.	
3/12/2016	Op.	
3/13/2016	Op.	
3/14/2016	Op.	
3/15/2016	Op.	
3/16/2016	Op.	
3/17/2016	Op.	
3/18/2016	Op.	
3/19/2016	Op.	
3/20/2016	Op.	
3/21/2016	Op.	
3/22/2016	Op.	
3/23/2016	Op.	
3/24/2016	Op.	
3/25/2016	Op.	
3/26/2016	Op.	
3/27/2016	Op.	
3/28/2016	Op.	
3/29/2016	Op.	
3/30/2016	Op.	
3/31/2016	Op.	
4/1/2016	Op.	
4/2/2016	Op.	
4/3/2016	Op.	
4/4/2016	Op.	
4/5/2016	Op.	
4/6/2016	Op.	
4/7/2016	Op.	
4/8/2016	Op.	
4/9/2016	Op.	
4/10/2016	Op.	
4/11/2016	Op.	
4/12/2016	Op.	
4/13/2016	Op.	
4/14/2016	Op.	
4/15/2016	Op.	
4/16/2016	Op.	
4/17/2016	Op.	
4/18/2016	Op.	
4/19/2016	Op.	
4/20/2016	No Op.	Stopped-debris
4/21/2016	No Op.	Stopped-debris
4/22/2016	Op.	
4/23/2016	Op.	
4/24/2016	Op.	
4/25/2016	Op.	
4/26/2016	Op.	
4/27/2016	Op.	
4/28/2016	Op.	
4/29/2016	Op.	
4/30/2016	Op.	
5/1/2016	Op.	
5/2/2016	Op.	
5/3/2016	Op.	
5/4/2016	No Op.	Stopped-debris
5/5/2016	Op.	
5/6/2016	Op.	
5/7/2016	Op.	
5/8/2016	Op.	
5/9/2016	Op.	
5/10/2016	Op.	
5/11/2016	Op.	
5/12/2016	Op.	
5/13/2016	Op.	
5/14/2016	Op.	
5/15/2016	Op.	
5/16/2016	Op.	
5/17/2016	Op.	
5/18/2016	Op.	
5/19/2016	Op.	
5/20/2016	Op.	
5/21/2016	Op.	
5/22/2016	Op.	
5/23/2016	Op.	
5/24/2016	Op.	
5/25/2016	Op.	
5/26/2016	Op.	
5/27/2016	Op.	
5/28/2016	Op.	
5/29/2016	Op.	

5/30/2016	Op.		7/19/2016	Op.	
5/31/2016	Op.		7/20/2016	Op.	
6/1/2016	Op.		7/21/2016	Op.	
6/2/2016	Op.		7/22/2016	Op.	
6/3/2016	Op.		7/23/2016	Op.	
6/4/2016	Op.		7/24/2016	Op.	
6/5/2016	Op.		7/25/2016	Op.	
6/6/2016	Op.		7/26/2016	Op.	
6/7/2016	Op.		7/27/2016	Op.	
6/8/2016	Op.		7/28/2016	Op.	
6/9/2016	Op.		7/29/2016	Op.	
6/10/2016	Op.		7/30/2016	Op.	
6/11/2016	Op.		7/31/2016	Op.	
6/12/2016	Op.		8/1/2016	Op.	
6/13/2016	Op.		8/2/2016	Op.	
6/14/2016	Op.		8/3/2016	Op.	
6/15/2016	Op.		8/4/2016	Op.	
6/16/2016	Op.		8/5/2016	No Op.	Stopped-debris
6/17/2016	Op.		8/6/2016	Op.	
6/18/2016	Op.		8/7/2016	Op.	
6/19/2016	Op.		8/8/2016	Op.	
6/20/2016	Op.		8/9/2016	Op.	
6/21/2016	Op.		8/10/2016	No Op.	Stopped-debris
6/22/2016	Op.		8/11/2016	Op.	
6/23/2016	Op.		8/12/2016	Op.	
6/24/2016	Op.		8/13/2016	Op.	
6/25/2016	Op.		8/14/2016	Op.	
6/26/2016	Op.		8/15/2016	Op.	
6/27/2016	Op.		8/16/2016	Op.	
6/28/2016	Op.		8/17/2016	Op.	
6/29/2016	Op.		8/18/2016	Op.	
6/30/2016	Op.		8/19/2016	Op.	
7/1/2016	Op.		8/20/2016	Op.	
7/2/2016	Op.		8/21/2016	Op.	
7/3/2016	Op.		8/22/2016	Op.	
7/4/2016	Op.		8/23/2016	No Op.	Stopped-out of pos.
7/5/2016	Op.		8/24/2016	Op.	
7/6/2016	Op.		8/25/2016	Op.	
7/7/2016	Op.		8/26/2016	Op.	
7/8/2016	Op.		8/27/2016	Op.	
7/9/2016	Op.		8/28/2016	Op.	
7/10/2016	Op.		8/29/2016	Op.	
7/11/2016	Op.		8/30/2016	Op.	
7/12/2016	Op.		8/31/2016	Op.	
7/13/2016	Op.		9/1/2016	Op.	
7/14/2016	Op.		9/2/2016	Op.	
7/15/2016	Op.		9/3/2016	Op.	
7/16/2016	Op.		9/4/2016	No Op.	Stopped-debris
7/17/2016	Op.		9/5/2016	Op.	
7/18/2016	Op.		9/6/2016	Op.	

9/7/2016	Op.		10/20/2016	Op.	
9/8/2016	Op.		10/21/2016	No Op.	Stopped-debris
9/9/2016	Op.		10/22/2016	Op.	
9/10/2016	Op.		10/23/2016	No Op.	Stopped-debris
9/11/2016	Op.		10/24/2016	Op.	
9/12/2016	Op.		10/25/2016	No Op.	Stopped-debris
9/13/2016	No Op.	Stopped-debris	10/26/2016	No Op.	Stopped-debris
9/14/2016	No Op.	Stopped-debris	10/27/2016	No Op.	Stopped-debris
9/15/2016	Op.		10/28/2016	Op.	
9/16/2016	Op.		10/29/2016	Op.	
9/17/2016	Op.		10/30/2016	Op.	
9/18/2016	No Op.	Stopped-debris	10/31/2016	Op.	
9/19/2016	No Op.	Stopped-debris	11/1/2016	Op.	
9/20/2016	Op.		11/2/2016	Op.	
9/21/2016	Op.		11/3/2016	Op.	
9/22/2016	Op.		11/4/2016	Op.	
9/23/2016	Op.		11/5/2016	Op.	
9/24/2016	No Op.	Stopped-debris	11/6/2016	Op.	
9/25/2016	No Op.	Stopped-debris	11/7/2016	Op.	
9/26/2016	No Op.	Stopped-debris	11/8/2016	Op.	
9/27/2016	No Op.	Stopped-debris	11/9/2016	No Op.	Stopped-debris
9/28/2016	No Op.	Stopped-debris	11/10/2016	Op.	
9/29/2016	Op.		11/11/2016	Op.	
9/30/2016	Op.		11/12/2016	Op.	
10/1/2016	No Op.	Stopped-debris	11/13/2016	Op.	
10/2/2016	Op.		11/14/2016	Op.	
10/3/2016	Op.		11/15/2016	Op.	
10/4/2016	Op.		11/16/2016	Op.	
10/5/2016	Op.		11/17/2016	Op.	
10/6/2016	No Op.	Stopped-debris	11/18/2016	Op.	
10/7/2016	No Op.	Stopped-debris	11/19/2016	Op.	
10/8/2016	No Op.	Stopped-debris	11/20/2016	Op.	
10/9/2016	No Op.	Stopped-debris	11/21/2016	Op.	
10/10/2016	Op.		11/22/2016	Op.	
10/11/2016	Op.		11/23/2016	Op.	
10/12/2016	No Op.	Stopped-debris	11/24/2016	Op.	
10/13/2016	Op.		11/25/2016	Op.	
10/14/2016	Op.		11/26/2016	Op.	
10/15/2016	Op.		11/27/2016	Op.	
10/16/2016	Op.		11/28/2016	Op.	
10/17/2016	No Op.	Stopped-debris	11/29/2016	Op.	
10/18/2016	Op.		11/30/2016	Op.	
10/19/2016	Op.				

APPENDIX C: Regression Models

Model: Chinook Yearlings (Spring '08-'15) Back Position, (r^2=0.569; p = 0.001)

Origin/Species/Stage	Date	Marked	Recaptured	Trap Efficiency	ASIN Transform	Discharge (m^3/s)
Wild Chinook Yearlings	4/10/2008	25	2	0.12	0.354	6
Wild Chinook Yearlings	3/26/2009	24	5	0.25	0.524	5
Wild Chinook Yearlings	3/30/2009	34	4	0.147	0.394	5
Wild Chinook Yearlings	4/2/2009	37	10	0.297	0.577	6
Wild Chinook Yearlings	4/5/2009	59	15	0.271	0.548	6
Wild Chinook Yearlings	4/10/2009	36	3	0.111	0.34	11
Wild Chinook Yearlings	3/12/2010	25	1	0.08	0.287	8
Wild Chinook Yearlings	3/16/2010	30	5	0.2	0.464	8
Wild Chinook Yearlings	3/20/2010	21	1	0.095	0.314	8
Wild Chinook Yearlings	4/5/2010	37	1	0.054	0.235	10
Wild Chinook Yearlings	4/9/2010	31	4	0.161	0.413	9
Wild Chinook Yearlings	4/12/2010	58	4	0.086	0.298	8
Wild Chinook Yearlings	4/16/2010	73	2	0.041	0.204	11
Wild Chinook Yearlings	4/14/2012	48	1	0.042	0.206	15

Model: Chinook Subyearlings (Fall '09-'15) Back Position, (r^2=0.130; p = 0.086)

Origin/Species/Stage	Date	Marked	Recaptured	Trap Efficiency	ASIN Transform	Discharge (m^3/s)
Wild Chinook Subyearlings	8/20/2009	20	2	15.00%	0.398	9
Wild Chinook Subyearlings	8/29/2009	34	4	14.71%	0.394	6
Wild Chinook Subyearlings	10/7/2009	22	2	13.64%	0.378	3
Wild Chinook Subyearlings	10/16/2009	34	6	20.59%	0.471	4
Wild Chinook Subyearlings	11/17/2009	35	3	11.43%	0.345	11
Wild Chinook Subyearlings	11/23/2009	21	0	4.76%	0.22	9
Wild Chinook Subyearlings	11/21/2011	39	2	7.69%	0.281	5
Wild Chinook Subyearlings	10/4/2012	33	5	18.18%	0.441	4
Wild Chinook Subyearlings	10/24/2012	87	6	8.05%	0.288	8
Wild Chinook Subyearlings	10/28/2012	36	1	5.56%	0.238	20
Wild Chinook Subyearlings	10/31/2013	46	7	17.39%	0.43	7
Wild Chinook Subyearlings	11/6/2013	38	9	26.32%	0.539	7
Wild Chinook Subyearlings	11/9/2013	40	6	17.50%	0.432	7
Wild Chinook Subyearlings	11/13/2013	29	2	10.34%	0.327	12
Wild Chinook Subyearlings	11/23/2013	25	3	16.00%	0.412	11
Wild Chinook Subyearlings	11/27/2013	24	0	4.17%	0.206	9
Wild Chinook Subyearlings	9/17/2015	39	4	12.82%	0.366	3

Appendix D. Historical Morphometric Data

Spring Chinook (2007-2016)

Trap Year	Brood Year	Origin/Species/Stage	Fork Length (mm)			Weight (g)			K-factor
			Mean	n	SD	Mean	n	SD	
2007	2005	Wild Yearling Smolt	93	173	8.5	8.6	173	2.2	1.1
2007	2005	Wild Yearling Precocial Parr	123	4	7.2	22.2	4	5.8	1.2
2007	2005	Hatchery Yearling Smolt*	76	208	17.9	5.4	203	4.2	1.2
2007	2005	Hatchery Yearling Precocial Parr	98	20	8.7	11.1	19	2.2	1.2
2007	2006	Wild Subyearling Fry	35	7	1.6	—	—	—	—
2007	2006	Wild Subyearling Parr	95	33	12.4	9.8	33	4.1	1.1
2008	2006	Wild Yearling Smolt	100	105	12.3	12.5	105	13.5	1.2
2008	2006	Wild Yearling Precocial Parr	126	9	8.4	22.8	9	4.1	1.1
2008	2006	Hatchery Yearling Smolt	117	229	12.7	18.7	228	9.8	1.2
2008	2006	Hatchery Yearling Precocial Parr	155	2	15.6	47.6	2	12.6	1.3
2008	2007	Wild Subyearling Fry	41	10	4.4	—	—	—	—
2008	2007	Wild Subyearling Parr	95	202	9.1	9.4	202	2.5	1.1
2009	2007	Wild Yearling Smolt	104	275	6.4	12.5	274	2.6	1.1
2009	2007	Wild Yearling Precocial Parr	134	5	7.0	28.5	2	2.7	1.2
2009	2007	Hatchery Yearling Precocial Parr	188	2	17.7	81.9	2	27.1	1.2
2009	2008	Wild Subyearling Fry	38	13	2.1	—	—	—	—
2009	2008	Wild Subyearling Parr	85	507	11.8	7.2	499	2.7	1.2
2010	2008	Wild Yearling Smolt	96	345	7.1	11.2	345	2.4	1.3
2010	2008	Wild Yearling Precocial Parr	130	15	10.3	26.4	15	6.6	1.2
2010	2009	Wild Subyearling Fry	40	31	3.6	—	—	—	—
2010	2009	Wild Subyearling Parr	87	166	12.6	7.7	166	3.0	1.2
2011	2009	Wild Yearling Smolt	99	64	7.7	11.3	64	2.8	1.2
2011	2009	Wild Yearling Precocial Parr	137	1	—	32.3	1	—	1.3
2011	2009	Hatchery Yearling Smolt	127	46	10.6	24.3	46	6.5	1.2
2011	2010	Wild Subyearling Fry	37	26	2.5	—	—	—	—
2011	2010	Wild Subyearling Parr	91	159	13.0	9.2	159	7.1	1.2
2012	2010	Wild Yearling Smolt	98	182	7.9	10.9	179	2.8	1.2
2012	2010	Wild Yearling Precocial Parr	123	13	12.7	22.4	13	6.5	1.2
2012	2011	Hatchery Subyearling Fry	84	29	4.4	6.5	2	2.3	1.1
2012	2011	Hatchery Subyearling Parr	110	25	7.4	14.6	25	3.3	1.1
2012	2011	Wild Subyearling Fry	35	18	2.7	—	—	—	—
2012	2011	Wild Subyearling Parr	91	315	10.1	8.8	288	2.8	1.2
2013	2011	Wild Yearling Smolt	103	20	7.0	12.3	20	3.0	1.1
2013	2011	Wild Yearling Precocial Parr	111	2	0.7	13.5	2	3.0	1.0
2013	2011	Hatchery Yearling Precocial Parr	155	4	17.4	43.4	4	17.8	1.2
2013	2012	Wild Subyearling Fry	40	77	8.1	—	—	—	—
2013	2012	Wild Subyearling Parr	84	445	12.3	6.7	444	4.7	1.1

2014	2012	Wild Yearling Smolt	94	43	7.0	9.4	43	2.2	1.1
2014	2012	Wild Yearling Precocial Parr	127	7	13.0	23.2	7	7.4	1.1
2014	2013	Wild Subyearling Fry	40	22	3.8	—	—	—	—
2014	2013	Wild Subyearling Parr	86	185	14.1	7.5	185	3.3	1.2
2015	2013	Wild Yearling Smolt	103	32	6.8	13.0	31	2.8	1.1
2015	2013	Wild Yearling Precocial Parr	145	2	13.4	35.2	2	11.4	1.1
2015	2014	Wild Subyearling Fry	38	11	3.3	0.5	10	0.2	0.9
2015	2014	Wild Subyearling Parr	96	151	7.5	10.4	148	6.3	1.2
2016	2014	Wild Yearling Smolt	106	3	1.5	12.4	3	0.3	1.1
2016	2015	Wild Subyearling Fry	38	50	3.0	0.46	49	0.3	0.8
2016	2015	Wild Subyearling Parr	89	147	10.7	8.29	147	2.8	1.1

[a] Includes residualized non-precocial smolts caught after June 30
[b] "Fry" classification based on age despite FL ≥ 50mm

Appendix N

Genetic Diversity of Upper Columbia River Summer Chinook Salmon

Genetic Structure of upper Columbia River Summer Chinook and Evaluation of the Effects of Supplementation Programs

by

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Abstract

We investigated genetic relationships among temporally replicated collections of summer Chinook from the Wenatchee River, Methow River, and Okanogan River in the upper Columbia River basin. Samples from the Eastbank Hatchery – Wenatchee stock, Eastbank Hatchery – MEOK stock, and Wells Hatchery were also included in the analysis. Samples of natural- and hatchery-origin summer Chinook were analyzed and compared to determine if the supplementation program has had any impacts to the genetic structure of these populations. We also calculated the effective number of breeders for collection locations of natural- and hatchery-origin summer Chinook from 1993 and 2008. In general, population differentiation was not observed among the temporally replicated collection locations. A single collection from the Okanogan River (1993) was the only collection showing statistically significant differences. The effective number of breeders was not statistically different from the early collection in 1993 in comparison to the late collection in 2008. Overall, these analyses revealed a lack of differentiation among the temporal replicates from the same locations and among the collection from different locations, suggesting the populations have been homogenized or that there has been substantial gene flow among populations. Additional comparisons among summer-run and fall-run Chinook populations in the upper Columbia River were conducted to determine if there was any differentiation between Chinook with different run timing. These analyses revealed pairwise F_{ST} values that were less than 0.01 for the collections of summer Chinook to collections of fall Chinook from Hanford Reach, lower Yakima River, Priest Rapids, and Umatilla. Collections of fall Chinook from Crab Creek, Lyons Ferry Hatchery, Marion Drain, and Snake River had pairwise F_{ST} values that were higher in comparison to the collections of summer Chinook. The consensus clustering analysis did not provide good statistical support to the groupings, but did show relationships among collections based on geographic proximity. Overall the summer and fall run Chinook that have historically been

spawned together were not differentiated while fall Chinook from greater geographic distances were differentiated.

Introduction

The National Marine Fisheries Service (NMFS) recognizes 15 Evolutionary Significant Units (ESU) for Chinook salmon (*Oncorhynchus tshawytscha*) (Myers et al. 1998). The summer Chinook from the upper Columbia River are included in the Upper Columbia River Summer- and Fall-Run ESU, which encompasses all late-run (summer and fall), ocean-type Chinook salmon from the mainstem Columbia River and its tributaries (excluding the Snake River) between Chief Joseph and McNary Dams (Waknitz et al. 1995). Waknitz et al. (1995) concluded that due to high total abundance this ESU was not likely to become at risk from extinction. Yet, a majority of natural spawning activity was in the vicinity of Hanford Reach, and it was unclear whether natural production was self-sustaining given the vast summer Chinook artificial propagation efforts (Waknitz et al. 1995). Additionally, the Biological Review Team expressed concern about potential consequences to genetic and life-history traits from an increasing contribution of hatchery fish to total spawning escapement (Waknitz et al. 1995).

Artificial propagation of ocean-type Chinook from the middle/upper Columbia has been continuous since the implementation of the Grand Coulee Fish Maintenance Project (GCFMP) in 1939 (Myers et al. 1998). The US Fish and Wildlife Service established three hatchery programs for summer/fall Chinook during the GCFMP, Leavenworth NFH, Entiat NFH, and Winthrop NFH. The Washington Department of Fisheries (now Washington Department of Fish and Wildlife) followed with hatchery programs at Rocky Reach (1964), Wells Dam (1967), Priest Rapids (1974), and Eastbank (1990) facilities. Currently, only Leavenworth NFH and Winthrop NFH are not producing summer/fall Chinook. Entiat NFH has resumed production of summer/fall Chinook (Wells FH Stock) in 2009 and released their first yearling summer Chinook smolts in 2010. Since

1941, over 200 million ocean-type Chinook salmon have been released into the middle Columbia River Basin (Myers et al. 1998). Initially, the hatchery programs differentiated between early returning fish (i.e., stream-type) and later returning fish (i.e., ocean-type), but no distinction was made regarding the "summer" and "fall" components of the ocean-type stocks (Waknitz et al. 1995). Therefore, all Chinook salmon now migrating above Rock Island Dam descend from not only a mixture between different stocks from the basin, but also a mixture between the endemic summer and fall life histories. While hatchery protocols have been modified of late to maintain discreet summer and fall Chinook hatchery stocks (Utter et al. 1995; see also HGMP), physical evidence and genetic data suggests that summer and fall Chinook may have become homogenized. During the 1970's and 80's, given coded-wire tag recoveries, summer-run Chinook originating from above Rock Island Dam were believed to have spawned extensively with Hanford Reach and Priest Rapids Hatchery fish (Chapman 1994). Stuehrenberg et al. (1995) reported that 10% of their radio tagged summer Chinook were occupying typical fall-run spawning habitat on the mainstem Columbia river, and 25% of fall fish released from Priest Rapids were recovered as summers at (or above) Wells Hatchery. Genetic data reported by Marshall et al. (1995) and Waknitz et al. (1995) corroborate these observations, as genetic distances observed between summer and fall Chinook within the Upper Columbia River Summer- and Fall-Run ESU were essentially zero.

In response to the need for evaluation of the supplementation hatchery programs, both a monitoring and evaluation plan (DCPUD 2005; Murdoch and Peven 2005) and the associated analytical framework (Hays et al. 2006) were developed for the Habitat Conservation Plan's Hatchery Committee through the joint effort of the fishery co-managers (CCT, NMFS, USFWS, WDFW, and YN) and Chelan County and Douglas County PUDs. These reports outline 10 objectives to be applied to various species assessing the impacts of hatchery operations mitigating the operation of Wells, Rocky Reach, and Rock Island hydroelectric projects. The present monitoring and evaluation study plan differs

in scope from previous monitoring and evaluation projects proposed by WDFW Molecular Genetics Lab, in that it does not investigate a single watershed, but instead will encompass all summer Chinook stocks from the upper Columbia River including the three supplementation (Wenatchee, Methow, and Okanogan) and the harvest augmentation program (Wells summer Chinook). The objectives of this study were to determine if genetic diversity, population structure, and effective population size have changed in natural spawning populations as a result of the hatchery programs.

Materials and Methods

Collections

A total of 2,416 summer Chinook were collected from tributaries in the upper Columbia River basin and were analyzed (Table 1). Two collections of natural-origin summer Chinook from 1993 (prior to the supplementation program) were taken from the Wenatchee River Basin and were compared to collections of hatchery and natural-origin from 2006 and 2008 that were post-supplementation. Two pre-supplementation collections from the Methow River (1991 and 1993) were compared to post-supplementation collections from 2006 and 2008. Three pre-supplementation collections from the Okanogan River Basin (1991, 1992, and 1993) were compared with post-supplementation collections from 2006 and 2008. A collection of natural-origin summer Chinook from the Chelan River was also analyzed. Additionally, hatchery collections from Eastbank Hatchery (Wenatchee and MEOK stock) and Wells Hatchery were analyzed and compared to the in-river collections. Summer Chinook data (provided by the USFWS) from the Entiat River was also used for comparison. Lastly, data from eight collections of fall Chinook was compared to the collections of summer Chinook.

Laboratory Analyses

All laboratory analyses were conducted at the WDFW Genetics Laboratory in Olympia, Washington. Genomic DNA was extracted by digesting a small piece of fin tissue using the nucleospin tissue kits obtained from Macherey-Nagel following the recommended conditions in the user manual. Extracted DNA was eluted with a final volume of 100 µL.

Genotype information was generated using thirteen microsatellite markers following standard laboratory protocols and analysis methods. Descriptions of the loci assessed in this study and polymerase chain reaction (PCR) conditions are given in Table 2. PCR reactions were run with a thermal profile consisting of: denaturation at 95^{o}C for 3 min, denaturation at 95^{o}C for 15 sec, anneal for 30 sec at the appropriate temperature for each locus (Table 2), extension at 72^{o}C for 1 min, repeat cycle (steps 2-4), final extension at 72^{o}C for 30 minutes. PCR products were then processed with an ABI-3730 DNA Analyzer. Genotypes were visualized with a known size standard (GS500LIZ 3730) using GENEMAPPER 3.7 software. Alleles were binned in GENEMAPPER using the standardized allele sizes established for the Chinook GAPS dataset (Seeb et al. 2007).

Within-collection Statistical Analyses

Allele frequencies were calculated with CONVERT (version 1.3, Glaubitz 2003). Hardy-Weinberg proportions for all loci within each collection were calculated using GENEPOP (version 3.4, Raymond and Rousset 1995). Heterozygosity (observed and expected) was computed for each collection group using GDA (Lewis and Zaykin 2001).

Allelic richness and F_{IS} (Weir and Cockerham 1984) inbreeding coefficient were calculated using FSTAT (version 2.9.3.2, Goudet 2001). Linkage disequilibrium for each pair of loci in each collection was calculated using GENEPOP v 3.4 (10,000 dememorizations, 100 batches, and 5,000 iterations per batch). Pairwise estimates of genetic differentiation between collection groups were

calculated using GENEPOP (version 3.4, Raymond and Rousset 1995). Statistical significance for the tests of Hardy-Weinberg proportions, linkage disequilibrium, and genotypic differentiation was evaluated using a Bonferroni correction of p-values to account for multiple, simultaneous tests (Rice 1989).

Between-collection Statistical Analyses

Pairwise F_{ST} estimates were computed to examine population structure among collections using GENETIX (version 4.03, Belkhir et al. 2001). This estimate uses allelic frequency data and departures from expected heterozygosity to assess differences between pairs of populations.

We used PHYLIP (version 3.5c, Felsenstein 1993) to calculate Cavalli-Sforza and Edwards (1967) pairwise chord distances between collections. Bootstrap calculations were performed using SEQBOOT followed by calculations of genetic distance using GENDIST. The NEIGHBOR-JOINING method of Saitou and Nei (1987) was used to generate the dendrograms and CONSENSE to generate a final consensus tree from the 1,000 replicates. The dendrogram generated in PHYLIP was plotted as an unrooted radial tree using TREEVIEW (version 1.6.6, Page 1996).

Effective Number of Breeders

The effective number of breeders (N_b) was estimated for pre- and post-supplementation program collections (where possible) to investigate whether hatchery programs had affected that genetic metric over the operational period. Wang (2009) derived an equation for effective size (N_e) as a function of the frequency of nested full-sib and half-sib families in a random collection of individuals.

$$\frac{1}{N_e} = \frac{1+3\alpha}{4}(Q_1 + Q_2 + 2Q_3) - \frac{\alpha}{2}\left(\frac{1}{N_1} + \frac{1}{N_2}\right) \qquad \text{(equation 10)}$$

Where α is a measure of the deviation of genotype frequencies from Hardy-Weinberg expectation (equivalent to Wright's (1969) F_{IS}), Q_i are the probabilities that a pair of offspring are paternal half sibs, maternal half sibs, or full sibs, respectively, and N_1 and N_2 are the number of male and female parents that generation, respectively. Genetic parameters (i.e., sibship distributions) were estimated for summer Chinook collections using algorithms implemented in COLONY (Jones and Wang 2009). To be clear, Wang's (2009) method as implemented here will estimate N_b, given multi-locus genotypes from each collection were partitioned by brood year for this analysis. To obtain an estimate of N_e each N_b value must be multiplied by the mean generation time of that population.

Results

Collections

A total of 2,350 individuals from 32 collections of temporally replicated samples (six locations) were analyzed (Table 1). Temporally replicated collections of hatchery and natural-origin samples were from the Wenatchee, Methow, and Okanogan Rivers. Temporally replicated hatchery-origin summer Chinook were from Wells Hatchery, Eastbank Hatchery - Wenatchee stock, and Eastbank Hatchery - Methow/Okanogan (MEOK) stock. A total of 232 of those individuals were excluded from any analyses because they failed to amplify at nine or more loci. Data for remaining 2,118 individuals were analyzed to assess differences between temporally replicated natural- and hatchery-origin summer Chinook for each location and to compare the differences among the different collection locations. Summer Chinook data from the temporally replicated collection locations were then combined and compared to fall Chinook data from the GAPS v.3.0 dataset.

Statistical Analyses

The population statistics (Hardy-Weinberg equilibrium and F_{IS}) calculated for each of the 32 temporally replicated collection locations were consistent with neutral expectations (i.e., no associations among alleles). Three collections did have a single locus that did not meet expectations (Wenatchee hatchery-origin 2006, Wells hatchery 2006, and Okanogan hatchery-origin 2009). Based on these results we suggest the collections represented randomly breeding groups and were not comprised of mixtures of individuals from different genetic source populations.

Population differentiation was assessed for each of the temporally replicated collections from within each location (Table 3). This analysis revealed the only significant difference observed within a collection location pertained to the collection from 1993 Okanogan River natural-origin samples. Because of the significant difference of this collection to the other temporal replicates it was not included in further analyses.

Given the absence of genetic differentiation observed among the temporally replicated collections, the 32 collections from the Wenatchee, Methow, and Okanogan River were combined to form three location-specific collections for analysis. Population differentiation metrics were compared among the composite Wenatchee, Methow, and Okanogan collections and eight other location-specific collections (11 locations total). Comparing all collections, there were a total of 39 significant genic test comparisons out of a total 496 (Table 4). Thirty-eight of the 39 statistically significant pairwise differences pertained to the Okanogan River and 2006 Wells Hatchery collections (Table 4). F_{ST} results are described further below.

Within-collection genetic metrics were estimated for the 11 location-specific collections of summer Chinook from the upper Columbia River, in addition to eight collections of fall Chinook (Table 1). The population statistics (Hardy-Weinberg equilibrium and F_{IS}) calculated for these collections of summer and fall

Chinook were also consistent with neutral expectations. The collection from Lyons Ferry Hatchery had one locus that did not meet expectations and the collections from Crab Creek and Marion Drain both had three loci that did not meet expectations.

The hatchery collections in general had a higher percentage of significantly linked loci; however the observed genetic diversity were similar for the natural and hatchery-origin collections. Analysis of allelic richness was based on 11 individuals per collection, the minimum number of individuals across all collections with complete multilocus genotypes. The largest number of linked loci occurred in the Crab Creek, Entiat River, and Okanogan natural-origin collections. Allelic richness was on average lower in the collections of summer Chinook (10.7) collections in comparison to the collections of fall Chinook (11.0).

Pairwise F_{ST} (Table 4) estimates revealed low levels of differentiation, where all observed F_{ST} values between the collections of summer Chinook were lower than 0.0096. There were 15 out of 28 comparisons between collections of summer Chinook that were significantly different from zero and occurred primarily from comparisons of the Okanogan River (hatchery and natural-origin) and Wells Hatchery to all other collections. The collection of Eastbank Hatchery – MEOK stock was differentiated from the Wenatchee River natural-origin and Entiat River collections. The collection from the Chelan River had a small sample size of 23 individuals and only differentiated from the Eastbank Hatchery – MEOK stock. F_{ST} estimates regarding pairwise comparisons between each of four fall Chinook collection locations (Crab Creek, Lyons Ferry Hatchery, Marion Drain, and Snake River) to all other collections were significantly different from zero (Table 5). Pairwise comparisons for three other fall Chinook collections (Hanford Reach, lower Yakima River, and Umatilla River) to the collections of summer Chinook were significantly different from zero (Table 6). The only fall Chinook collection that was not significantly differentiated from all of the summer Chinook was Priest Rapids.

The relative genetic relationships among the test groups were assessed using the consensus clustering analysis (Figure 1). Statistical support for the dendrogram topology (i.e., tree shape) was low regarding the branching that separated the collections of summer Chinook from the upper Columbia River. The collections of fall Chinook; however were supported with bootstrap support over 76% with the exception of three collections (lower Yakima River, Crab Creek, and Umatilla River). In other words, 760 of the 1000 bootstrap replicates supported the placement of the node separating summer and fall collections. The collection from the Chelan River had bootstrap support of 68%; however the sample size for that collections was small (N = 23). Even though the bootstrap support was low among the collections of summer Chinook there was concordance between geography and genetic distance.

Where comparisons were possible between pre- and post-supplementation program collections, the effective number of breeders (N_b) estimated to have comprised those collections were slightly lower for contemporary (2008) collections; however in all cases the 95% confidence intervals overlapped between historical and contemporary collections, suggesting statistical equivalency. Regarding Wenatchee River collections, the point estimates of N_b ranged from 134 (08FU) to 190 (93DD), where all collections had overlapping confidence intervals (Table 7). The upper bound of the 1989 brood year for collection 93DD was very large, suggesting the sample size was insufficient for properly inferring the sibship distribution within the collection. Comparing the Okanogan natural collections 93ED and 08GA, the estimated N_b were 142 (CI 102 – 203) and 127 (CI 92 – 180), respectively. For the Eastbank Hatchery MEOK stock comparisons, the N_b estimated for the 93DF collection was 171 (CI 129 – 229), as compared to the 166 (CI 126 – 226) estimated for collection 08MO. In all cases, the estimated N_b can be converted to effective population size (N_e) by multiplying the estimate by the mean generation time.

Discussion

The collections of summer Chinook populations from the upper Columbia River are of interest because census sizes are reduced below historic levels and are the subject of mitigation and supplementation hatchery programs. Concern over the impacts of hatchery supplementation programs on the genetic integrity of natural-origin populations led to our primary objective, which was to evaluate genetic metrics for temporally replicated collections of summer Chinook in the upper Columbia River pre and post hatchery supplementation. A similar analysis by Kassler and Dean (2010) was conducted on spring Chinook in the Tucannon River to evaluate the effects of a supplementation and captive brood program on natural-origin stocks. Additionally, upper Columbia River spring Chinook supplementation programs (Blankenship et al. 2007; Small et al. 2007), spring and fall Chinook populations in the Yakima Basin (Kassler et al. 2008), and a potentially unique population of fall Chinook in Crab Creek (Small et al. 2010) have been evaluated. In the present analysis of summer Chinook populations, collections of pre- and post- supplementation summer Chinook were collected from the Wenatchee River, Methow River, and Okanogan River Basins and analyzed to determine if the genetic profile has changed as a result of the supplementation program. Analysis was then conducted on the collections of summer run to compare the fall run Chinook collections in the upper Columbia River basin.

Allozyme analyses of these three summer run Chinook stocks in the upper Columbia River have identified that each stock was distinct, with a closer relationship detected between the Wenatchee and Methow Rivers (WDF and WDW 1993, Marshall 2002). Wenatchee summer Chinook are thought to be a mixture of native summer Chinook and Chinook from the Grand Coulee Fish Maintenance Project (GCFMP). The goal of the GCFMP project between 1939 and 1943 was to trap migrating Chinook salmon at Rock Island dam (75 miles below Grand Coulee) and homogenize the populations, which reduced the

genetic uniqueness of the distinct tributary populations present in the upper Columbia River.

We found allele frequencies for individual temporally replicated hatchery- and natural-origin collection locations of adult summer Chinook were not significantly different from that expected of a single underlying population, except for one collection (1993 Okanogan natural-origin; Table 3). This collection was differentiated to the Okanogan collections in 2006 and 2008; however it was not differentiated from the collection in 1992. The Okanogan collection from 1992 was also not differentiated to any other collection; therefore the difference in the collection from Okanogan 1993 was likely not an indication of genetic change from pre supplementation to post supplementation. The collection was however dropped from further analyses so as to not confuse interpretation of results. The lack of allelic differentiation observed among the temporally replicated collections was interpreted as the genetic metrics from each location in the early 1990's did not differ from the samples collected in 2008. Spanning a few generations, allele frequencies are not expected to change for large populations at genetic equilibrium. In contrast, changes in allele frequencies of small populations may occur due to the stochastic sampling of genes from one generation to the next (i.e., genetic drift).

A second round of analyses was conducted to evaluate the genetic relationships of the summer run collections (temporal collections were combined) with data from the Entiat River, Chelan River, and eight collections of fall Chinook. Assessment of the relationship between the summer run collections in comparison to each other provided very little evidence of genetic differentiation between these collections. While population differentiation did show some significant differences between the Okanogan River and Wells Hatchery collections, all of the pairwise F_{ST} values were below 0.003. Meaning that a very small proportion of the observed genetic variation could be attributed to restrictions in gene flow (i.e., population structure)

The comparison of the hatchery-origin collections revealed a lack of differentiation between the Eastbank Hatchery – Wenatchee stock, Eastbank Hatchery – MEOK stock, and the Wells Hatchery (with exception of the 2006 collection). The genetic similarity or low level of genetic differentiation among these stocks suggests that there has been an integration of natural- and hatchery-origin summer Chinook in the upper Columbia River or a lack of ancestral genetic difference. The difference of the 2006 Wells Hatchery collection to the other collections is most likely a result of sampling effect because of the lack of differentiation among the stocks in the basin. If the 2006 collection had been mixed from different sources of summer Chinook there would not be a detectable level of differentiation as was seen with the 2006 sample.

The analyses to compare summer and fall Chinook collections provided some understanding on the genetic relationships of Chinook with different run timings in the upper Columbia River basin. Historically, the hatchery programs in the upper Columbia River were separated into groups of the early returning fish (i.e., stream-type) and later returning fish (i.e., ocean-type), but the programs did not sort individuals identified as “summer” or “fall” stocks (Waknitz et al. 1995). Now all Chinook salmon that are migrating above Rock Island Dam descend from a mixture of different stocks from the upper Columbia River basin, but also a mixture between the endemic summer and fall life histories.

Small et al. (2010) conducted an analysis on summer run and fall run Chinook in the upper Columbia River and concluded that Crab Creek Chinook in the upper Columbia River were genetically distinct to all other fall and summer run Chinook stocks that were analyzed. They did note a departure from Hardy Weinberg expectation as a result of a null allele at the microsatellite locus *Ogo-4* and a higher linkage disequilibrium value due to the inclusion of family groups in one of their samples. Kassler et al. (2008) found differentiation among spring and fall Chinook populations in the Yakima River.

The tests of pairwise F_{ST} indicated a very low level of genetic differentiation (less than one percent difference) between collections of summer-run Chinook and fall-run Chinook. The range of pairwise F_{ST} values for comparisons between the summer run and fall run collections was 0.0016 – 0.0248. The larger values from the range were associated to the collections from Crab Creek, Lyons Ferry Hatchery, and Marion Drain. Studies by Kassler et al. (2008) and Small et al. (2010) have documented differences among the populations of these collections to others within the upper Columbia River basin. The low pairwise F_{ST} values between Priest Rapids and Hanford Reach collections and the summer run collections were not surprising because summer-run Chinook originating from above Rock Island Dam were believed to have spawned extensively with Hanford Reach and Priest Rapids Hatchery fish during the 1970's and 80's (Chapman 1994). The lack of differentiation among the summer and fall stocks in the Columbia River was also identified by Utter et al. (1995) and the HGMP where they state physical evidence and genetic data suggests that summer and fall Chinook may have become homogenized.

Despite low levels of statistical bootstrap support for dendrogram topology (i.e., tree shape), there was concordance observed between geographic location and the genetic relationships among the summer and fall Chinook populations. The collections from the Okanogan (hatchery and natural-origin) did separate out with collections from Wells Dam Hatchery, Entiat River, and Eastbank Hatchery – MEOK stock, and were next to a group of the Methow and Wenatchee collections. The fall Chinook populations are also separated to the summer collections and the position of all but three of these collections (lower Yakima River, Crab Creek, and Umatilla River) were statistically supported. The geographic proximity of the fall collections seemed to follow the observed pattern in this dendrogram. The relationship of the Snake River and Lyons Ferry Hatchery in proximity to the collection from Marion Drain was not surprising while

the relationship between Priest Rapids and Hanford Reach was easily a result of the stocking practices of fall Chinook in the 1970 and 1980's.

A secondary objective of this study was to determine if the effective population size of upper Columbia River summer Chinook populations had changed over time due to supplementation efforts. We observed that the number of effective breeders in the collections from 1993 and 2008 has not changed thus providing reason to believe that the genetic diversity of summer Chinook in the upper Columbia River has not been altered through the supplementation program.

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Table 1. Samples of adult hatchery- and natural-origin summer and fall Chinook that were analyzed from the upper Columbia River. Total number of individuals that were analyzed / individuals with data for 9 or more loci that were included in the analysis. Collection statistics (allelic richness, linkage disequilibrium (before and after Bonferroni correction), F_{IS}, heterozygosity (H_O and H_E)) and p-values for deviations from Hardy-Weinberg equilibrium (HWE). P-values were defined as significant after implementation of Bonferroni correction for multiple tests (Rice 1989).

WDFW GSI code[a]	Collection location	N =	Allelic Richness[b]	Linkage Disequilibrium[c]	F_{IS} (p-value)[d]	H_O	H_E
93DD	Wenatchee River upstream of Tumwater Dam - natural origin	51 / 45					
93DE	Wenatchee River downstream of Tumwater Dam - natural origin	88 / 88					
06CQ	Wenatchee River upstream of Tumwater Dam - natural origin	95 / 86					
06CR	Wenatchee River downstream of Tumwater Dam - natural origin	95 / 82					
08FV	Wenatchee River upstream of Tumwater Dam - natural origin	95 / 82					
08FW	Wenatchee River downstream of Tumwater Dam - natural origin	95 / 87					
	Wenatchee River - Natural origin combined	**519 / 470**	**10.7**	**17 / 4**	**0.001 (0.403)**	**0.8504**	**0.8513**
06CP	Wenatchee River - hatchery origin	95 / 70					
08FU	Wenatchee River - hatchery origin	95 / 83					
	Wenatchee River - Hatchery origin combined	**190 / 153**	**10.6**	**18 / 6**	**0.018 (0.013)**	**0.8409**	**0.8561**
93EC	Methow River - natural origin	27 / 27					
06CT	Methow River - natural origin	95 / 90					
08FY	Methow River - natural origin	95 / 88					
09CO	Methow River - natural origin	91 / 80					
	Methow River - Natural origin combined	**308 / 285**	**10.7**	**4 / 1**	**0.006 (0.160)**	**0.8506**	**0.8554**
06CS	Methow River - hatchery origin	14 / 8					
08FX	Methow River - hatchery origin	21 / 18					
09CP	Methow River - hatchery origin	19 / 18					
	Methow River - Hatchery origin combined	**54 / 44**	**10.8**	**11 / 2**	**-0.003 (0.593)**	**0.8553**	**0.8523**

Table 1 continued.							
92FM	Okanogan River - natural origin	49 / 46					
93ED*	Okanogan River - natural origin	103 / 87					
06CV	Okanogan River - natural origin	95 / 88					
08GA	Okanogan River - natural origin	95 / 92					
09CN	Okanogan River - natural origin	133 / 126					
	Okanogan River - Natural origin combined	**475 / 439**	**10.8**	**9 / 4**	**0.003 (0.304)**	**0.8563**	**0.8596**
* - not included in the combined dataset							
06CU	Okanogan River - hatchery origin	58 / 49					
08FZ	Okanogan River - hatchery origin	19 / 18					
09CM	Okanogan River - hatchery origin	117 / 107					
	Okanogan River - hatchery origin combined	**194 / 174**	**10.8**	**31 / 10**	**-0.011 (0.920)**	**0.8678**	**0.8586**
91FL	Wells Hatchery	68 / 42					
92FK	Wells Hatchery	25 / 23					
93DG	Wells Hatchery	11 / 9					
06DM	Wells Hatchery	95 / 91					
08HY	Wells Hatchery	95 / 91					
	Wells Hatchery combined	**294 / 256**	**10.7**	**8 / 3**	**-0.001 (0.529)**	**0.8670**	**0.8665**
08MN	**Eastbank Hatchery - Wenatchee River stock**	**95 / 90**	**10.7**	**6 / 1**	**0.020 (0.024)**	**0.8326**	**0.8498**
92FO	Eastbank Hatchery - Methow / Okanogan (MEOK) stock	36 / 33					
93DF	Eastbank Hatchery - Methow / Okanogan (MEOK) stock	90 / 86					
08MO	Eastbank Hatchery - Methow / Okanogan (MEOK) stock	95 / 88					
	Eastbank Hatchery - MEOK stock combined	**221 / 207**	**10.7**	**2 / 0**	**-0.005 (0.782)**	**0.8647**	**0.8604**
		2,350 / 2,118					

Table 1 continued.							
06KN	**Chelan River**	**70 / 23**	**10.3**	**11 / 0**	**0.027 (0.118)**	**0.8334**	**0.8556**
Data provided by USFWS							
	Entiat River - summer Chinook	**190**	**10.9**	**33 / 10**	**0.008 (0.119)**	**0.8553**	**0.8625**
Data from Small et al. (2010)							
08EH	Crab Creek	108					
09AZ	Crab Creek	291					
	Crab Creek	**399**	**10.5**	**35 / 14**	**0.018 (0.000)**	**0.8519**	**0.8676**
GAPS v.3.0 data							
	Priest Rapids Hatchery - fall Chinook	**81**	**11.1**	**3 / 2**	**0.015 (0.079)**	**0.8591**	**0.8723**
	Hanford Reach - fall Chinook	**220**	**11.3**	**4 / 0**	**0.010 (0.068)**	**0.8661**	**0.8746**
	Umatilla - fall Chinook	**96**	**11.2**	**17 / 6**	**-0.003 (0.623)**	**0.8719**	**0.8693**
	lower Yakima River - fall Chinook	**103**	**11.0**	**3 / 1**	**0.000 (0.511)**	**0.8724**	**0.8721**
	Marion Drain - fall Chinook	**190**	**10.8**	**9 / 4**	**0.022 (0.001)**	**0.8586**	**0.8782**
	Lyons Ferry Hatchery - fall Chinook	**186**	**10.6**	**7 / 4**	**0.013 (0.033)**	**0.8527**	**0.8641**
	Snake River - fall Chinook	**521**	**11.1**	**0 / 0**	**-0.001 (0.634)**	**0.8720**	**0.8708**
		NA / 2,009					
[a] - Year that samples were collected is identifed by the two numbers in the WDFW GSI code							
[b] - based on a minimum of 11 diploid individuals							
[c] - adjusted alpha p-value = 0.0006							
[d] - adjusted alpha p-value = 0.0002							

Table 2. PCR conditions and microsatellite locus information (number alleles/locus and allele size range) for multiplexed loci used for the analysis of Chinook. Also included are the observed and expected heterozygosity (H_o and H_e) for each locus.

PCR Conditions			Locus statistics		Heterozygosity		
Poolplex	Locus	Dye Label	# Alleles/ Locus	Allele Size Range (bp)	H_o	H_e	References
Ots-M	*Ots-201b*	blue	49	137 - 334	0.9474	0.9544	Unpublished
	Ots-208b	yellow	56	154 - 378	0.9523	0.9672	Greig et al. 2003
	Ssa-408	red	32	184 - 308	0.9177	0.9214	Cairney et al. 2000
Ots-N	*Ogo-2*	red	22	206 - 260	0.8526	0.8673	Olsen et al. 1998
Ots-O	*Ogo-4*	blue	20	128 - 170	0.6694	0.7028	Olsen et al. 1998
	Ots-213	yellow	45	178 - 370	0.9430	0.9525	Greig et al. 2003
	Ots-G474	red	16	152 - 212	0.6816	0.6838	Williamson et al. 2002
Ots-R	*Ots-3M*	blue	15	128 - 158	0.7854	0.7938	Banks et al. 1999
	Omm-1080	green	54	162 - 374	0.9517	0.9670	Rexroad et al. 2001
Ots-S	*Ots-9*	red	9	99 - 115	0.6531	0.6543	Banks et al. 1999
	Ots-212	blue	33	123 - 251	0.9205	0.9360	Greig et al. 2003
Ots-T	*Oki-100*	blue	50	164 - 361	0.9500	0.9567	Unpublished
	Ots-211	red	34	188 - 327	0.9325	0.9414	Greig et al. 2003

Table 3. Tests of population differentiation for temporal collections of summer Chinook from natural and hatchery-origin populations in the upper Columbia River. P-values that are highlighted grey are significantly different after Bonferroni correction (Rice 1989). Adjusted alpha p-value was 0.0001 . The H and W in the collection identifier is for wild or hatchery-origin and the two digit number identifes the year samples were collected.

Wenatchee River

	WenW93U	WenW93D	WenH06	WenW06U	WenW06D	WenH08	WenW08U	WenW08D
WenW93U	****							
WenW93D	0.0162	****						
WenH06	0.0033	0.0102	****					
WenW06U	0.3039	0.1642	0.4795	****				
WenW06D	0.0261	0.0160	0.0678	0.5300	****			
WenH08	0.1126	0.0708	0.0073	0.4359	0.0893	****		
WenW08U	0.2115	0.1148	0.4191	0.7243	0.3830	0.8856	****	
WenW08D	0.1915	0.0014	0.7047	0.4928	0.1671	0.7755	0.7665	****

D - collection was downstream of Tumwater Dam; U - collection was upstream of Tumwater Dam

Methow River

	MetW93	MetH06	MetW06	MetH08	MetW08	MetW09	MetH09
MetW93	****						
MetH06	0.3962	****					
MetW06	0.5481	0.4688	****				
MetH08	0.1408	0.1192	0.2052	****			
MetW08	0.8219	0.8937	0.6156	0.3779	****		
MetW09	0.2564	0.4282	0.2502	0.0328	0.7309	****	
MetH09	0.1543	0.5678	0.0547	0.0017	0.0098	0.0073	****

Okanogan River

	OkanW92	OkanW93	OkanH06	OkanW06	OkanH08	OkanW08	OkanH09	OkanW09
OkanW92	****							
OkanW93	0.0066	****						
OkanH06	0.0193	0.0000	****					
OkanW06	0.2843	0.0082	0.0031	****				
OkanH08	0.1290	0.1106	0.0652	0.7329	****			
OkanW08	0.0106	0.0029	0.0082	0.4075	0.7396	****		
OkanH09	0.0187	0.0001	0.0094	0.0551	0.2214	0.0281	****	
OkanW09	0.0527	0.0000	0.0024	0.7130	0.0262	0.0065	0.0002	****

Table 3 continued.

Wells Dam Hatchery

	Wells91	Wells92	Wells93	Wells06	Wells08
Wells91	****				
Wells92	0.5863	****			
Wells93	0.0490	0.0784	****		
Wells06	0.0089	0.0100	0.0542	****	
Wells08	0.0819	0.1088	0.2552	0.0256	****

Eastbank Hatchery - Wenatchee and MEOK stocks

	EBHWen08	EBHME92	EBHME93	EBHME08
EBHWen08	****			
EBHME92	0.8681	****		
EBHME93	0.0251	0.8661	****	
EBHME08	0.0086	0.9563	0.1895	****

Table 4. F_{ST} pairwise comparisons and genotypic tests of differentiation for hatchery- and natural-origin summer Chinook from the upper Columbia River. Above the diagonol are the F_{ST} values and below are p-values for the test of genotypic differentiation. Non-significant p-values for the result of the genotypic differentiation test are in bold type and F_{ST} values that are not significantly different from zero are in bold type.

	Wenatchee Hatchery	Wenatchee Natural	Methow Hatchery	Methow Natural	Okanogan Hatchery	Okanogan Natural	Wells Hatchery	Eastbank Wenatchee stock	Eastbank MEOK stock	Entiat River	Chelan River
Wenatchee Hatchery	****	**0.0000**	**0.0011**	**0.0000**	**0.0013**	**0.0010**	0.0015	**0.0004**	**0.0007**	**0.0004**	**0.0072**
Wenatchee Natural	**0.4351**	****	**0.0016**	**0.0000**	0.0014	0.0016	0.0024	**0.0006**	0.0012	0.0009	**0.0068**
Methow Hatchery	**0.3800**	**0.0205**	****	**0.0012**	0.0029	**0.0008**	**0.0027**	**0.0014**	**0.0022**	**0.0019**	**0.0078**
Methow Natural	**0.2237**	**0.6566**	**0.1502**	****	**0.0011**	0.0011	0.0013	**0.0007**	**0.0007**	**0.0008**	**0.0053**
Okanogan Hatchery	0.0001	0.0000	**0.0364**	**0.0008**	****	**0.0010**	**0.0014**	0.0029	**0.0000**	**0.0007**	**0.0055**
Okanogan Natural	0.0000	0.0000	**0.1755**	0.0000	**0.0003**	****	0.0016	0.0023	**0.0005**	**0.0008**	**0.0049**
Wells Hatchery	0.0000	0.0000	**0.0129**	0.0000	0.0000	0.0000	****	0.0036	**0.0006**	**0.0008**	**0.0041**
Eastbank Wenatchee	**0.5261**	**0.4102**	**0.1215**	**0.8404**	**0.0015**	0.0000	0.0000	****	**0.0018**	0.0030	0.0096
Eastbank MEOK stock	**0.0485**	0.0000	**0.4246**	**0.0009**	**0.5786**	**0.0051**	0.0000	**0.0065**	****	**0.0005**	**0.0039**
Entiat River	**0.0565**	0.0000	**0.1795**	**0.0044**	**0.0005**	0.0000	**0.0032**	**0.0039**	**0.0042**	****	**0.0052**
Chelan River	**0.0091**	**0.0026**	**0.0182**	**0.0156**	**0.0048**	**0.0030**	**0.0066**	**0.0059**	**0.0493**	**0.0617**	****

Table 5. F_{ST} pairwise comparisons and genotypic tests of differentiation for fall Chinook. Above the diagonol are the F_{ST} values and below are p-values for the test of genotypic differentiation. Non-significant p-values for the result of the genotypic differentiation test are in bold type and F_{ST} values that are not significantly different from zero are in bold type.

	Crab Creek	Hanford Reach Fall	Lyons Ferry Hatchery Fall	lower Yakima River Fall	Marion Drain Fall	Priest Rapids Fall	Umatilla River Fall	Snake River Fall
Crab Creek	****	0.0087	0.0134	0.0079	0.0143	0.0107	0.0073	0.0097
Hanford Reach Fall	0.0000	****	0.0077	**0.0000**	0.0064	**0.0000**	**0.0000**	0.0022
Lyons Ferry Hatchery Fall	0.0000	0.0000	****	0.0063	0.0074	0.0092	0.0062	0.0029
lower Yakima River Fall	0.0000	**0.4140**	0.0000	****	0.0054	**0.0000**	**0.0000**	0.0018
Marion Drain Fall	0.0000	0.0000	0.0000	0.0000	****	0.0067	0.0061	0.0060
Priest Rapids Fall	0.0000	**0.0695**	0.0000	**0.0083**	0.0000	****	**0.0000**	0.0027
Umatilla River Fall	0.0000	**0.4879**	0.0000	**0.4896**	0.0000	**0.2539**	****	0.0011
Snake River Fall	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	****

Table 6. F_{ST} pairwise comparisons and genotypic tests of differentiation for hatchery- and natural-origin summer Chinook from the upper Columbia River and fall Chinook. Above the diagonol are the F_{ST} values and below are p-values for the test of genotypic differentiation. Non-significant p-values for the result of the genotypic differentiation test are in bold type and F_{ST} values that are not significantly different from zero are in bold type.

Population Differentiation											
	Wenatchee Hatchery	Wenatchee Natural	Methow Hatchery	Methow Natural	Okanogan Hatchery	Okanogan Natural	Wells Hatchery	Eastbank Wenatchee stock	Eastbank MEOK stock	Entiat River	Chelan River
Crab Creek	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Hanford Reach Fall	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0349**
Lyons Ferry Hatchery Fall	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
lower Yakima River Fall	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0074**
Marion Drain Fall	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Priest Rapids Fall	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0642**
Umatilla River Fall	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0579**
Snake River Fall	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Table 6 continued.

Pairwise F_{ST}

	Crab Creek	Hanford Reach Fall	Ferry Hatchery	Yakima River	Marion Drain Fall	Priest Rapids Fall	Umatilla River Fall	Snake River Fall
Wenatchee Hatchery	0.0158	0.0054	0.0180	0.0056	0.0153	**0.0025**	0.0053	0.0103
Wenatchee Natural	0.0162	0.0059	0.0185	0.0063	0.0157	0.0030	0.0059	0.0102
Methow Hatchery	0.0191	0.0104	0.0248	0.0095	0.0220	0.0069	0.0107	0.0165
Methow Natural	0.0148	0.0057	0.0182	0.0051	0.0148	0.0033	0.0055	0.0101
Okanogan Hatchery	0.0146	0.0041	0.0166	0.0042	0.0151	**0.0016**	0.0041	0.0082
Okanogan Natural	0.0163	0.0064	0.0187	0.0062	0.0170	0.0035	0.0068	0.0113
Wells Hatchery	0.0120	0.0051	0.0135	0.0044	0.0120	0.0028	0.0046	0.0077
Wenatchee stock	0.0184	0.0073	0.0203	0.0074	0.0167	0.0047	0.0084	0.0128
Eastbank MEOK stock	0.0128	0.0036	0.0143	0.0038	0.0135	**0.0019**	0.0038	0.0079
Entiat River	0.0147	0.0059	0.0176	0.0057	0.0156	0.0028	0.0056	0.0100
Chelan River	0.0074	**0.0046**	0.0110	**0.0040**	0.0160	**0.0047**	**0.0035**	0.0072

Table 7. Effective number of breeders per brood year with the largest number of samples of summer Chinook in the upper Columbia River. Brood years with sample size less than 19 individuals (shown in bold type) were not analyzed with exception of the 2008 Wells Hatchery collection. A comparison could not be made between an early and late collection from Wells Hatchery.

WDFW Code	Collection Location	Sample Size	Nb =	CI95(L) =	CI95(U) =
93DD[A]	Wenatchee Natural - upstream	23 / 19	152 / 190	77 / 87	616 / 2,147,483,647
08FV	Wenatchee Natural - upstream	56	162	112	249
93DE[A]	Wenatchee Natural - downstream	39 / 34	145 / 152	94 / 95	256 / 302
08FW	Wenatchee Natural - downstream	67	140	105	199
08FU	Wenatchee Hatchery	60	134	90	213
93EC[A]	Methow Natural	**10 / 15**	---	---	---
08FY	Methow Natural	62	150	106	218
08FX	Methow Hatchery	**9**	---	---	---
93ED	Okanogan Natural	69	142	102	203
08GA	Okanogan Natural	59	127	92	180
08FZ	Okanogan Hatchery	**16**	---	---	---
93DG	Wells Hatchery	**6**	---	---	---
08HY[B]	Wells Hatchery	24 / 39	---	---	---
08MN	Eastbank Hatchery - Wenatchee	88	190	144	263
93DF	Eastbank Hatchery - MEOK	84	171	129	229
08MO	Eastbank Hatchery - MEOK	88	166	126	226

[A] - calculations were made for samples from brood year 1988 / brood year 1989

[B] - samples were collected from brood year 2003 / brood year 2004

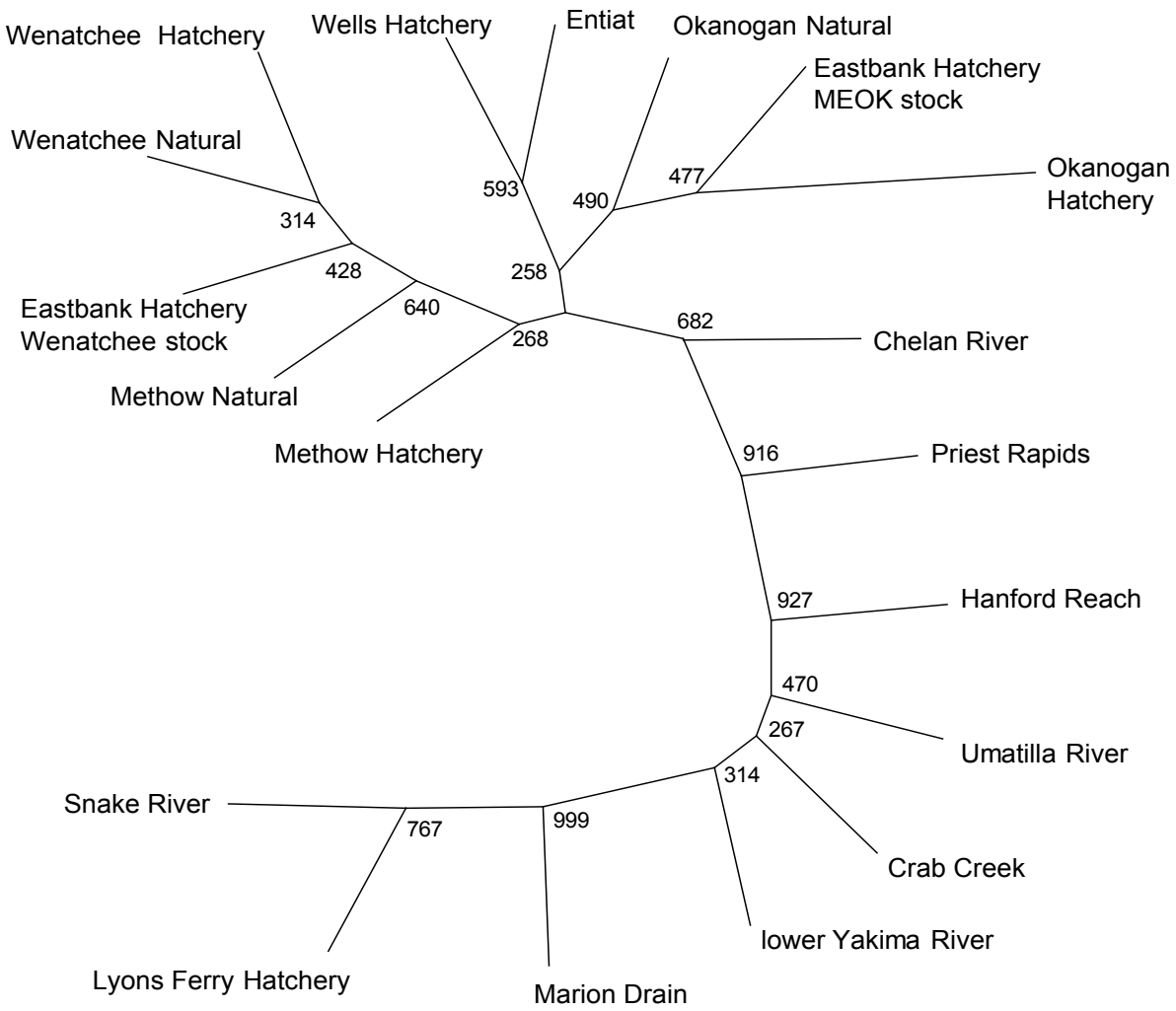

Figure 1. Relationship of natural- and hatchery-origin Chinook collections from the upper Columbia River basin using Cavalli-Sforza and Edwards (1967) chord distance. Bootstrap values are shown at each node.

Appendix O

Summer Chinook Spawning Ground Surveys in the Methow River Basin and Chelan River, 2016

BioAnalysts, Inc.℠
4725 North Cloverdale Road, Ste. 102
Boise ID 83713

March 10, 2017

To: Chelan and Grant Public Utility Districts

From: Denny Snyder and Mark Miller

Re: 2016 Summer Chinook Spawning Ground Surveys in the Methow Basin and Chelan River.

The purpose of this memo is to provide information on the supplemented natural spawning population of summer Chinook in the Methow and Chelan River basins. This work is part of a larger effort focused on monitoring and evaluating Grant and Chelan PUDs' hatchery supplementation programs. The tasks and objectives associated with implementing Grant and Chelan PUDs' Hatchery M&E Plan for 2016 are outlined in Hillman et al. (2013). In 2016, The Okanogan Basin was surveyed by the Colville Confederated Tribes (CCT).

METHODS

Spawning ground surveys were conducted by foot and raft beginning the third week of September and ending late-November. We did not use aerial surveys on the Methow River because past work has demonstrated that ground counts were more accurate than aerial surveys (Miller and Hillman 1997). Ground surveys were used to provide more accurate counts and a complete census of Chinook redds within their spawning distribution. Observers floated or walked through sampling reaches and recorded the location and numbers of redds each week (see Figures 1 and 2). Observers recorded the date, water temperature, river mile, and prepared a drawing of the area where redds were located. A different symbol was used each week to record the number of new and incomplete redds.

To maintain consistency, at least one observer surveyed the same stream reach on successive dates. In areas where numerous summer Chinook spawn, we constructed detailed maps of the river and used the cell-area-method (Hamilton and Bergersen 1984) to identify the number of redds within each cell. Cells were bound by noticeable landmarks along the banks (e.g., bridges or trees) or at stream habitat boundaries (e.g., transitions between pools and riffles). The number of redds were then recorded in the corresponding grid on the map. When possible, observers estimated the number of redds in a large disturbed area by counting females that defended redds. We assumed that the area or territory defended by a female was one redd.

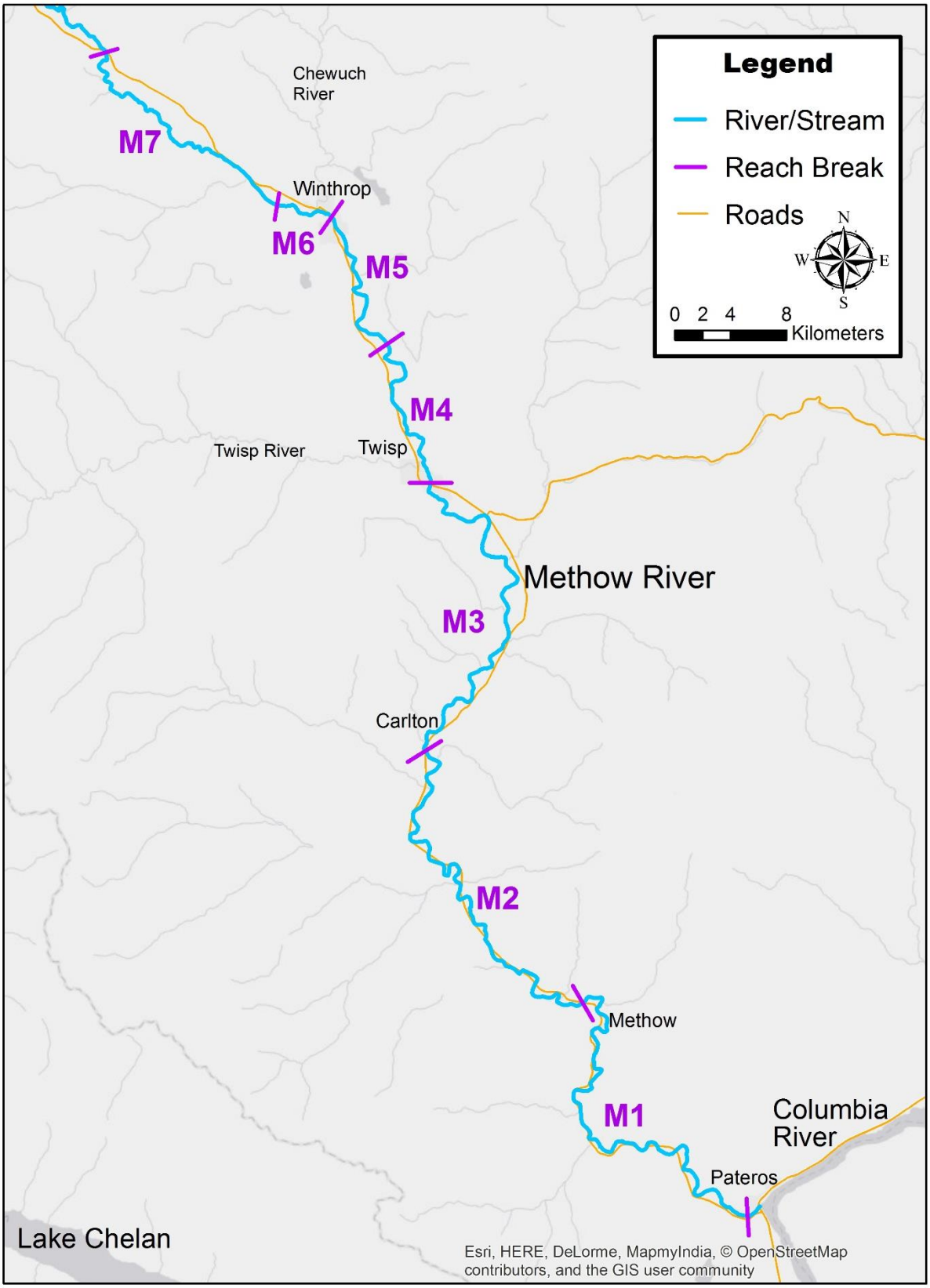

Figure 1. Summer Chinook survey reaches on the Methow River, 2016,

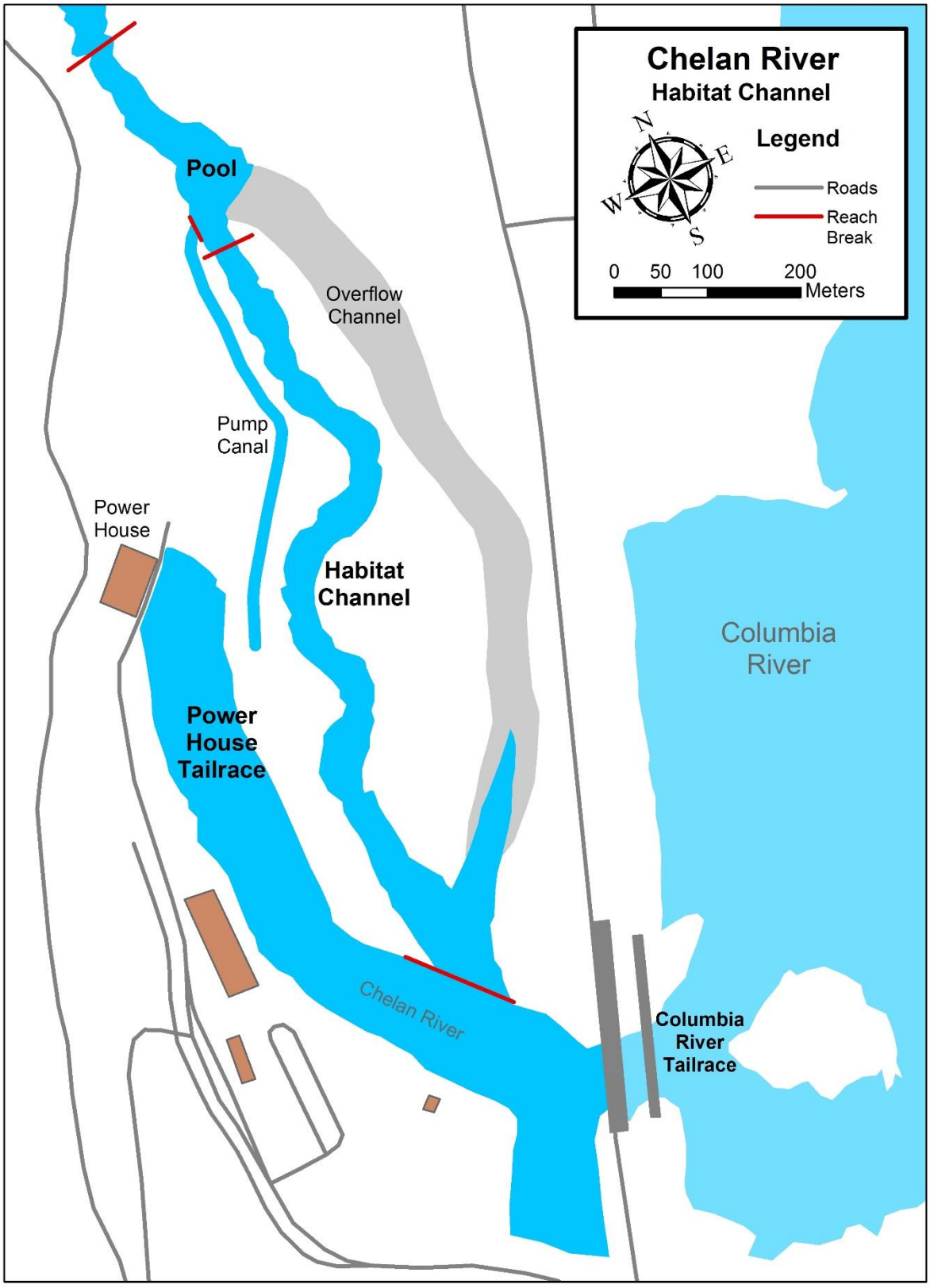

Figure 2. Summer Chinook survey areas on the Chelan River, 2016.

Spawning escapement was estimated as the number of redds times the sex ratio observed at Wells Dam during broodstock collection. In 2016, reach M1 experienced some clarity issues during spawning surveys. Turbidity noticeably increased during rainstorm events and was probably influenced by the Carlton Complex Fires and landslides that occurred in 2014.

Carcasses of summer Chinook were sampled to describe the spawning population. Biological data collection included: scale samples for age analysis, length measurements (POH and FKL), sex, egg voidance, marks, and presence of PIT tags. These data will be used to assess length-at-age, size-at-age, egg voidance, origin (hatchery or naturally produced), and stray rates. No DNA samples were collected on summer Chinook this year. In this report, we only report the number of redds counted in the Okanogan Basin.

RESULTS

Methow

There were 1,115 summer Chinook redds counted within seven reaches on the Methow River (Table 1). Most redds (81%) were located in reaches from the mouth to the town of Twisp (M1-M3). Estimated escapement based on expansion of redd counts from the sex-ratio observed at Wells Dam during broodstock collection indicates that 2,241 summer Chinook (1,115 redds x 2.01 fish/redd) spawned in the Methow River.

Table 1. Number of summer Chinook redds observed each week within the Methow River, 2016. Dashes (--) indicate that no survey occurred.

Reach	Location (Rkm)	Sep		Oct				Nov				Dec	Total	Percent
		18-24	25-1	2-8	9-15	16-22	23-29	30-5	6-12	13-19	20-26	27-3		
		39	40	41	42	43	44	45	46	47	48	49		
M1	0.0-23.8	--	0	3	47	13	54	42	22	1	0	--	182	16
M2	23.8-43.8	--	5	71	146	64	11	8	4	0	--	--	309	28
M3	43.8-63.7	--	6	131	208	48	16	1	--	--	--	--	410	37
M4	63.7-72.3	--	0	12	31	14	0	--	--	--	--	--	57	5
M5	72.3-80.1	--	0	51	70	26	0	--	--	--	--	--	147	13
M6	80.1-83.0	--	0	0	1	0	--	--	--	--	--	--	1	0
M7	83.0-96.1	--	4	5	0	0	0	--	--	--	--	--	9	1
Total:		**--**	**15**	**273**	**503**	**165**	**81**	**51**	**26**	**1**	**0**	**--**	**1,115**	**100**

Time of spawning was assessed as the number of new redds counted each week in the Methow River. Spawning began the last week of September, peaked in early October, and ended the third week of November (Figure 3). Stream temperatures in the Methow River varied from 10.5-11.0°C in September when spawning began. Spawning peaked the first week of October in Reach M7, while peak spawning occurred in reaches M2-M6 the second week of October. Spawning peaked

the fourth week of October in reach M1 (Table 1). This was the sixth highest redd count observed in the last 26 years for the Methow River (Appendix A).

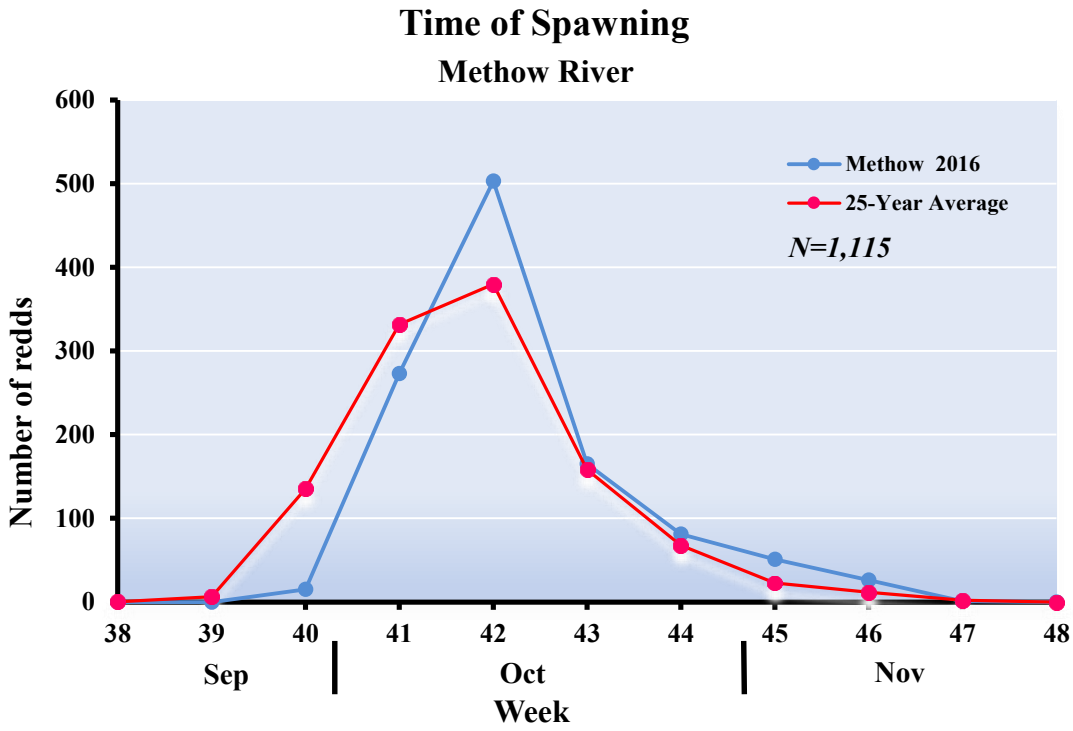

Figure 3. Number of new redds counted each week from late September to late-November in the Methow River, 2016. The figure shows the beginning, peak, and end of spawning for summer Chinook in the Methow River compared to a 25-year average (1991-2015).

There were 587 summer Chinook salmon carcasses sampled within the seven reaches on the Methow River (Table 2). The presence or absence of an adipose fin could not be determined on one fish. Twenty-six percent of the fish returning to the Methow River were sampled based on the estimated escapement of 2,241 summer Chinook. Ad-clipped hatchery fish made up 32% and naturally produced fish (adipose fin present) made up 68% of the fish sampled (Table 2).

Table 2. Number and percent of hatchery (ad-clipped) and naturally produced (ad-present) summer Chinook sampled in the Methow River, 2016.

Reach	Location (Rkm)	Ad-Clipped Hatchery				Naturally Produced				Reach Total
		Male	Female	Total	Percent	Male	Female	Total	Percent	
M1	0.0-23.8	15	14	29	35	26	27	53	65	**82**[1]
M2	23.8-43.8	40	17	57	34	64	47	111	66	**168**
M3	43.8-63.7	12	70	82	38	44	90	134	62	**216**
M4	63.7-72.3	5	6	11	25	20	13	33	75	**44**
M5	72.3-80.1	0	7	7	10	15	48	63	90	**70**
M6	80.1-83.0	0	0	0	0	0	1	1	100	**1**
M7	83.0-96.1	0	0	0	0	3	2	5	100	**5**
Total		**72**	**114**	**186**	**32**	**172**	**228**	**400**	**68**	**586**

[1] **Origin of one female carcass in Reach 1 could not be determined.**

Most (90%) of the ad-clipped hatchery fish were located in reaches M1-M3, while naturally produced fish were sampled within all survey reaches (Figure 4). Naturally produced fish made up 100% of the fish sampled in upper reaches (M6 and M7). Female summer Chinook accounted for 58% of the fish sampled in 2016 (Table 2).

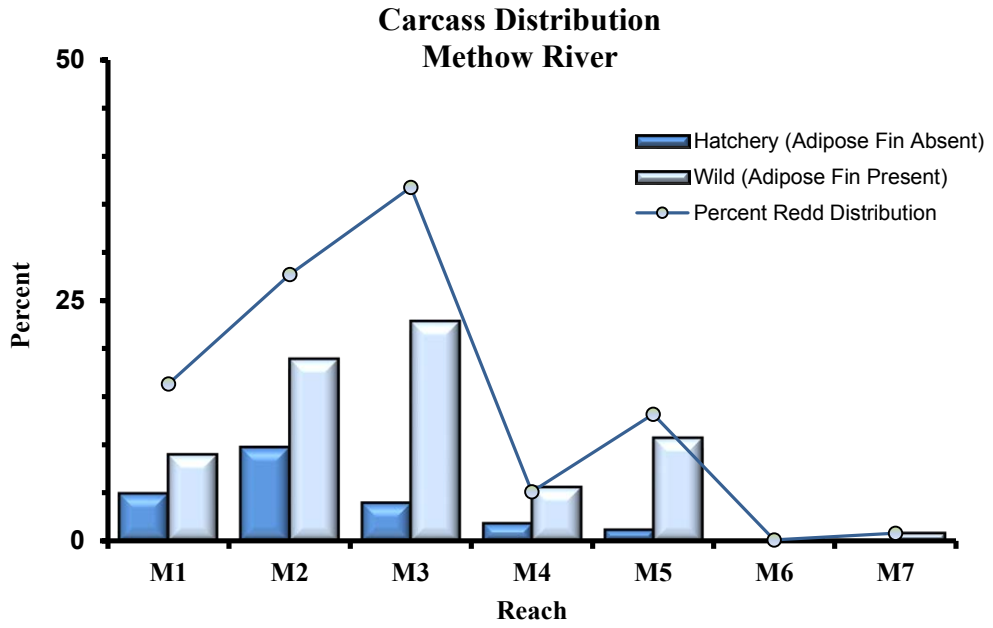

Figure 4. Percent distribution of ad-clipped hatchery and naturally produced fish plotted against the percent distribution of redds observed in reaches on the Methow River, 2016.

Egg voidance was assessed by sampling spawned-out female carcasses. Based on 343 sampled female carcasses, average egg voidance was 98%. Four females (1%) died before spawning (i.e., they retained all their eggs).

Chelan River

There were 448 redds counted in the Chelan River. This is the second highest redd count observed for summer Chinook in the Chelan River since 2000. The majority of spawning occurred in the powerhouse tailrace (46%), habitat channel (24%), and in the Columbia River tailrace (16%) (Table 3). Estimated escapement based on expansion of counts from the sex-ratio observed at Wells Dam during broodstock collection indicates that 900 summer Chinook (448 redds x 2.01 fish/redd) spawned in the Chelan River.

Table 3. Number of summer Chinook redds observed each week within the Chelan and Columbia rivers, 2016. Dashes (--) indicate that no survey occurred.

Reach	Location (Rkm)	Sep		Oct				Nov				Dec	Total	Percent
		18-24	25-1	2-8	9-15	16-22	23-29	30-5	6-12	13-19	20-26	27-3		
		39	40	41	42	43	44	45	46	47	48	49		
Powerhouse Tailrace		--	0	2	28	85	62	21	8	1	0	0	207	46
Columbia R. Tailrace		--	0	0	1	30	31	10	2	0	0	0	74	16
Pool		--	0	1	22	16	14	5	2	1	0	0	61	14
Habitat Channel		--	0	2	21	38	30	11	3	1	0	0	106	24
Total:		**--**	**0**	**5**	**72**	**169**	**137**	**47**	**15**	**3**	**0**	**0**	**448**	**100**

Time of spawning was assessed as the number of new redds counted each week in the Chelan River. Spawning activity began the first week of October and peaked two weeks later (Figure 5). Spawning ended the third week of November. An exceptionally high redd count in 2013 (792 redds) and late spawning in 2014 currently influence the average time of spawning. As more years of information are collected, average time of spawning will likely not appear bimodal.

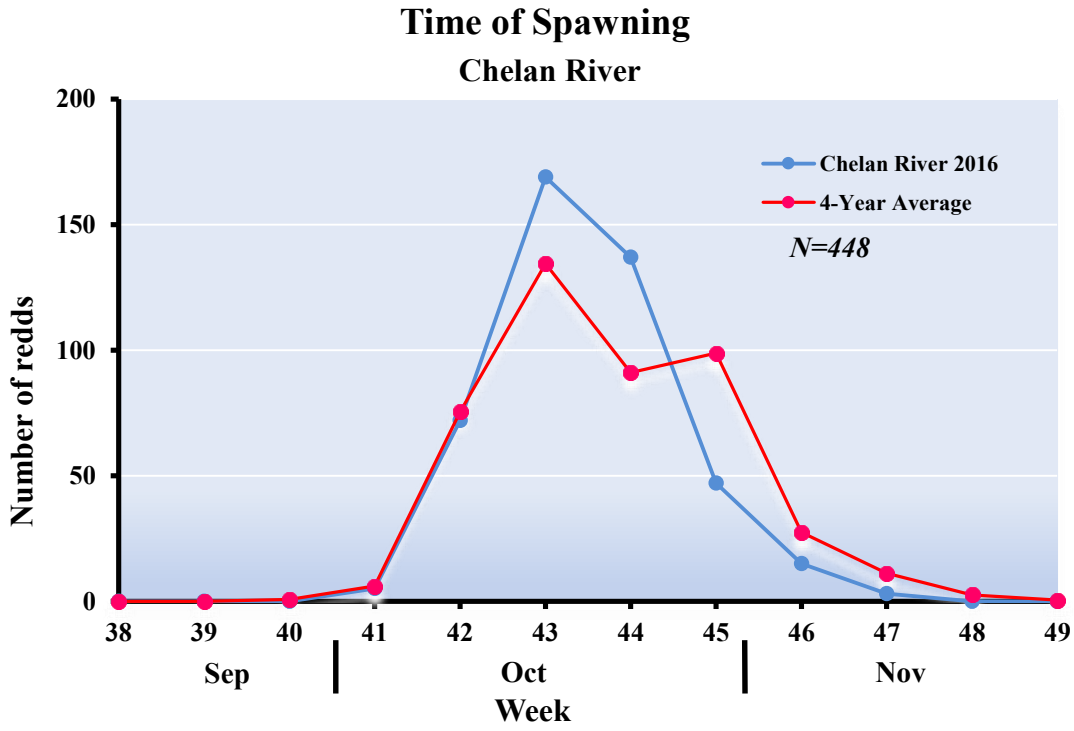

Figure 5. Number of new summer Chinook redds counted each week in the Chelan River from late September to mid-November. The figure displays the beginning, peak, and end of spawning for summer Chinook in the Chelan River in 2016 compared to a 4-year average (2012-2015).

There were 253 summer Chinook carcasses sampled in the Chelan River (Table 4). Twenty-eight percent of the summer Chinook returning to the Chelan River were sampled based on the estimated spawning escapement of 900 fish. Based on the absence of their adipose fin, hatchery fish made up 52% and naturally produced (ad-present) fish made up 48% of the fish examined. Females made up 73% of the carcasses examined (Table 4).

Table 4. Number and percent of hatchery (ad-clipped) and naturally produced (ad-present) summer Chinook collected in the Chelan River, 2016. The origin of one fish sampled could not be determined in the Chelan River.

Reach	Location (Rkm)	Ad-Clipped Hatchery				Naturally Produced				Reach Total
		Male	Female	Total	Percent	Male	Female	Total	Percent	
Powerhouse Tailrace		0	8	8	30	4	15	19	70	**27**
Columbia R. Tailrace		21	43	64	50	12	52	64	50	**128**
Pool		10	15	25	73	3	6	9	27	**34**
Habitat Channel		9	26	35	56	9	19	28	44	**63[1]**
Total		**40**	**92**	**132**	**52**	**28**	**92**	**120**	**48**	**253**

[1] **Origin of one female carcass in habitat channel could not be assigned.**

The distribution of ad-clipped hatchery fish and naturally produced fish varied within the Chelan River (Figure 6). A disproportionate number of fish (compared to redds counts) were sampled in the Columbia River tailrace. This likely occurred because carcasses drifted from upstream spawning areas and settle in the Columbia River tailrace. More hatchery fish were sampled in the habitat channel and pool upstream. Conversely, more wild fish were sampled in the powerhouse tailrace than hatchery summer Chinook.

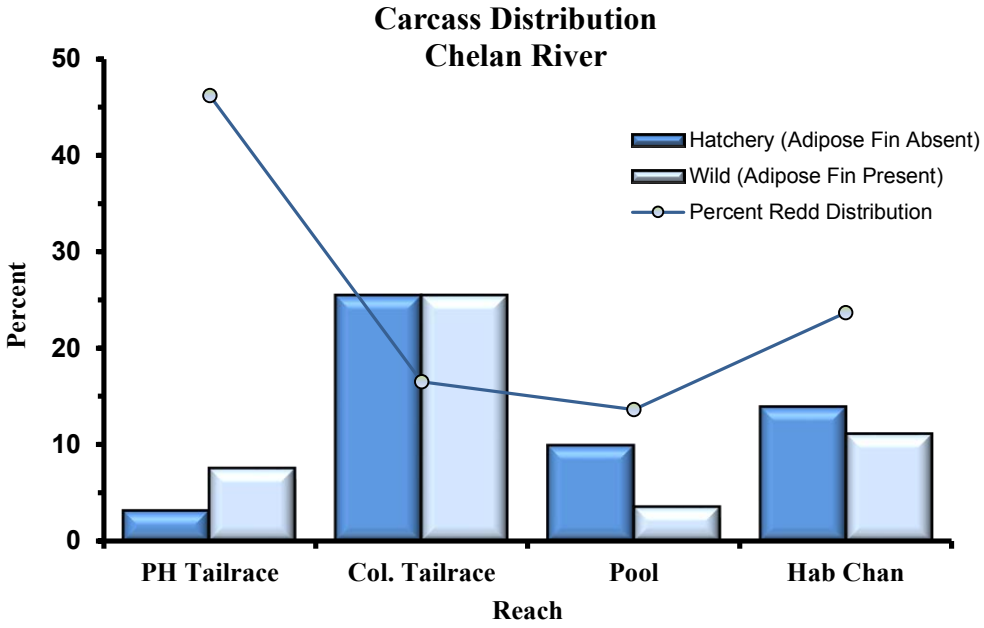

Figure 6. Percent distribution of ad-clipped hatchery and naturally produced fish plotted against the percent distribution of redds observed in reaches on the Chelan River, 2016.

In 2016, about 50 summer Chinook were collected as broodstock from the pool area upstream from the habitat channel.

Mean egg voidance assessed from 181 female carcasses was 81%. Egg voidance from four females could not be determined and seventeen females (17%) died before spawning. No Coho were sampled in 2016.

Okanogan Basin

In 2016, CCT conducted summer Chinook surveys in the Okanogan River basin. A total of 5,276 redds were counted in the Okanogan Basin (Personal Communication, Andrea Pearl, CCT).

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Appendix A. Historical aerial and ground redd counts of summer Chinook in the Methow, Chelan, Okanogan, and Similkameen rivers, 1956-2016.

Year	Methow		Okanogan		Similkameen		Chelan	
	Aerial	Ground	Aerial	Ground	Aerial	Ground	Aerial	Ground
1956	109	--	37	--	30	--	--	--
1957	451	--	53	--	30	--	--	--
1958	335	--	94	--	31	--	--	--
1959	130	--	50	--	23	--	--	--
1960	194	--	29	--	--	--	--	--
1961	120	--	--	--	--	--	--	--
1962	678	--	--	--	17	--	--	--
1963	298	--	9	--	51	--	--	--
1964	795	--	112	--	67	--	--	--
1965	562	--	109	--	154	--	--	--
1966	1,275	--	389	--	77	--	--	--
1967	733	--	149	--	107	--	--	--
1968	659	--	232	--	83	--	--	--
1969	329	--	103	--	357	--	--	--
1970	705	--	656	--	210	--	--	--
1971	562	--	310	--	55	--	--	--
1972	325	--	182	--	64	--	--	--
1973	366	--	138	--	130	--	--	--
1974	223	--	112	--	201	--	--	--
1975	432	--	273	--	184	--	--	--
1976	191	--	107	--	139	--	--	--
1977	365	--	276	--	268	--	--	--
1978	507	--	195	--	268	--	--	--
1979	622	--	173	--	138	--	--	--
1980	345	--	118	--	172	--	--	--
1981	195	--	55	--	121	--	--	--
1982	142	--	23	--	56	--	--	--
1983	65	--	36	--	57	--	--	--
1984	162	--	235	--	301	--	--	--
1985	164	--	138	--	309	--	--	--
1986	169	--	197	--	300	--	--	--
1987	211	--	201	--	164	--	--	--
1988	123	--	113	--	191	--	--	--
1989	126	--	134	--	221	370	--	--
1990	229	--	88	47	94	147	--	--
1991	--	153	55	64	68	91	--	--
1992	--	107	35	53	48	57	--	--
1993	--	154	144	162	152	288	--	--
1994	--	310	372	375	463	777	--	--
1995	--	357	260	267	337	616	--	--

Year	Methow		Okanogan		Similkameen		Chelan	
	Aerial	Ground	Aerial	Ground	Aerial	Ground	Aerial	Ground
1996	--	181	100	116	252	419	--	--
1997	--	205	149	158	297	486	--	--
1998	--	225	75	88	238	276	--	--
1999	--	448	222	369	903	1,275	--	--
2000	--	500	384	549	549	993	--	196
2001	--	675	883	1,108	865	1,540	--	240
2002	--	2,013	1,958	2,667	2,000	3,358	--	253
2003	--	1,624	1,099	1,035	103	378	--	173
2004	--	973	1,310	1,327	2,127	1,660	--	185
2005	--	874	1,084	1,611	1,111	1,423	--	179
2006	--	1,353	1,857	2,592	1,337	1,666	--	208
2007	--	620	1,265	1,301	523	707	--	86
2008	--	599	1,019	1,146	673	1,000	--	153
2009	--	692	1,109	1,672	907	1,298	--	246
2010	--	887	688	1,011	642	1,107	--	398
2011	--	941	1,203	1,714	1,047	1,409	--	413
2012	--	960	1,170	1,613	762	1,066	--	426
2013	--	1,551	NA	2,267	NA	1,280	--	729
2014	--	591	NA	2,231	NA	2,022	--	400
2015	--	1,231	NA	4,276[1]	NA	--	--	448
2016	--	1,115	729	2757	141	1649		448

[1.] The redd count is for the entire Okanogan Basin (Similkameen + Okanogan rivers).