Appendix A

Abundance and Total Numbers of Chinook Salmon and Trout in the Chiwawa River basin, Washington, 2015

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TO: HCP Hatchery Committee

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Subject: Abundance and Total Numbers of Chinook Salmon and Trout in the Chiwawa River basin, Washington, 2015

The Chelan County Public Utility District (PUD) hatchery program is operated through a habitat conservation program (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 and September 2015. This was the 23rd 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-

[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.

8.55), respectively (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 199 randomly selected sites.

During sampling in August 2015, discharge in the Chiwawa River averaged 108 cubic feet per second (cfs) and ranged from 89-137 cfs (Figure 2). Stream temperatures during the study period ranged from 8.0 to 20.0ºC. Fish species observed in the Chiwawa River basin and reference areas during the 1992-2015 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 2015 survey were produced from 485 redds counted in the fall of 2014 (Hillman et al. 2015). Assuming a mean fecundity of 4,045 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 1,961,825 eggs in 2014 (Appendix A).

In 2015, riffles made up the largest fraction of habitat types in reaches of the Chiwawa River basin (53% of the total stream surface area) (Table 1). Pools (24%), glides (7%), and multiple channels (16%) constituted the remaining 47% 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 111,224 (±7% of the estimated total) in the Chiwawa River basin in August 2015 (Table 2). Extrapolating based on volume of habitat types, age-0 Chinook numbered 97,358 (±7%) in the Chiwawa River basin. About 7% of the juvenile Chinook were in tributaries to the Chiwawa River. During the 1992-2015 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 1992-2014 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 2014 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 1992-2015 includes only 23 years of sampling because there was no sampling in 2000.

associated with woody debris in multiple channels (multiple channel use index = 2.80)[3]. These sites (multiple channels) made up 16% of the total surface area of the Chiwawa River basin, but they provided habitat for 63% of all the age-0 Chinook in the basin in 2015 (Appendix C). In contrast, riffles made up 53% of the total surface area, but provided habitat for only 5% of all age-0 Chinook in the Chiwawa River basin (riffle use index = 0.25). Pools made up 24% of the total surface area and provided habitat for 31% of all age-0 Chinook in the basin (pool use index = 1.58). Few Chinook used glides that lacked woody debris (glide use index = 0.26).

As noted earlier, we assumed that the Chiwawa River was seeded with 1,961,825 Chinook eggs (485 redds times 4,045 eggs/female) in fall, 2014, and that at least 111,224 of those survived to August 2015. This means that the egg-to-parr survival was at least 5.7% (95% confidence bound 5.2-6.1%). During 1992-2015, egg-to-parr survival averaged 8.1% (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 620 (±43% of the estimated total) age-1+ Chinook salmon in the Chiwawa River basin in August 2015 (Table 3). In August 1992-2015, 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 Rock 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 of the use index 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:

$$Juveniles = \frac{(148{,}410 \times Redds)}{(184 + Redds)}$$

where the bootstrap estimated standard errors for the two parameters were 17,021 and 55, respectively. The adjusted $R^2 = 0.84$. The second-best model was the smooth hockey stick model, which was 1.64 AIC_c units from the best model (Table 4; Figure 6). The estimated parameters for this model were:

$$LN(Juveniles) = 11.6 + LN\left(1 - e^{-\left(\frac{723.8}{113{,}413}\right)Redds}\right)$$

where the bootstrap estimated standard errors of the two parameters were 0.1 and 136, 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 would limit the capacity of juvenile Chinook to about 180,000 parr in the basin (bootstrap upper 95% CI of $\boldsymbol{\alpha}$ in the Beverton-Holt model). This equates to about 1,621 Chinook parr per hectare. In contrast, the smooth hockey stick model, which fit the data as well as the Beverton-Holt model, would limit the carrying capacity for juvenile Chinook to about 140,000 parr (bootstrap upper 95% CI of $\boldsymbol{J_\infty}$ in the smooth hockey stick model). This equates to about 1,261 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 10,208 (±11% of the estimated total) age-0 steelhead/rainbow (<4 in) in reaches of the Chiwawa River basin in August 2015 (Table 5). During the 1992-2015 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 1992-2015, 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 754 (±26% of the estimated total) age-1+ steelhead/rainbow (4-8 in) lived in reaches of the Chiwawa River basin in August 2015 (Table 6). This was the lowest number of age-1+ steelhead/rainbow that we recorded during the more than 20-year survey period. During the survey period 1992-2015, 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

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

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 18 (±106% of the estimated total) in the Chiwawa River basin in August 2015 (Table 7). During the period 1992-2015, 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 239 (±17% of the estimated total) juvenile (2-8 in) bull trout lived in reaches of the Chiwawa River basin in August 2015 (Table 8). We found most of these fish in the upper-most reaches of the Chiwawa River and in Rock Creek. During 1992-2015, 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.[5] 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. Of the reaches we surveyed, they were most numerous in Reaches 7-10 on the Chiwawa River. In 2015, they occurred 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 themselves. As a result, she found it difficult to accurately estimate their numbers. Although this implies that we underestimated numbers of juvenile bull trout in the Chiwawa River, the relative

[5] 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.

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 2,286 (±14% of the estimated total) adult (>8 in) bull trout in reaches of the Chiwawa River basin in August 2015 (Table 9). This was the greatest number of adult bull trout that we recorded during the more than 20-year survey period. In previous years, numbers ranged from 76 to 900 (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 2015, we estimated that at least 28 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. Brook trout occurred in the lower seven reaches on the Chiwawa River. In both the Chiwawa and Little Wenatchee rivers, brook trout usually used multiple channels. 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 294 westslope cutthroat trout occurred in the Chiwawa River, Phelps Creek, Nason Creek, and Little Wenatchee River survey areas in August 2015. 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 6,861 adult and 2,145 juvenile whitefish lived in these streams in August 2015. We found few whitefish in most tributaries to the Chiwawa River.

Conclusion

This was the 23rd 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 23-year period. Numbers of juveniles in 2001, 2002, and 2009-2015 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 capacity of the Chiwawa River basin

is between 140,000 to 180,000 spring Chinook parr. This equates to an overall density of about 1,300-1,600 parr per hectare. These densities can be achieved with about 470 redds. Assuming that a female Chinook produces only one redd (Murdoch et al. 2009), a spawning escapement of about 470 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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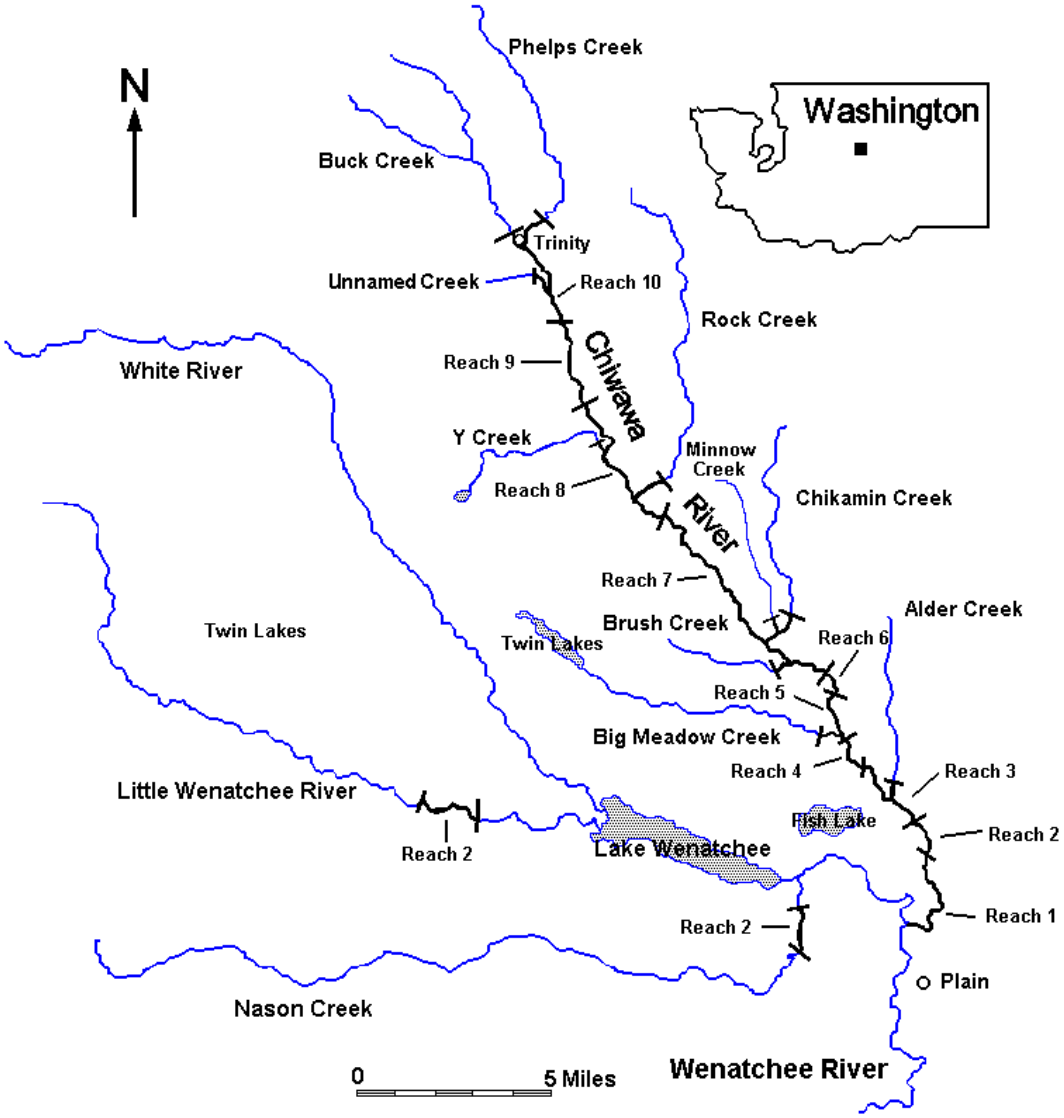

Figure 1. Location of study reaches on the Chiwawa River, and Chikamin, Rock, Big Meadow, Unnamed, Alder, Brush and Phelps creeks, Chelan County, Washington. Reach 2 on Nason Creek and Reach 2 on the Little Wenatchee River were matched with Reaches 3 and 8 on the Chiwawa River, respectively.

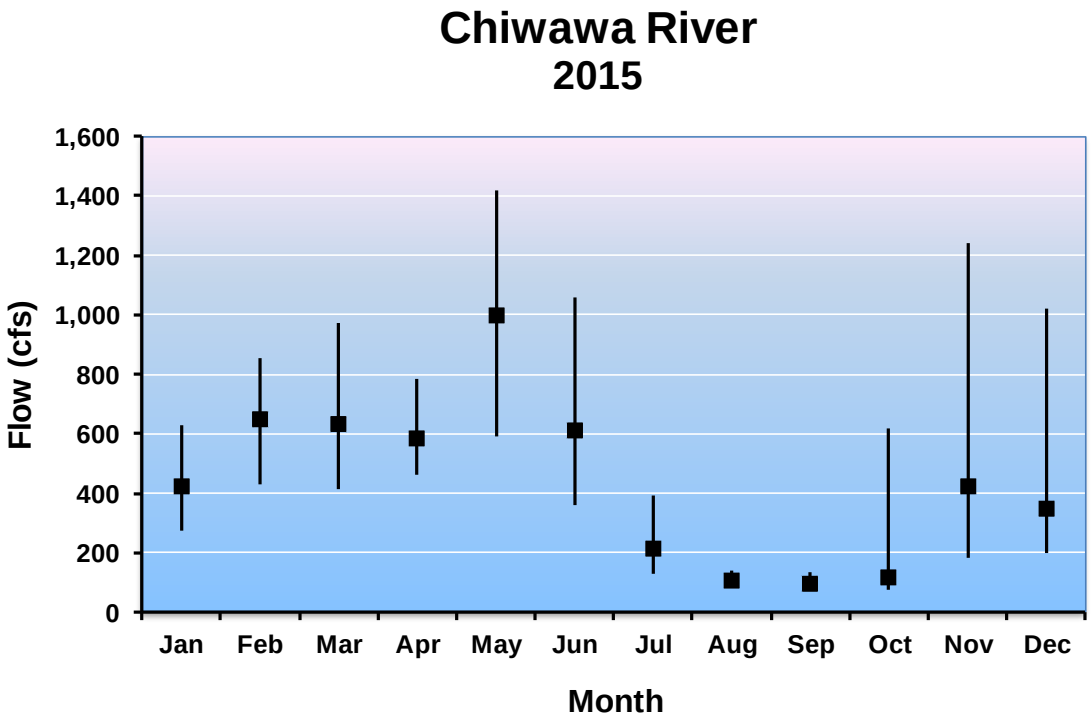

Figure 2. Mean, minimum, and maximum monthly flows in the Chiwawa River for 2015.

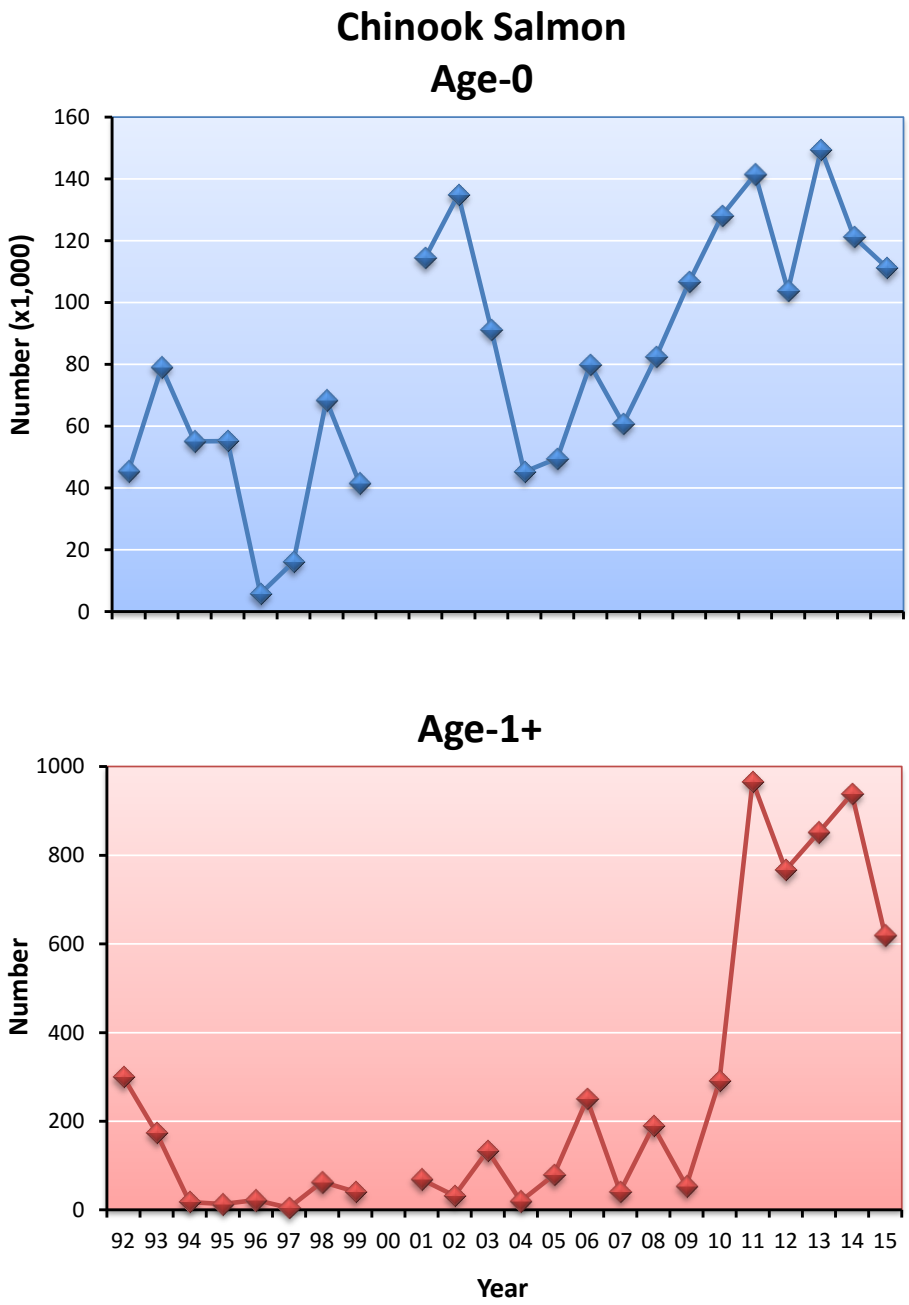

Figure 3. Numbers of age-0 and age-1+ Chinook salmon within the Chiwawa River basin in August 1992-2015; ND = no data.

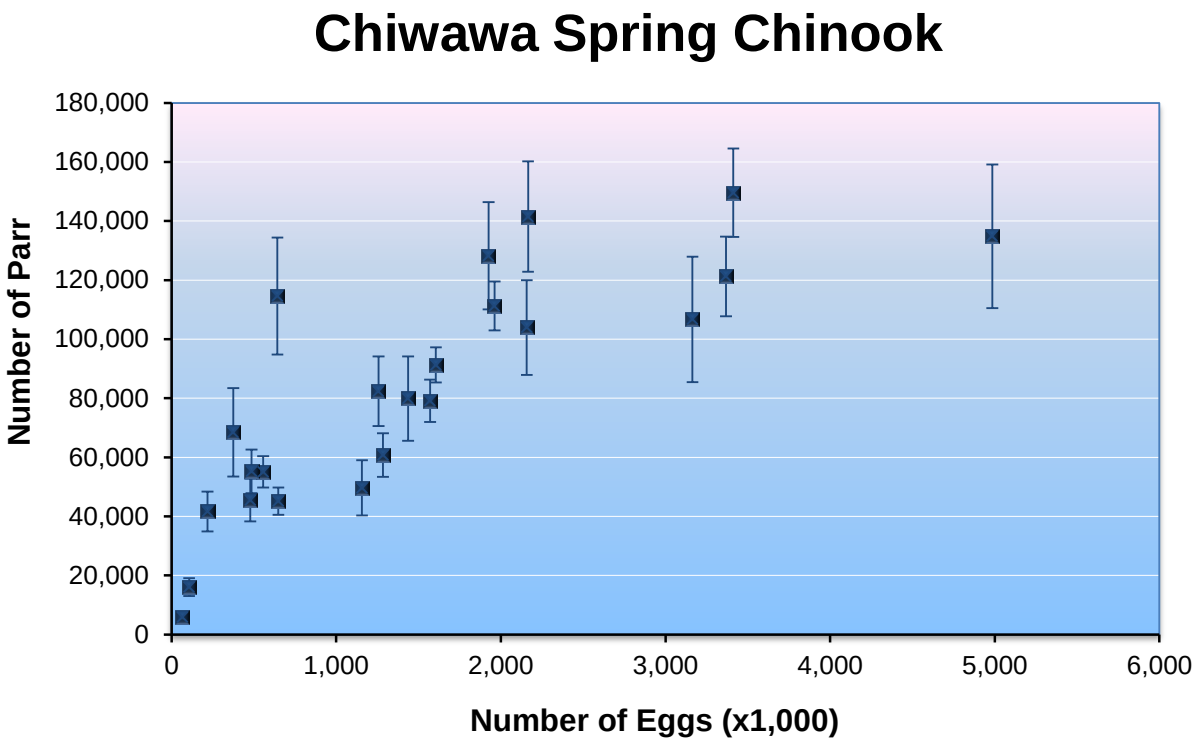

Figure 4. Relationship between total numbers of age-0 Chinook salmon (based on fish/ha) and numbers of eggs in the Chiwawa River basin. Vertical bars indicate 95% confidence bounds.

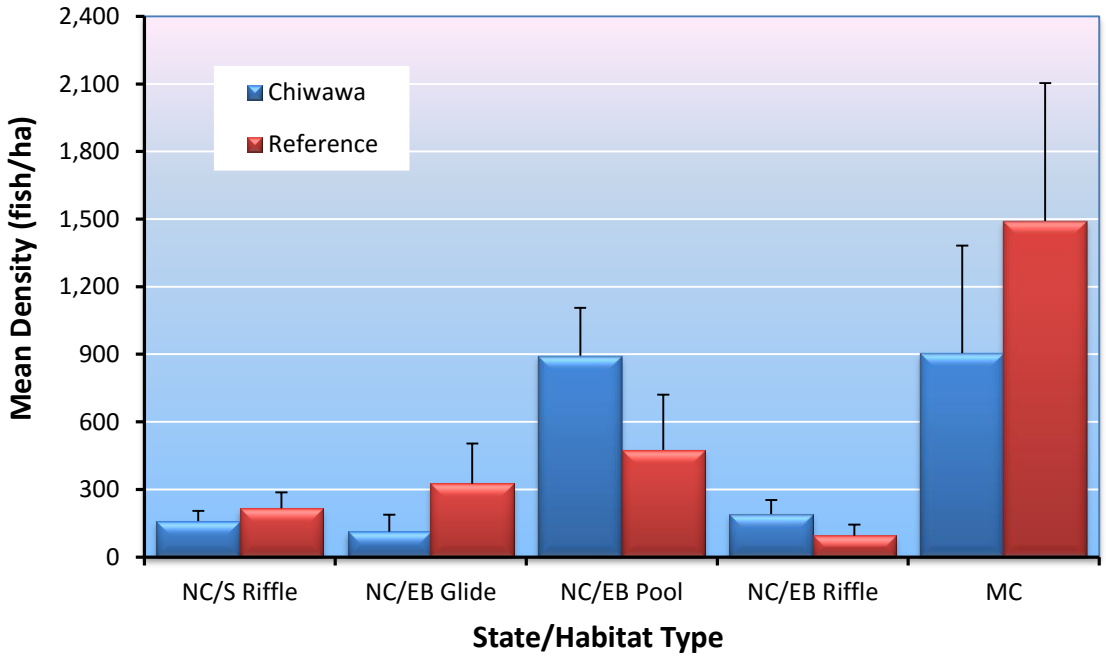

Figure 5. Comparison of the means (95% CI) of age-0 Chinook salmon densities (fish/ha) within state/habitat types in Reaches 3 and 8 of the Chiwawa River and their matched reference areas on Nason Creek and the Little Wenatchee River. There was no sampling in 2000 and no sampling in reference areas in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

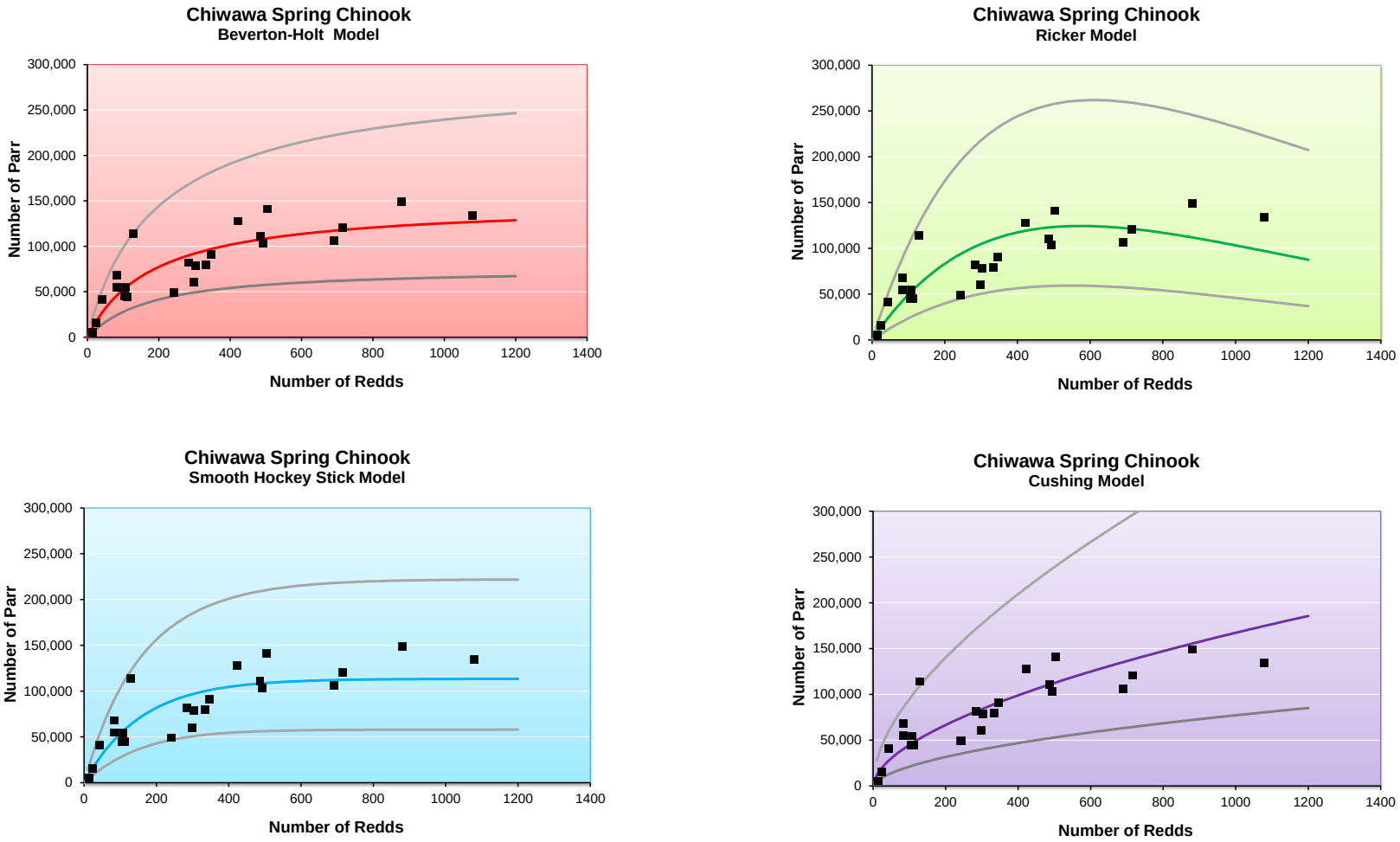

Figure 6. Relationship between numbers of juvenile (age-0) Chinook and redds in the Chiwawa River basin, 1992-2015 (no sampling occurred in 2000). Figures show the fit of the Beverton-Holt model, smooth hockey stick, Ricker model, and the Cushing model to the data. Gray lines indicate the upper and lower 95% C.B.

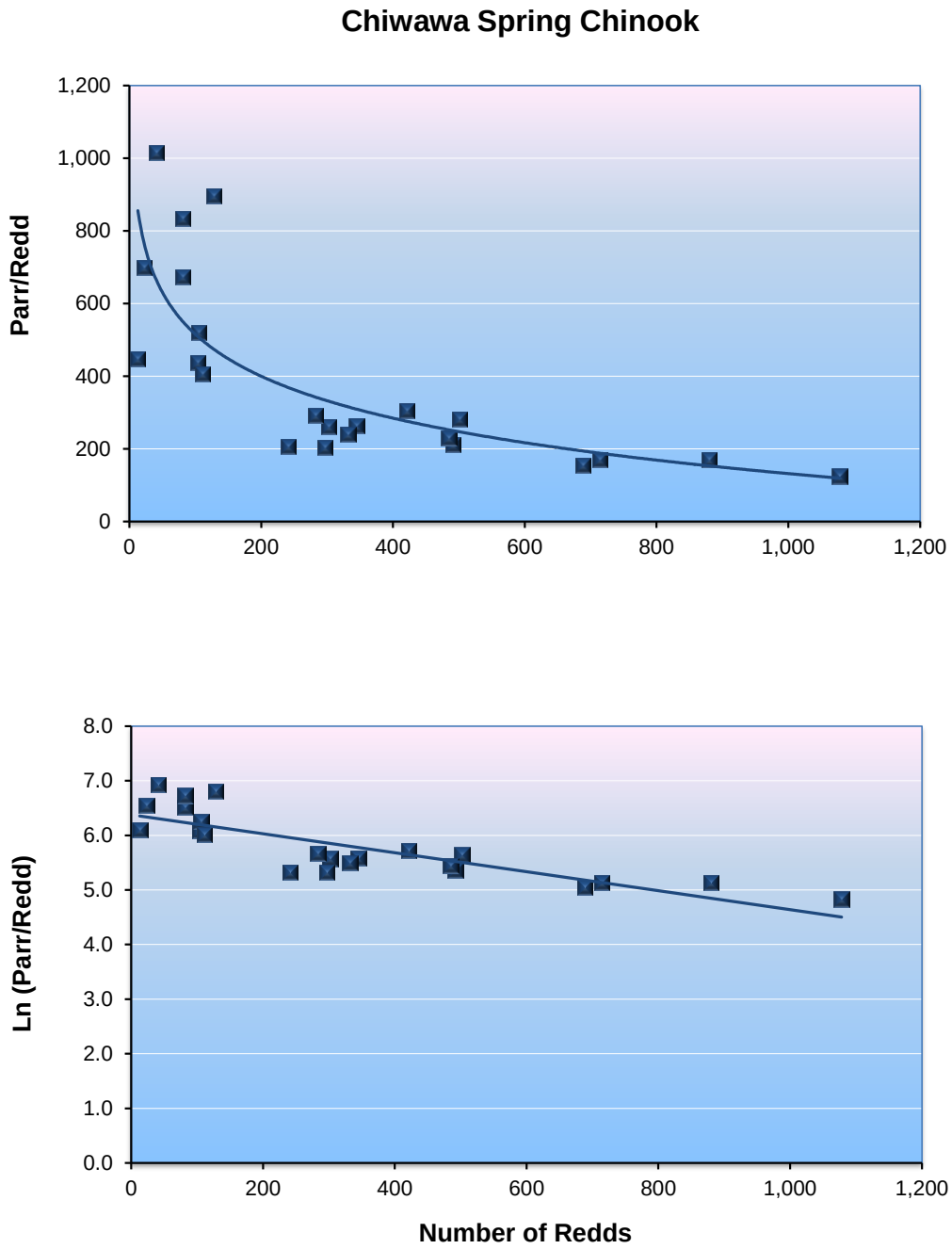

Figure 7. Relationship between parr/redd and numbers of redds (top figure) and natural log parr/redd and numbers of redds (bottom figure) in the Chiwawa River basin, 1992-2015. No sampling was conducted in 2000. Estimates for 1993-2015 included the Chiwawa River and its tributaries; the 1992 estimate 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.

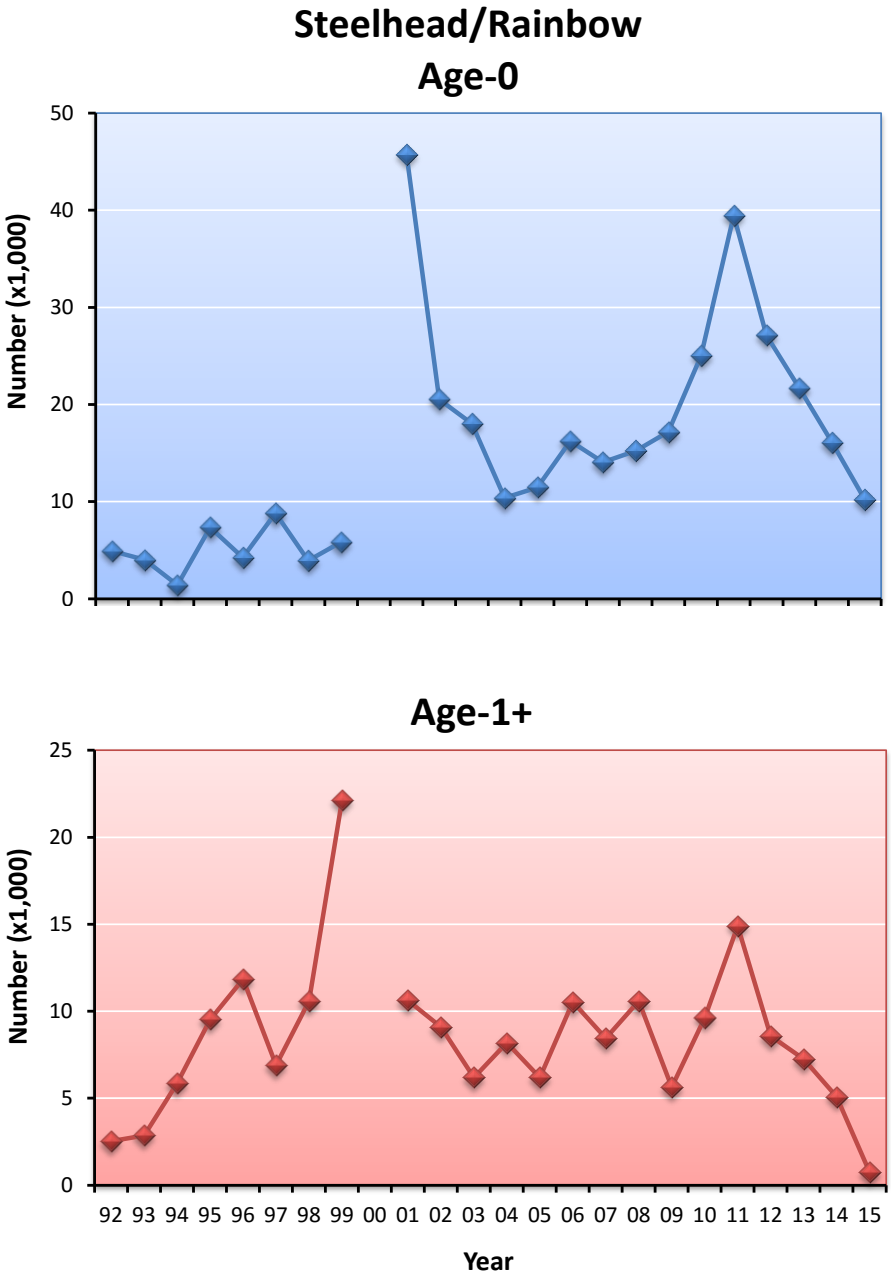

Figure 8. Numbers of age-0 (<4 in) and age-1+ (4-8 in) steelhead/rainbow within the Chiwawa River basin in August 1992-2015; ND = no data.

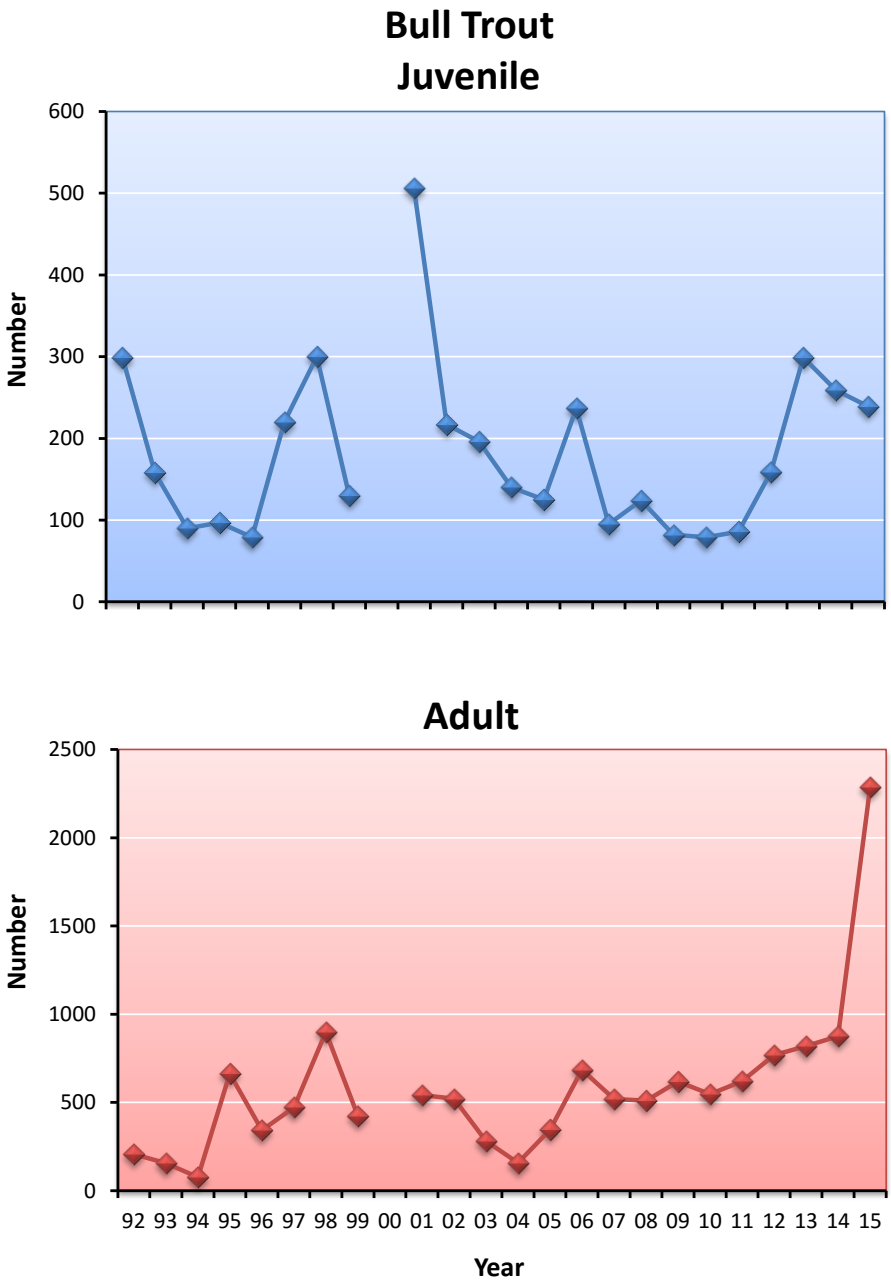

Figure 9. Numbers of juvenile (2-8 inches) and adult (>8 inches) bull trout within the Chiwawa River basin in August 1992-2015; ND = no data.

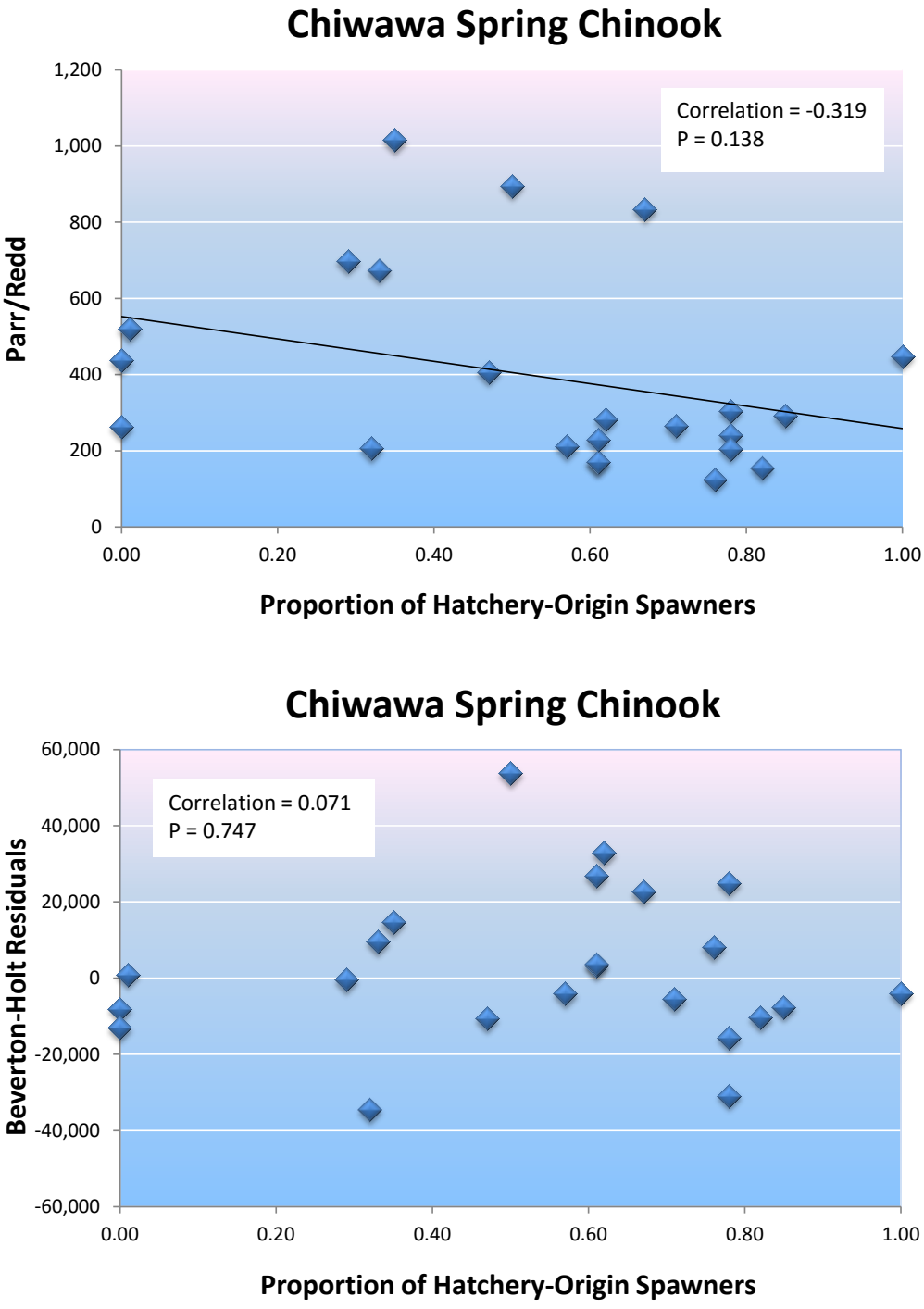

Figure 10. Relationship between juvenile productivity (parr/redd) and the proportion of hatchery-origin spawners (pHOS) (top figure) and the relationship between the residuals from the Beverton-Holt stock/recruitment relationship and pHOS (bottom figure).

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, 2015. 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	0.49	0.49
						NC/EB	P	1.17	0.88
						NC/EB	R	16.60	1.57
2	3.77-5.51	0.010	Glacial Drift over Chumstick Formation	Glacial Canyon	CC Fluvial	NC/EB	G	0.29	0.25
						NC/EB	P	0.70	0.24
						NC/EB	R	6.08	0.58
3	5.51-7.88	0.009	Glacial Drift over Chumstick Formation	Glacial Valley	MCV Alluvial	NC/S	R	4.45	0.70
						NC/EB	G	0.11	0.11
						NC/EB	R	4.13	0.48
						MC	MC	0.38	0.38
4	7.88-8.90	0.007	Glacial Drift over Chumstick Formation	Glacial Canyon	CC Fluvial	NC/EB	P	0.34	0.26
						NC/EB	R	2.34	0.33
						MC	MC	0.39	0.39
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	7.63	0.92
6	10.83-11.80	0.008	Glacial Drift over Chumstick Formation	Glacial Canyon	CC Fluvial	NC/EB	P	0.35	0.35
						NC/EB	R	3.72	0.93
						MC	MC	0.36	0.36
7	11.80-20.03	0.001	Glacial Drift over Chumstick Formation	Glacial Valley	UCV Alluvial	NC	G	1.89	0.44
						NC	P	5.11	0.49
						NC	R	0.71	0.17
						NC/EB	G	2.30	1.20
						NC/EB	P	5.83	1.50
						NC/EB	R	4.20	0.47
						MC	MC	4.05	1.77
8	20.03-25.42	0.003	Glacial Drift over Swakane Gneiss	Glacial Valley	UCV Alluvial	NC/EB	G	2.09	0.85
						NC/EB	P	7.01	2.02
						NC/EB	R	4.46	0.81
						EB	P	0.22	0.22
						EB	R	0.34	0.34
						MC	MC	5.90	2.34
9	25.42-28.81	0.007	Glacial Drift over Swakane Gneiss	Glacial Valley	MCV Alluvial	NC	P	3.92	0.43
						NC	R	2.20	0.47
						MC	MC	2.58	1.10
10	28.81-31.11	0.011	Pre-upper Jurassic Gneiss	Glacial Valley	MCV Alluvial	NC	P	0.47	0.24
						NC	R	1.87	0.27
						MC	MC	3.92	0.28

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	0.40	0.08
						NC	R	0.14	0.06
						NC	MC	0.07	0.07
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	0.14	0.14
Chikamin Creek[1]									
1	0.00-0.94	0.013	Glacial Drift over Chumstick Formation	Glacial Valley	UCV Alluvial	NC	G	0.05	0.05
						NC	P	0.19	0.06
						NC	R	0.32	0.10
						MC	MC	0.14	0.14
Rock Creek									
1	0.00-0.73	0.020	Glacial Drift over Swakane Gneiss	Glacial Valley	UCV Alluvial	NC	P	0.20	0.05
						NC	R	0.37	0.08
						MC	MC	0.10	0.10
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	0.02	0.02
						NC	P	0.10	0.05
						NC	R	0.07	0.02
						NC	MC	0.00	0.00
Alder Creek									
1	0.00-0.01		Glacial Drift over Chumstick Formation	Glacial Valley	MCV Alluvial	NC	P	0.001	0.001
						NC	R	0.006	0.006
Brush Creek									
1	0.00-0.01		Glacial Drift over Chumstick Formation	Glacial Valley	UCV Alluvial	NC	P	0.002	0.002
						NC	R	0.003	0.003
Clear Creek									
1	0.00-0.05		Glacial Drift over Chumstick Formation	Glacial Valley	UCV Alluvial	NC	P	0.003	0.003
						NC	R	0.002	0.002
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 2015.

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	70.3	0.025	1,285	±437	0.34	1,250	±384	0.31
2	111.7	0.027	790	±135	0.17	690	±75	0.11
3	47.5	0.014	431	±22	0.05	471	±21	0.05
4	368.7	0.082	1,132	±66	0.06	1,137	±89	0.08
5	44.2	0.012	343	±27	0.08	377	±21	0.06
6	58.7	0.020	260	±45	0.17	252	±37	0.15
7	728.6	0.113	17,553	±3,979	0.23	15,333	±2,998	0.20
8	743.2	0.135	14,878	±5,167	0.35	13,792	±4,405	0.32
9	1,953.8	0.343	16,998	±4,623	0.27	14,448	±1,710	0.12
10	7,283.8	1.992	50,040	±1,852	0.04	41,690	±2,185	0.05
Phelps Creek								
1	2,035.7	2.074	285	±0	0.00	285	±0	0.00
Chikamin Creek[1]								
1	2,738.6	1.947	1,917	±626	0.33	2,467	±560	0.23
Rock Creek								
1	6,205.9	2.524	4,158	±564	0.14	4,110	±1,875	0.46
Unnamed Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Big Meadow Creek								
1	5,446.2	2.688	1,013	±545	0.54	915	±373	0.41
Alder Creek								
1	10,142.9	11.270	71	±0	0.00	71	±0	0.00
Brush Creek								
1	12,400.00	22.963	62	±0	0.00	62	±0	0.00
Clear Creek								
1	1,600.0	1.404	8	±0	0.00	8	±0	0.00
Y Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Grand Total	**1,001.6**	**0.206**	**111,224**	**±8,280**	**0.07**	**97,358**	**±6,342**	**0.07**

[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 2015.

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	0.9	0.000	16	±21	1.31	15	±10	0.67
2	4.5	0.001	32	±37	1.16	26	±24	0.92
3	0.0	0.000	0	±0	0.00	0	±0	0.00
4	7.5	0.002	23	±0	0.00	23	±0	0.00
5	0.5	0.000	4	±0	0.00	3	±0	0.00
6	0.0	0.000	0	±0	0.00	0	±0	0.00
7	11.9	0.002	286	±227	0.79	244	±140	0.57
8	3.3	0.001	67	±78	1.16	61	±53	0.87
9	6.0	0.001	52	±72	1.38	42	±53	1.26
10	1.2	0.001	8	±11	1.38	10	±6	0.60
Phelps Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Chikamin Creek[1]								
1	31.4	0.025	22	±33	0.00	32	±33	0.00
Rock Creek								
1	164.2	0.066	110	±67	0.61	108	±144	1.33
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	**5.6**	**0.001**	**620**	**±265**	**0.43**	**564**	**±218**	**0.39**

[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 23. 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	-123.272	0.000	1.000	0.663	0.838
Smooth Hockey Stick	3	-121.632	1.640	0.440	0.292	0.826
Gamma[b]	4	-116.473	6.799	0.033	0.022	0.799
Ricker	3	-115.227	8.046	0.018	0.012	0.778
Cushing	3	-115.186	8.087	0.018	0.012	0.770

[a] 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 2015.

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	52.2	0.017	953	±90	0.09	852	±91	0.11
2	58.0	0.015	410	±114	0.28	388	±139	0.36
3	102.4	0.030	929	±17	0.02	988	±9	0.01
4	61.6	0.014	189	±42	0.22	190	±32	0.17
5	46.1	0.013	358	±38	0.11	433	±32	0.07
6	18.7	0.006	83	±15	0.18	78	±11	0.14
7	65.4	0.011	1,575	±689	0.44	1,448	±700	0.48
8	1.7	0.000	35	±42	1.20	31	±28	0.90
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	2,841.4	1.913	1,989	±585	0.29	2,424	±571	0.24
Rock Creek								
1	2,500.0	1.064	1,675	±391	0.23	1,732	±683	0.39
Unnamed Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Big Meadow Creek								
1	9,467.7	4.780	1,761	±446	0.25	1,627	±179	0.11
Alder Creek								
1	24,285.7	26.984	170	±0	0.00	170	±0	0.00
Brush Creek								
1	12,400.0	22.963	62	±0	0.00	62	±0	0.00
Clear Creek								
1	3,800.0	3.333	19	±0	0.00	19	±0	0.00
Y Creek								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Grand Total	**91.9**	**0.022**	**10,208**	**±1,093**	**0.11**	**10,442**	**±1,160**	**0.11**

[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 2015.

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	7.4	0.003	135	±46	0.34	123	±38	0.31
2	2.5	0.001	18	±24	1.33	16	±19	1.19
3	19.3	0.006	175	±31	0.18	206	±22	0.11
4	12.7	0.003	39	±10	0.26	39	±7	0.18
5	14.4	0.004	112	±14	0.13	130	±9	0.07
6	8.1	0.003	36	±14	0.39	33	±11	0.33
7	4.1	0.001	99	±108	1.09	95	±118	1.24
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	57.1	0.032	40	±0	0.00	40	±0	0.00
Rock Creek								
1	149.3	0.060	100	±149	1.49	98	±178	1.82
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	**6.8**	**0.002**	**754**	**±195**	**0.26**	**780**	**±219**	**0.28**

[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 2015.

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	0.7	0.000	13	±19	1.46	15	±5	0.33
2	0.0	0.000	0	±0	0.00	0	±0	0.00
3	0.1	0.000	1	±0	0.00	1	±0	0.00
4	0.3	0.000	1	±0	0.00	1	±0	0.00
5	0.4	0.000	3	±0	0.00	3	±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	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	**0.2**	**0.000**	**18**	**±19**	**1.06**	**20**	**±5**	**0.25**

[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 2015.

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	0.0	0.000	0	±0	0.00	0	±0	0.00
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	0.0	0.000	0	±0	0.00	0	±0	0.00
9	6.9	0.001	60	±34	0.57	55	±27	0.49
10	21.8	0.006	150	±21	0.14	120	±14	0.12
Phelps Creek								
1	35.7	0.036	5	±0	0.00	5	±0	0.00
Chikamin Creek[1]								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Rock Creek								
1	35.8	0.002	24	±6	0.25	24	±17	0.71
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	**2.2**	**0.000**	**239**	**±41**	**0.17**	**204**	**±35**	**0.17**

[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 2015.

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	1.3	0.000	24	±16	0.67	20	±6	0.30
2	7.1	0.002	50	±21	0.42	42	±1	0.02
3	0.1	0.000	1	±0	0.00	1	±0	0.00
4	3.9	0.001	12	±10	0.83	12	±7	0.58
5	1.4	0.000	11	±0	0.00	10	±0	0.00
6	1.1	0.000	5	±0	0.00	5	±0	0.00
7	16.3	0.003	392	±204	0.52	352	±128	0.36
8	9.2	0.002	185	±159	0.86	184	±55	0.30
9	37.4	0.007	325	±64	0.20	283	±47	0.17
10	185.4	0.051	1,274	±169	0.13	1,072	±177	0.17
Phelps Creek								
1	42.9	0.044	6	±0	0.00	6	±0	0.00
Chikamin Creek[1]								
1	0.0	0.000	0	±0	0.00	0	±0	0.00
Rock Creek								
1	1.5	0.001	1	±0	0.00	1	±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	**20.6**	**0.004**	**2,286**	**±316**	**0.14**	**1,988**	**±230**	**0.12**

[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 1991-2014; 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	3,412,184	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
Average	**324**	**1,466,346**	**82,078**	**244**	**8.1**

APPENDIX B. Estimated numbers of salmonids (based on fish/ha) in the Chiwawa River basin, Washington, 1992-2015; 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

[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 1992-2015; 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									**0.08**
Pool	0.22	0.24									**0.19**
Riffle	0.54	0.53									**0.53**
M. Chan	0.17	0.16									**0.20**
Fraction of all age-0 Chinook within habitat types											
Glide	0.01	0.01									**0.02**
Pool	0.37	0.31									**0.30**
Riffle	0.11	0.05									**0.13**
M. Chan	0.51	0.63									**0.55**
Densities of age-0 Chinook within habitat types (fish/ha)											
Glide	133	66									**171**
Pool	1,569	1,300									**1,048**
Riffle	190	98									**163**
M. Chan	2,957	3,768									**1,855**
Number of age-0 Chinook within habitat types											
Glide	1,120	518									**1,745**
Pool	44,321	34,993									**24,908**
Riffle	13,085	6,017									**10,899**
M. Chan	62,713	69,969									**45,515**

Appendix B

Fish Trapping at the Chiwawa and Wenatchee Smolt Traps during 2015

Monitoring Juvenile Salmonids in the Wenatchee River Subbasin: Activities in the Chiwawa River and Lower Wenatchee River during 2015

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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 gathers 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 subbasin 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 screw 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 (cms); 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 at river kilometer (rkm) 76, about 9 km downstream of Lake Wenatchee (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.

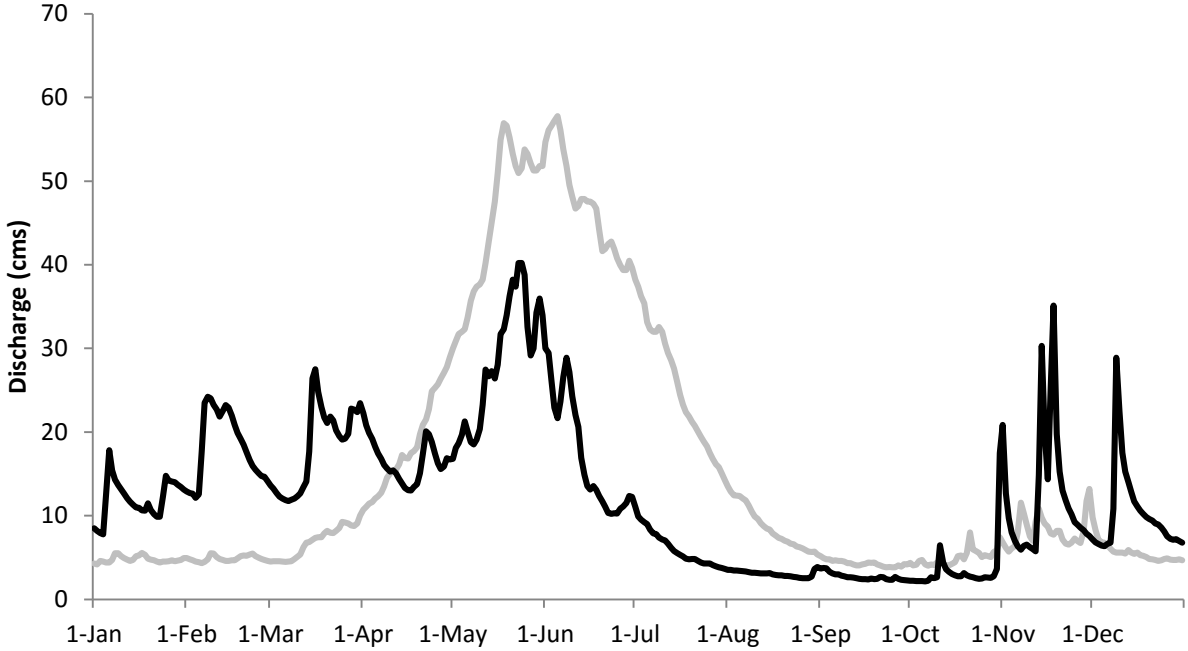

Figure 1. Discharge of the Chiwawa River at Plain, USGS gauge # 12456500. Black line represents 2015 discharge and grey line represents mean discharge from 1990-2014.

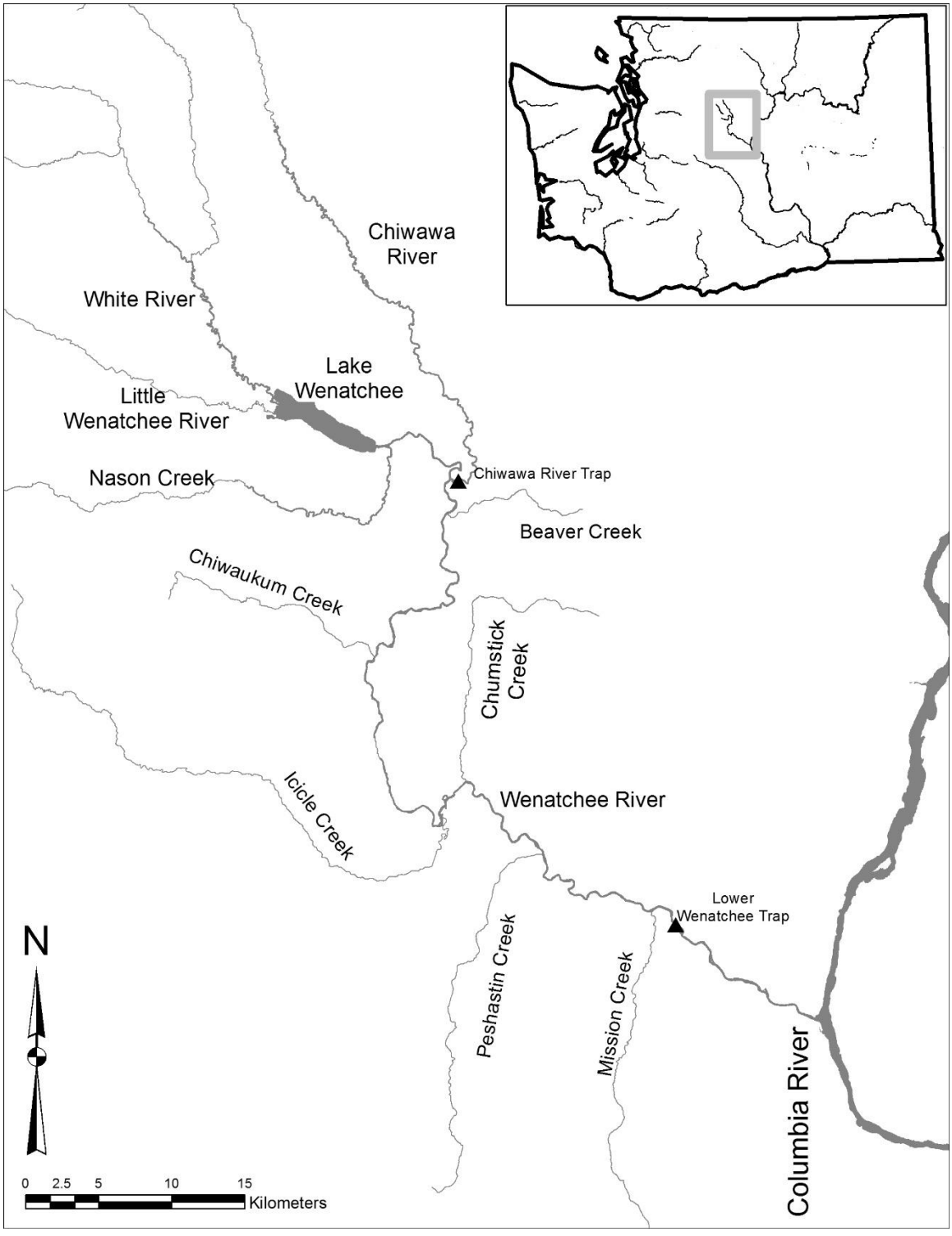

Figure 2. Wenatchee River subbasin (with rotary screw trap locations).

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 cms. 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.

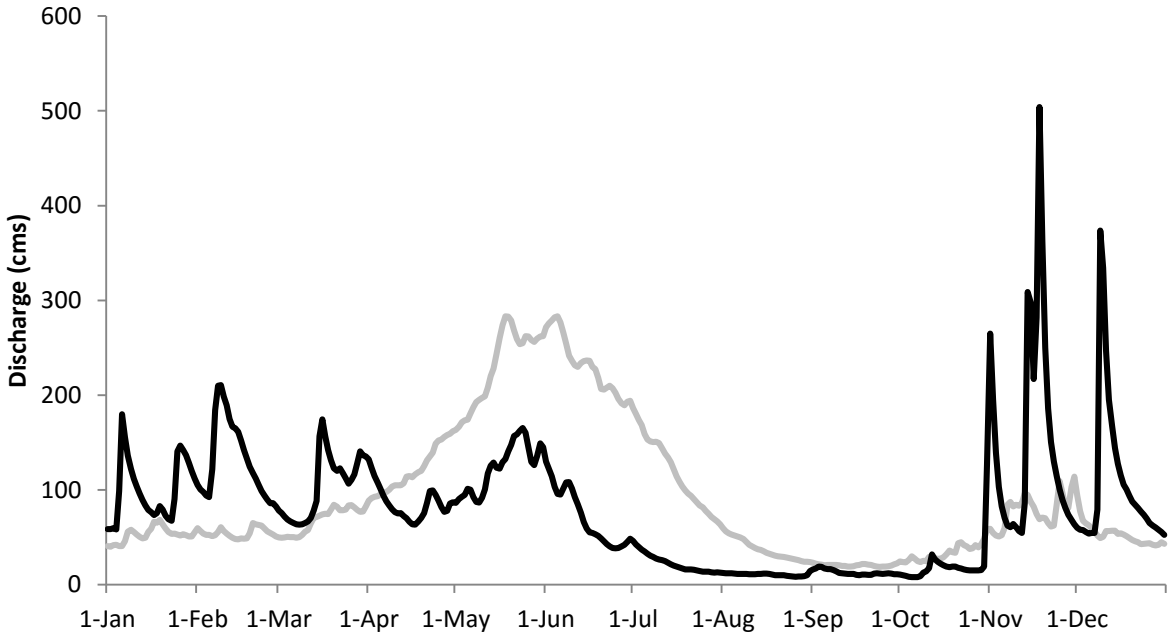

Figure 3. Discharge of the Wenatchee River at Monitor, USGS gauge # 12462500. Black line represents 2015 discharge and grey line represents mean discharge from 1990-2014.

METHODS

Rotary Screw 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 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 age 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. 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.

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. All estimates of emigrating spring Chinook do not include fry 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. 2014). 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 Screw Traps – Chiwawa

Trap Operation

The Chiwawa trap operated between 25 February and 24 November 2015. During that time the trap was inoperable for 29 days as a result of low or high discharge, debris and hatchery fish releases. The trap was operated in two positions based on season (i.e., lower position through June 30 and upper position after July 1).

Fish Sampling

A total of 60,302 individual fish were collected, with wild spring Chinook Salmon and steelhead comprising 62% and 5% of the total catch, respectively. Additionally, 7,162 hatchery spring Chinook, 3,151 hatchery steelhead, and 38 wild coho were collected. Throughout the sampling period 18,470 PIT tag were deployed into wild spring Chinook and steelhead (16,675 and 1,795 respectively). Spring Chinook mortality for the season totaled 42 yearling, 390 subyearling parr, and 31 fry (0.7%, 2.1%, and 0.24%, respectively). Mortality of steelhead throughout the season totaled 45 (1.38%). The mean fork length (SD) of captured yearling and subyearling spring Chinook Salmon (fry excluded) was 93 (9.0) mm and 71 (10.7) mm, respectively (Table 1).

Table 1. Mean fork length (mm) and weight (g) of spring Chinook Salmon captured in the Chiwawa River smolt trap during 2015.

	Yearling transitional/smolts			Subyearling parr		
	Mean	SD	*N*	Mean	SD	*N*
Fork length	92.5	9.0	6,304	71.1	10.7	15,241
Weight	8.8	2.9	6,244	4.2	1.7	14,660

Yearling Spring Chinook (Brood Year 2013)

Wild yearling spring Chinook Salmon were primarily captured between 25 February and 14 June (Figure. 4). A total of 6,350 yearling Chinook Salmon were captured and an estimated 6,891 would have been captured if the trap had operated without interruption. Nine mark/recapture efficiency trials using PIT tags were conducted when the trap was in the lower position producing a mean trap efficiency of 19%. In 2015, mark/recapture trials were conducted at all desired discharge levels but a statistically significant flow-efficiency regression model could not

be obtained (R^2 = 0.22, P < 0.069). Thus, a pooled estimate combining the 2014 and 2015 mark/recapture trials was developed. The estimated number (95% C.I.) of yearling spring Chinook Salmon that emigrated from the Chiwawa River in 2015 was 39,396 (±8,399).

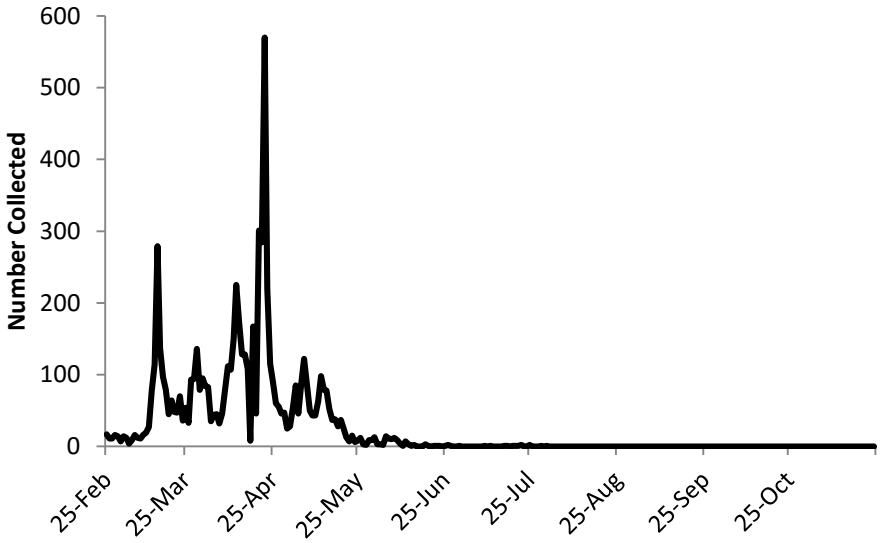

Figure 4. Daily catch of yearling spring Chinook Salmon at the Chiwawa River rotary screw trap.

Subyearling Spring Chinook (Brood Year 2014)

Wild subyearling spring Chinook Salmon were captured throughout the sampling period, with peak catches of parr in October and November and fry occurring in March and April (Figures 5 and 6, respectively). A total of 18,190 subyearling parr and 12,962 fry were captured with an estimated 19,435 subyearling parr and 13,936 fry had the trap operated without interruption. Four mark/recapture efficiency trials were conducted (three PIT and one Bismarck Brown) with a mean trap efficiency of 25.4%. A combination of mark/recapture efficiency trials from 2014 and 2015 were used to create a regression model for the upper trap position (R^2 = 0.58, *P* = 0.002). Data from 2002, 2003, 2013 and 2015 were combined to create a regression model (R^2 = 0.83, *P* < 0.001) for subyearling Chinook captured at the lower trap position. In 2015, the estimated number of subyearling spring Chinook Salmon (excluding fry < 50 mm FL) emigrating from the Chiwawa River during the sampling period was 77,510 (± 9,074).

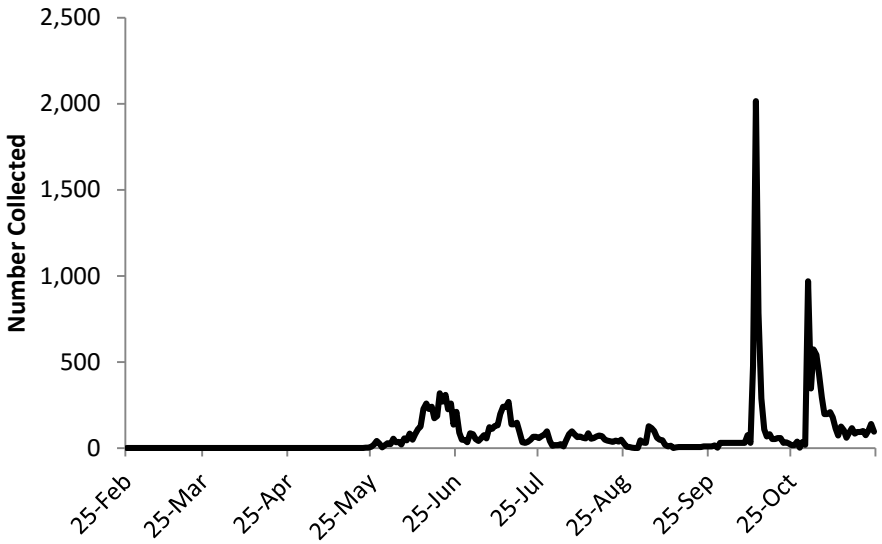

Figure 5. Daily catch of wild spring Chinook subyearling parr at the Chiwawa River rotary screw trap.

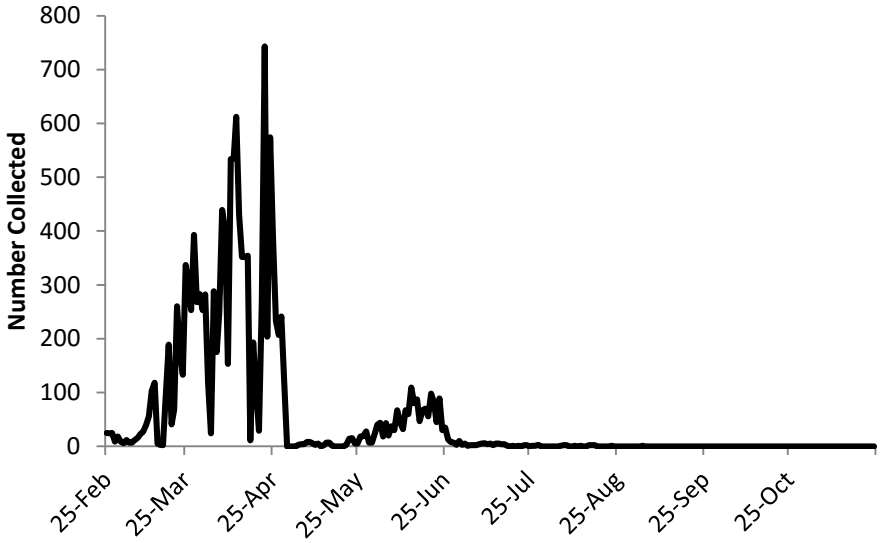

Figure 6. Daily catch of wild spring Chinook fry at the Chiwawa River rotary screw trap.

Summer Steelhead

During the trapping period, 259 steelhead transitional/smolts and 3,004 steelhead/rainbow parr and fry were captured. While collections occurred in moderate numbers throughout the year, peak collections occurred during October (Figure 7). The mean fork length (SD) of steelhead parr and transitional/smolts captured was 75.8 (23.1) and 167.1 (21.8) mm, respectively (Table. 2).

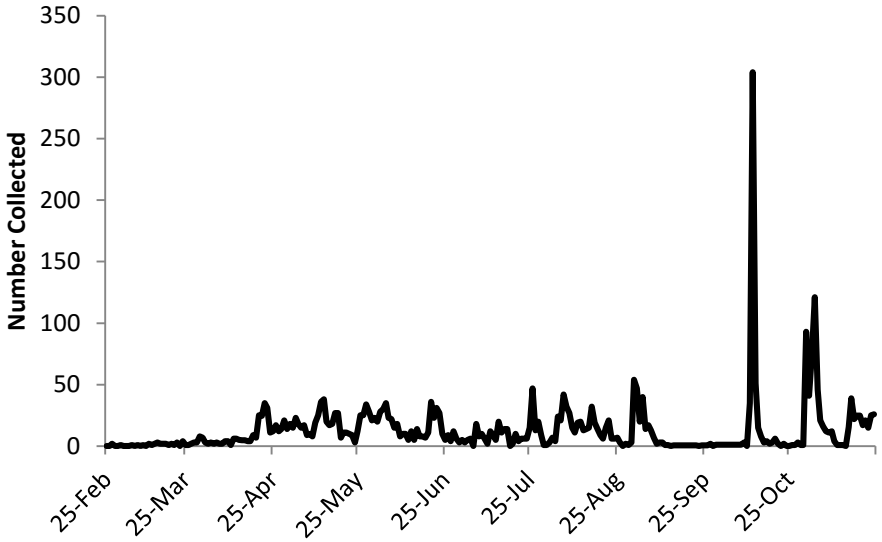

Figure 7. Daily catch of all wild steelhead at the Chiwawa River rotary screw trap.

Table 2. Mean fork length (mm) and weight (g) and of steelhead/rainbow captured in the Chiwawa River smolt trap during 2015.

	Transitional/smolts			Parr		
	Mean	SD	*N*	Mean	SD	*N*
Fork length	167.1	21.8	256	75.8	23.1	2,570
Weight	50.1	19.2	252	6.0	7.88	2,557

Egg-to-emigrant Survival

For BY 2013, 714 redds were counted in the Chiwawa River with an estimated 3,367,224 eggs being deposited. A total of 113,091 emigrants were estimated resulting in an egg-to-emigrant survival of 3.4% (Table 3). This is down slightly 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	--	--	--	--

[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 298 bull trout (32 ≥ 300 mm FL and 266 <300 mm FL) were captured. Additionally, a total of 72 western cutthroat trout (*O. clarki lewisi*), 2 resident rainbow (*O. mykiss*) and 8 Eastern brook trout (*S. fontinalis*) were collected. In all, 260 bull trout, and 65 western cutthroat trout were released with PIT tags. Monthly and annual totals of all fish captured are presented in Appendix D and Appendix E, respectively.

Rotary Screw Traps – Lower Wenatchee

Trap Operation

The Lower Wenatchee trap operated from 30 January through 27 June 2015. During this time the trap was inoperable for a total of 5 days due to high/low flows, high temperatures, heavy debris and major hatchery releases. Extreme river temperatures and low flows resulted in trapping operations being suspended for the season on 28 June. Throughout the season, the trap cones were operated in the lower position.

Fish Sampling

A total of 282,976 individual fish were collected, with wild summer Chinook Salmon comprising 89% of the total catch. Additionally, 1,559 wild yearling spring Chinook Salmon, 9,920 hatchery yearling Chinook Salmon, 4,178 wild sockeye, 331 wild steelhead, and 2,288 hatchery steelhead were captured. Throughout the sampling period 5,513 PIT tag were deployed into wild yearling spring Chinook, sockeye and steelhead (1,301; 3,922; and 290 respectively). Mortality for the season totaled 17 yearling spring Chinook, 282 subyearling summer Chinook, 64 sockeye, and 2 steelhead (1.1%, 0.1%, 1.5%, and 0.6%, respectively).

Wild Yearling Spring Chinook (Brood Year 2013)

Wild yearling spring Chinook Salmon were primarily captured in March and April (Figure 8). Throughout the trapping period 1,559 spring Chinook were collected and an estimated 1,654 would have been collected had the trap operated without interruption. One mark/recapture efficiency trial was carried out using caudal fin clipped yearling hatchery spring Chinook Salmon. 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 58,595 (±6,731). The mean fork length (SD) of captured yearling Chinook was 96 (9.7) mm (Table 4).

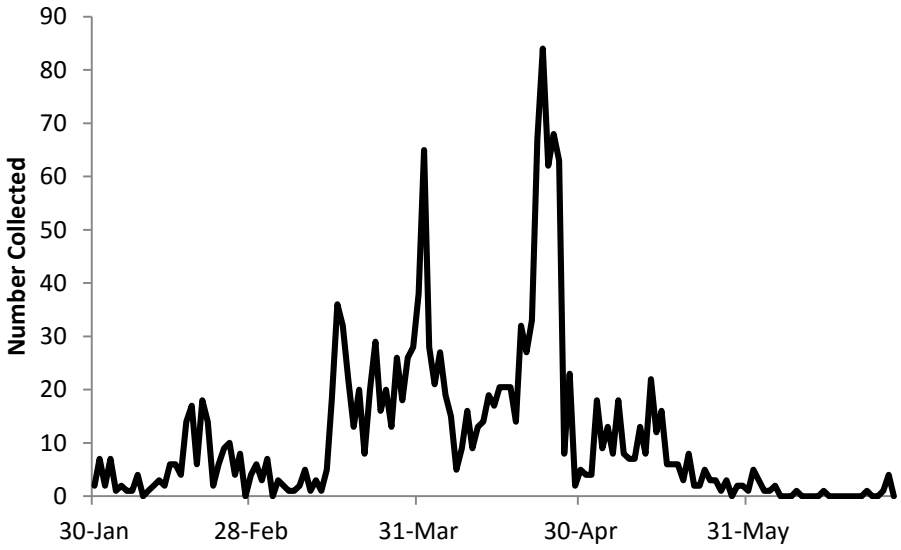

Figure 8. Daily capture of wild yearling Chinook Salmon at the Lower Wenatchee smolt trap.

Table 4. Average length and weight for wild yearling spring Chinook Salmon sampled at the Lower Wenatchee trap.

	Mean	SD	N
Fork length	96	9.8	1,491
Weight	9.4	3.7	1,473

Wild Subyearling Summer Chinook (Brood Year 2014)

Wild subyearling summer Chinook dominated the catch with 252,293 fish being processed,

most being collected in April and May (Figure 9). An estimated 274,346 would have been captured had the trap operated without interruption. Over the season, eight mark/recapture efficiency trials were carried out using Bismarck Brown during the 2015 trapping season. When combined with trials from the previous trapping season a significant discharge efficiency relationship was developed ($R^2 = 0.61$, $P < 0.001$) and an emigrant estimate (95% C.I.) of 14,157,778 (±2,125,578) was calculated. The mean fork length (SD) for captured subyearling parr and fry summer Chinook was 63 (9.7) and 41 (3.3), respectively (Table 5). No PIT tags were deployed in summer Chinook.

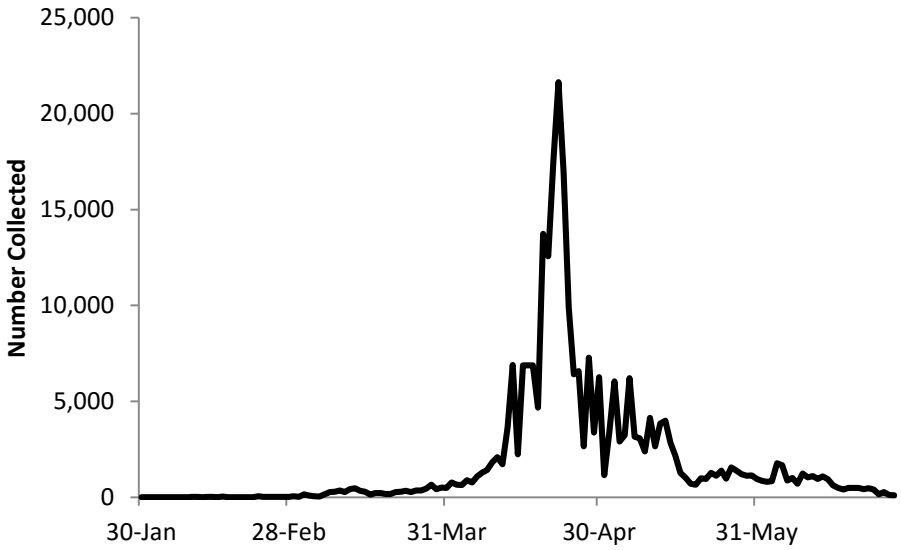

Figure 9. Daily capture of wild summer Chinook Salmon at the Lower Wenatchee River trap.

Table 5. Fork length and weight of subyearling Summer Chinook Salmon sampled at the lower Wenatchee smolt trap.

	Transition / Smolt			Parr			Fry		
	Mean	SD	N	Mean	SD	N	Mean	SD	N
Fork length	75.3	7.2	8	62.8	9.7	2,011	41.0	3.3	6,267
Weight	4.36	1.3	7	3.07	1.5	1,690	0.62	0.3	2,863

Wild Sockeye

A total of 4,178 juvenile sockeye were collected in the 2015 season and an estimated 5,239 had the trap operated without interruption. Almost all of these fish (96%) were collected in April (Figure 10). Three mark/recapture efficiency trials were carried out using PIT tagged juvenile sockeye Salmon. When combined with efficiency trials from the 2014 and 2013 season a significant discharge efficiency model ($R^2 = 0.52$, $P < 0.043$) was developed. This model produced an estimate (95% C.I.) of the 2015 emigrant population of juvenile sockeye at 1,065,614 (±238,901). Smolt survival (SE) to McNary of those tagged fish was 45% (5%) using a

Cormack Jolly Seber estimator. Over 90% of sockeye in run year 2013 and 2014 migrated as Age 1+ with the remaining being Age 2+ (Table 6). Mean fork length (SD) for captured sockeye was 86 (9.4) mm (Table 7).

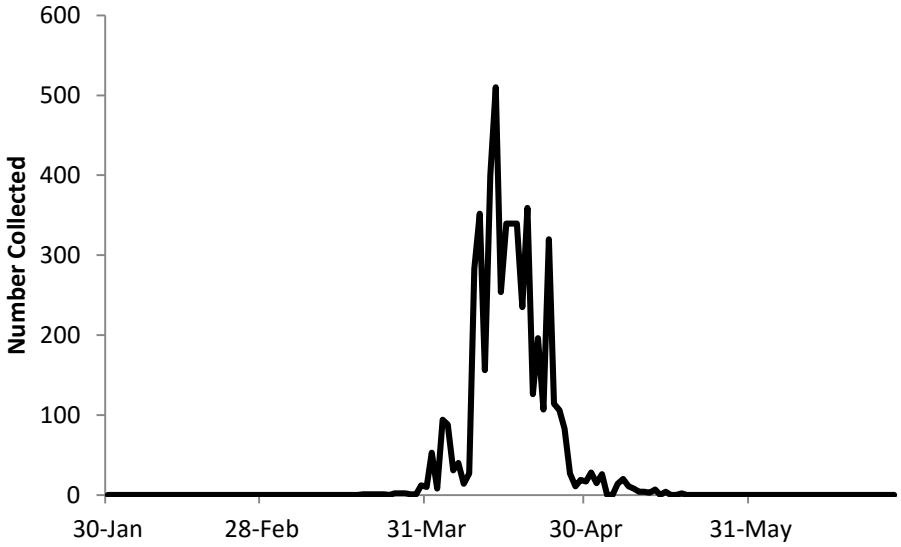

Figure 10. Daily capture of wild sockeye Salmon at the Lower Wenatchee River trap.

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	0.000	873,096
2014	0.924	0.076	0.000	1,275,027
2015	NA	NA	NA	1,065,614

Table 7. Fork length and weight of wild sockeye Salmon smolts sampled at the Lower Wenatchee smolt trap.

	Mean	SD	N
Fork length	86.0	9.4	4,067
Weight	5.37	3.0	4,049

Wild Summer Steelhead

Capture of wild steelhead at the Lower Wenatchee site for all life stages was low, totaling 331 smolts, parr, and fry combined and an estimated 339 collected had the trap operated without interruption. Peak catches of steelhead occurred in May (Figure 11). Due to the low captures no mark/recapture trials were conducted in 2015. In 2014 however, two trials using hatchery steelhead transitional/smolts were piloted. Based on these two trials a pooled efficiency of

0.036 was used to estimate (95% C.I.) the emigrant population at 8,632 (±45,053) parr and smolt emigrant steelhead. However, due to the small 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 179 (24.8) and 94 (22.7) mm, respectively (Table 8).

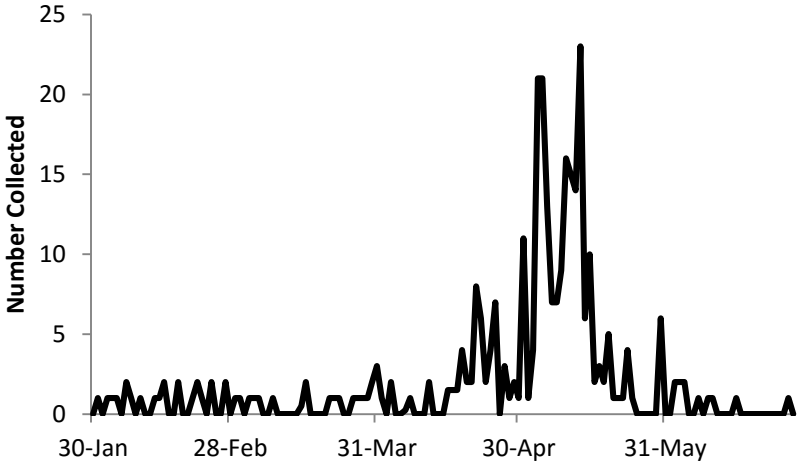

Figure 11. Daily capture of wild steelhead at the Lower Wenatchee River trap.

Table 8. Fork length and weight of wild steelhead sampled at the lower Wenatchee smolt trap.

	Transitional/Smolt			Parr		
	Mean	SD	N	Mean	SD	N
Fork length	179	24.8	227	94	22.7	74
Weight	60.24	25.6	226	10.39	9.4	71

Survival

For BY 2013, 1,159 spring Chinook Salmon redds were surveyed in the Wenatchee Basin producing an estimated 5,512,204 eggs. An estimate of 58,595 emigrants results in an estimated egg-to-emigrant survival of 1.06%. This is down from the last two year average of 1.65% (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

Brood Year	Number of redds	Estimated egg deposition	Estimated number	
			Total emigrants	Egg-to-emigrant survival (%)
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

For BY 2014, 3,458 summer Chinook Salmon redds were surveyed in the Wenatchee Basin, 95.9% being upstream of the Lower Wenatchee smolt trap. After extrapolating by the proportion of redds above the trap a total emigrant population of 14,763,064 was estimated resulting in an egg-to-emigrant survival of 89.17%. This is up from the last two year average of 80.73% (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
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

Brood year	Peak total redd expansion	Estimated egg deposition	Redds above trap / total redds	Estimated number		
				Trap estimate	Total emigrants	Egg-to-emigrant survival (%)
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

Non-target Taxa

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

Backpack Electrofishing

Fish Sampling

Between 1 October and 17 November 2014, WDFW personnel sampled the Chiwawa River over a 13-day span for a total of 55,895 seconds. During this sampling 1,019 subyearling spring Chinook received a PIT tag. The majority of the sampling (95%) occurred between rkm 35 and 55. The greatest concentration of juvenile Chinook occurred between rkm 50 and 53 which had a mean sample rate of one Chinook collected for every 53 seconds of sampling. Over the sample period 14 Chinook died resulting in a mortality rate of 1.3%. Additionally, 121 juvenile bull trout and 94 steelhead were collected, with 67 bull trout and 23 steelhead receiving PIT tags. Highest catch rates for bull trout were between rkm 42 and 47 while the lowest site sampled (rkm 11) had the highest catch rate of steelhead. There was no mortality associated with bull trout or steelhead.

Detections and Calculations

Between the non-trapping season of 18 November 2014 through 24 February 2015, a total of 16 detections of remotely tagged Chinook were recorded at the lower Chiwawa antenna array. During the trapping season of 17 October and 6 November 2014, and 13 March and 6 June 2015, the Chiwawa rotary smolt trap collected 17 and 47 remotely tagged Chinook, respectively. Due to uneven distribution of effort throughout the Chiwawa River and poor sample size, no emigrant estimate for the non-trapping period was calculated for the BY 2013.

DISCUSSION

Chiwawa River Smolt Trap

Over the last five years the Chiwawa River smolt trap has had an average installation date of 3 March. With the relatively mild spring in 2015, the smolt trap was installed almost a week earlier on 25 February. The 2015 trapping season provided relatively good trapping conditions with two minor stoppages in the spring (due to hatchery releases) and two minor stoppages in the fall (due to high discharge and debris). The Chiwawa River smolt trap is considered operable

between discharges of 90 and 1,500 cfs, and the only significant stoppages occurred between mid-September and mid-October when flow periodically dropped below 90 cfs.

A significant discharge efficiency model was produced for subyearling Chinook and a pooled estimate was used for yearling Chinook. 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 2013 was 119,615 and comprises estimates of subyearling emigrants in 2014 and yearling emigrants in 2015. Unfortunately, high flows and the inability to electrofish the Chiwawa River due to spawning bull trout concerns resulted in an abbreviated sampling window and prevented the completion of 2014 remote tagging efforts. This resulted in no estimate being calculated for the 2014 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.

Abnormally low discharge levels also limited the number of mark/recapture trials that could be done at the Chiwawa River smolt trap and reliance on historical data was necessary. Further complicating estimates, emigrating yearling and subyearling Chinook were collected when the trap was operating at both the upper and lower cone positions. However, insufficient numbers were present to produce a trap efficiency model for both life stages at each cone positions. In an effort to expand operational condition and reduce the dependence on historic data, 2016 trap operations will eliminate the lower cone position and a single upper cone positon will be used.

Lower Wenatchee River 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, 2015 proved to be a difficult trapping season for the Lower Wenatchee trap. Up until late June the Lower Wenatchee trap only had three minor stoppages due to hatchery fish releases and debris. However, the Lower Wenatchee trap is considered operable between discharges of 1,300 and 10,000 cfs and summer proved to be a substantial departure from normal discharge and river temperature. From late June through July water temperatures at our Lower Wenatchee trapping site fluctuated between 18 and 26 degrees Celsius and discharge was about 25% of normal. The culmination of these factors resulted in trapping operations terminating at its earliest known date of 28 June.

The early removal of the lower Wenatchee trap proved to be the most difficult part of the 2015 trapping season. To account for the early removal of the trap, historical run timing was used to extrapolate what the catch would have been had the trap been able to operate as normal. Historical emigration timing showed no sockeye, and only a small percentage of spring and

summer Chinook emigrated after 28 June (0.4% and 3.5%, respectively). Emigration estimates used these percentages to extrapolate to a total estimate of emigrants had the trap been able to operate further into the season.

Discharge efficiency models were obtained for three of the four target species at the lower Wenatchee trap during the 2015 trapping season (wild spring and summer Chinook Salmon and sockeye Salmon). Collections of wild steelhead continue to be inadequate for conducting a mark/recapture trial. In 2016, 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 on 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 and cold air and water temperatures hinder sampling efforts. In 2014, early high water events halted 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:

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

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

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

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

Appendix B. Bailey Population and Variance Equations.

Trap efficiency was calculated using the following formula:

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

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

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. Monthly collection information for the Chiwawa River smolt trap.

	2015											
Species/Origin	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Total
Chinook												
Wild yearling	--	55	1,839	3,072	1,277	94	13	0	0	0	0	6,350
Wild subyearling	--	83	3,516	7,639	352	5,509	3,058	1,423	641	5,340	3,591	31,152
Hatchery yearling	--	0	0	7,141	1	2	4	8	6	0	0	7,162
Steelhead												
Wild												
Smolt	--	0	9	59	163	8	6	12	2	0	0	259
Parr and fry	--	2	45	200	416	447	283	453	168	538	452	3,004
Hatchery	--	0	1	630	2,433	63	4	12	3	4	1	3,151
Coho												
Wild												
Smolt	--	0	0	0	0	0	0	0	0	0	0	0
Parr and fry	--	0	1	2	8	22	3	2	0	0	0	38
Hatchery	--	0	0	0	0	0	0	0	0	0	0	0
Bull trout												
Juvenile	--	0	9	1	4	7	18	13	14	147	53	266
Adult	--	0	0	0	0	0	2	5	10	14	1	32
Westslope cutthroat	--	0	3	0	6	8	22	24	8	0	1	72
Eastern brook trout	--	0	0	1	4	1	0	0	0	1	1	8
Rainbow trout	--	0	0	0	0	1	1	0	0	0	0	2
Mountain whitefish	--	0	3	17	6	44	2,407	2,619	355	42	51	5,544
Longnose dace	--	1	21	33	636	661	197	369	255	415	75	2,663
Northern pikeminnow	--	0	0	0	1	16	157	150	7	0	0	331
Sculpin spp.	--	0	8	0	13	40	48	23	13	58	22	225
Sucker spp.	--	0	0	0	0	0	11	16	1	2	0	30
Redside shiner	--	0	0	0	0	0	1	11	0	1	0	13

Appendix E. Annual collection information from the Chiwawa River smolt trap.

Species origin	2015	2014	2013	2012	2011	2010
Chinook						
Wild yearling	6,350	5,419	3,199	7,626	4,848	6,482
Wild subyearling	31,152	23,755	27,621	14,831	20,561	13,344
Hatchery yearling	7,162	5,293	15,909	30,751	25,620	22,481
Steelhead						
Wild	3,263	1,938	2,034	1,921	1,176	1,226
Smolt	259	49	85	183	195	210
Parr and Fry	3,004	1,889	1,949	1,738	981	1,016
Hatchery	3,151	290	1,539	1,664	8,250	9,921
Coho						
Wild yearling	0	0	1	1	3	4
Wild subyearling	38	12	0	0	4	5
Hatchery yearling	0	1	10	3	0	3
Bull trout						
Juvenile	266	260	310	488	351	499
Adult	32	75	51	31	7	45
Westslope cutthroat trout	72	59	86	60	38	54
Eastern brook trout	8	12	13	66	3	0
Mountain whitefish	5,544	2,970	2,108	3,291	990	778
Longnose dace	2,663	2,633	2,257	1,762	1,526	1,393
Northern pikeminnow	331	5	71	34	20	5
Sculpin spp.	225	131	91	157	129	51
Sucker spp.	30	4	6	0	0	0
Redside shiner	13	0	0	0	0	0
Yellow perch	0	0	0	0	0	0

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

	2015											
Species/Origin	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Total
Chinook												
Wild yearling	9	154	405	751	220	20	--	--	--	--	--	1,559
Wild subyearling	5	418	8,418	154,499	69,035	19,918	--	--	--	--	--	252,293
Hatchery yearling	0	15	0	8,973	931	1	--	--	--	--	--	9,920
Steelhead												
Wild												
Smolt	0	3	4	33	186	5	--	--	--	--	--	231
Parr and fry	1	18	16	15	19	31	--	--	--	--	--	100
Hatchery	0	0	7	247	1,991	43	--	--	--	--	--	2,288
Sockeye												
Wild	0	0	35	3,997	146	0	--	--	--	--	--	4,178
Coho												
Wild												
Smolt	1	6	10	5	0	0	--	--	--	--	--	22
Fry and parr	2	280	313	968	2,153	1,256	--	--	--	--	--	4,972
Hatchery	0	0	76	4,653	1,794	43	--	--	--	--	--	6,566
Unknown	0	0	0	16	121	6	--	--	--	--	--	143
Bull trout												
Juvenile	0	0	0	0	0	0	--	--	--	--	--	0
Adult	0	0	0	0	0	0	--	--	--	--	--	0
Westslope cutthroat trout	0	0	0	0	1	0	--	--	--	--	--	1
Mountain whitefish	0	0	0	1	3	5	--	--	--	--	--	9
Lamprey spp.	1	77	64	12	13	116	--	--	--	--	--	283
Longnose dace	1	29	6	5	49	152	--	--	--	--	--	242
Sculpin spp.	0	16	7	5	8	16	--	--	--	--	--	52
Sucker spp.	1	11	2	2	24	11	--	--	--	--	--	51
Redside shiner	0	0	0	4	2	13	--	--	--	--	--	19
Stickleback (3-spined)	0	0	0	0	2	11	--	--	--	--	--	13
Northern pikeminnow	0	2	0	2	5	3	--	--	--	--	--	12
Chiselmouth	0	0	0	0	0	6	--	--	--	--	--	6
Peamouth	0	0	0	0	0	3	--	--	--	--	--	3

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

Species/Origin	2015	2014	2013
Chinook			
Wild yearling	1,559	1,700	1,854
Wild subyearling	252,293	81,445	52,652
Hatchery yearling	9,920	31,290	13,979
Steelhead			
Wild	331	182	710
Smolt	231	80	173
Parr	100	102	537
Hatchery	2,288	494	819
Sockeye			
Wild	4,178	7,678	4,520
Hatchery	0	0	72
Coho			
Wild yearling	22	220	597
Wild subyearling	4,972	393	923
Hatchery yearling	6,566	16,908	12,960
Unknown yearling	143	NA	NA
Bull trout			
Juvenile	0	3	6
Adult	0	0	0
Westslope cutthroat trout	1	3	0
Mountain whitefish	9	27	110
Lamprey spp.	283	292	762
Longnose dace	242	541	1,382
Sculpin spp.	52	128	242
Sucker spp.	51	134	240
Redside shiner	19	94	423
Stickleback (3-spined)	13	66	196
Northern pikeminnow	12	37	39
Chiselmouth	6	69	10
Peamouth	3	9	10

Appendix C

Summary of PIT-Tagging Activities in the Wenatchee Basin, 2015

Appendix C. Numbers of fish captured, PIT tagged, lost, and released in the Wenatchee River basin during February through November, 2015.

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	31,152	169	10,471	414	0	10,471	1.33
	Wild Yearling Chinook	6,350	218	6,204	44	0	6,204	0.69
	Wild Steelhead/Rainbow	3,262	6	1,795	23	0	1,795	0.71
	Hatchery Steelhead/Rainbow	3,152	2	1	0	0	1	0
	Wild Coho	38	0	0	0	0	0	0
	Total	**43,954**	**395**	**18,471**	**481**	**0**	**18,471**	**1.09**
Chiwawa Remote (Electrofishing)	Wild Subyearling Chinook	1,103	0	1,054	20	0	1,054	1.81
	Wild Yearling Chinook	0	0	0	0	0	0	0
	Wild Steelhead/Rainbow	0	0	0	0	0	0	0
	Hatchery Steelhead/Rainbow	0	0	0	0	0	0	0
	Wild Coho	0	0	0	0	0	0	0
	Total	**1,103**	**0**	**1,054**	**20**	**0**	**1,054**	**1.81**
Nason Creek Trap	Wild Subyearling Chinook	548	0	219	9	0	219	1.64
	Wild Yearling Chinook	152	0	142	5	0	142	3.29
	Wild Steelhead/Rainbow	444	1	383	2	1	383	0.45
	Hatchery Steelhead/Rainbow	448	0	0	1	0	0	0.22
	Wild Coho	0	0	0	0	0	0	0
	Total	**1,592**	**1**	**744**	**17**	**1**	**744**	**1.07**
Nason Creek Remote (Electrofishing)	Wild Subyearling Chinook	1,143	10	1,089	46	0	1,089	4.02
	Wild Yearling Chinook	0	0	0	0	0	0	0
	Wild Steelhead/Rainbow	0	0	0	0	0	0	0
	Hatchery Steelhead/Rainbow	0	0	0	0	0	0	0
	Wild Coho	152	2	120	0	0	2	0
	Total	**1,295**	**12**	**1,209**	**46**	**0**	**1,091**	**3.55**
White River Trap	Wild Subyearling Chinook	162	1	150	0	1	149	0
	Wild Yearling Chinook	34	0	34	0	0	34	0
	Wild Steelhead/Rainbow	6	0	6	0	0	6	0
	Hatchery Steelhead/Rainbow	0	0	0	0	0	0	0
	Wild Coho	0	0	0	0	0	0	0
	Total	**202**	**1**	**190**	**0**	**1**	**189**	**0.00**
Lower Wenatchee Trap	Wild Subyearling Chinook	252,293	83	0	282	0	0	0.11
	Wild Yearling Chinook	1,559	1	1,301	17	0	1,301	1.09
	Wild Steelhead/Rainbow	311	0	290	2	0	290	0.64
	Hatchery Steelhead/Rainbow	2,288	0	1	0	0	1	0
	Wild Coho	4,994	1	1	20	0	1	0.4
	Wild Sockeye	4,178	3	3,922	64	0	3	1.53
	Total	**265,623**	**88**	**5,515**	**385**	**0**	**1,596**	**0.14**

Sampling Location	Species and Life Stage	Number collected	Number of recaptures	Number tagged	Number died	Shed tags	Total tags released	Percent mortality
Total:	Wild Subyearling Chinook	286,401	263	12,983	771	1	12,982	0.27
	Wild Yearling Chinook	8,095	219	7,681	66	0	7,681	0.82
	Wild Steelhead/Rainbow	4,023	7	2,474	27	1	2,474	0.67
	Hatchery Steelhead/Rainbow	5,888	2	2	1	0	2	0.02
	Wild Coho	5,184	3	121	20	0	3	0.39
	Wild Sockeye	4,178	3	3,922	64	0	3,922	1.53
Grand Total:		313,769	497	27,183	949	2	27,064	0.30

Appendix D

Wenatchee Steelhead Spawning Escapement Estimates, 2015

Wenatchee Steelhead Spawning Escapement Estimates in 2015

Kevin See

March 15, 2016

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 09, 2015 - May 28, 2015) 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 two 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 2015 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 modifications 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 led to many redds not disappearing before the end of the survey season, the estimates of redd life are biased low for this year. 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 5, any violation 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-occupancy 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 1.78 above Tumwater dam, and 1.73 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 0.6 above Tumwater dam, and 0.34 below Tumwater (Table 2).

Tributary areas

Estimates of escapement to various tributaries in the Wenatchee were made using a branching patch-occupancy model 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 (W2, 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 reaches 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).

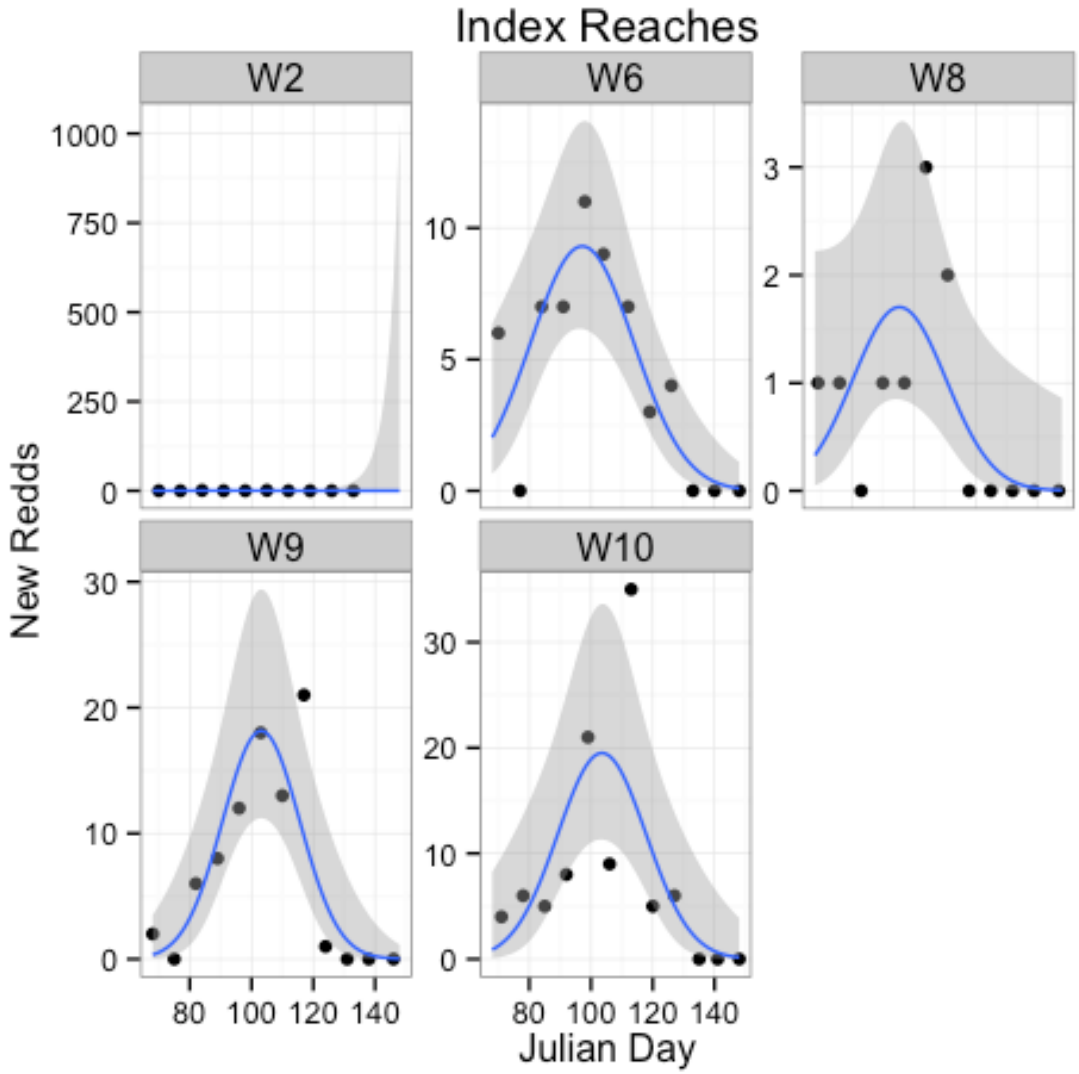
Results

Redd estimates

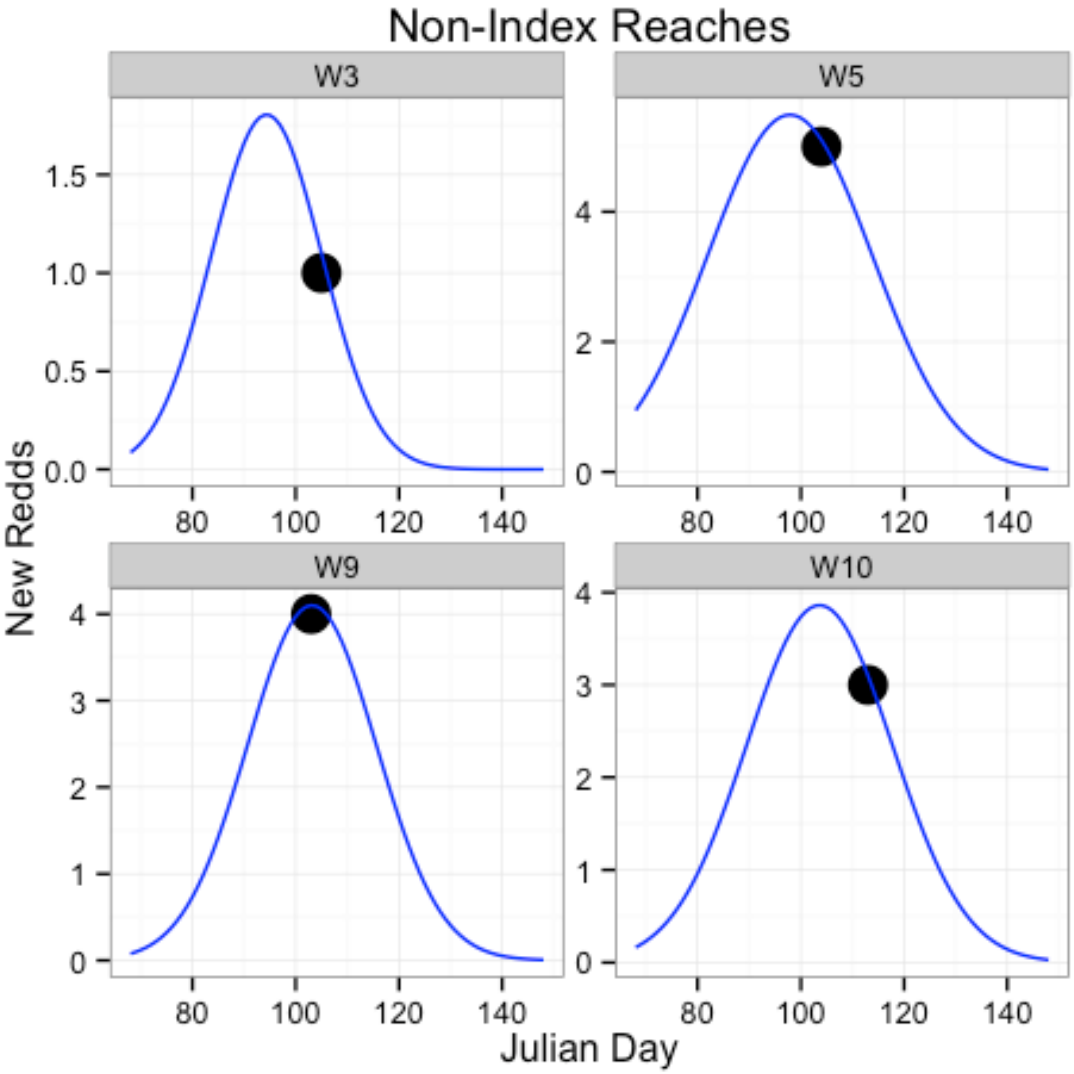
It should be noted that the GAUC parameters from index reaches were not used to estimate total redds in the associated non-index reaches. Figure 4 does illustrate that the non-index reach surveys were conducted close to the period of peak spawning (as determined by the associated index reaches), thus 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
W1	Non-Index	W2	0.55	0.24	0	0	NA
W2	Index	-	0.59	1.40	2	3	1.50
W3	Non-Index	W2	0.44	0.30	1	2	0.30
W4	Non-Index	W6	0.46	0.23	0	0	NA
W5	Non-Index	W6	0.50	0.22	5	10	0.22
W6	Index	-	0.99	0.85	54	53	0.88
W6	Non-Index	W6	0.46	0.15	0	0	NA
W8	Index	-	0.92	0.90	9	10	0.95
W9	Index	-	0.79	0.89	81	102	0.91
W9	Non-Index	W9	0.63	0.15	4	6	0.15
W10	Index	-	0.83	0.61	99	120	0.65
W10	Non-Index	W10	0.59	0.13	3	5	0.13
Total		NA	NA	NA	258	311	0.63



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	1.777	0.059	0.599	0.026
Below TUF	1.728	0.089	0.343	0.040

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

Area	Type	Hatchery	Natural
W1	Non-Index	0 (--)	0 (--)
W2	Index	2 (1.51)	4 (1.51)
W3	Non-Index	1 (0.32)	3 (0.31)
W4	Non-Index	0 (--)	0 (--)
W5	Non-Index	6 (0.25)	11 (0.23)
W6	Index	32 (0.89)	60 (0.88)
W6	Non-Index	0 (--)	0 (--)
W8	Index	10 (0.95)	7 (0.95)
W9	Index	108 (0.92)	73 (0.92)
W9	Non-Index	7 (0.16)	5 (0.16)
W10	Index	127 (0.65)	85 (0.66)
W10	Non-Index	5 (0.14)	4 (0.15)
Icicle	Trib	52 (0.32)	83 (0.25)
Peshastin	Trib	40 (0.37)	206 (0.16)
Mission	Trib	23 (0.49)	71 (0.28)
Chumstick	Trib	0 (--)	38 (0.39)
Chiwaukum	Trib	12 (0.72)	48 (0.34)
Chiwawa	Trib	168 (0.23)	168 (0.21)
Nason	Trib	68 (0.29)	237 (0.15)
Little Wenatchee	Trib	0 (--)	0 (--)
White River	Trib	0 (--)	0 (--)
Total		661 (0.45)	1103 (0.3)

Discussion

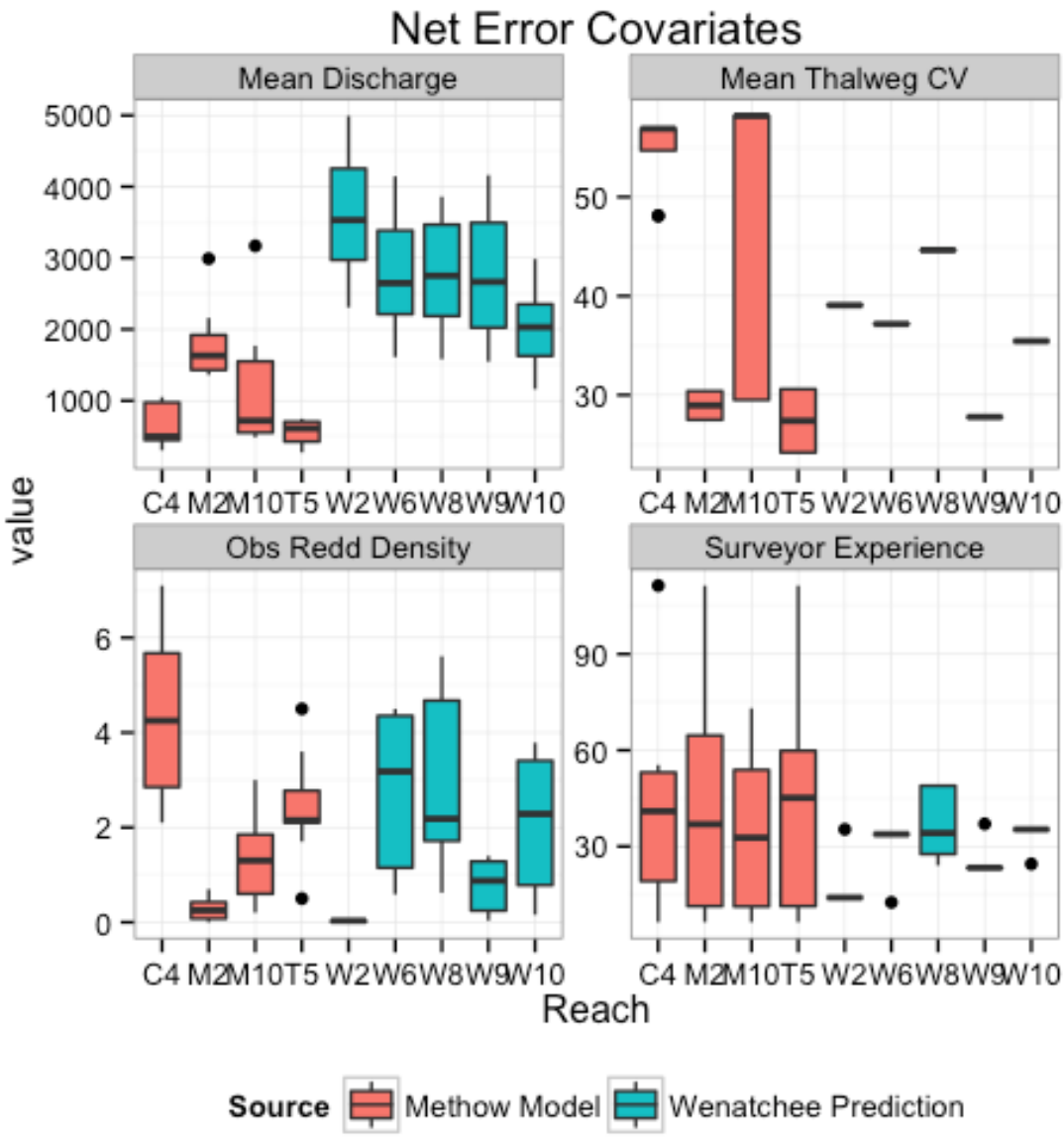
We have estimated the number of steelhead redds based on redd surveys, while incorporating potential observation error. After translating these to estimates of spawners by origin, we can then compare the spawner estimates to escapement estimates made using PIT tags, and estimate a pre-spawn mortality rate (Table 4). Taking the total PIT-tag based escapement estimate to the Wenatchee (after subtracting the number of hatchery fish removed at Tumwater), 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 hatchery fish had a higher pre-spawn mortality rate, although the difference is not statistically significant.

Table 4: Wenatchee pre-spawn mortality rates.

Origin	Pre-spawn_Mort	CV
Hatchery	0.25	0.0016
Natural	0.16	0.0013

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 2680 in the Wenatchee reaches in 2015. That difference alone would change net error predictions by 0.29, 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 2015 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.

Acknowledgements

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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.

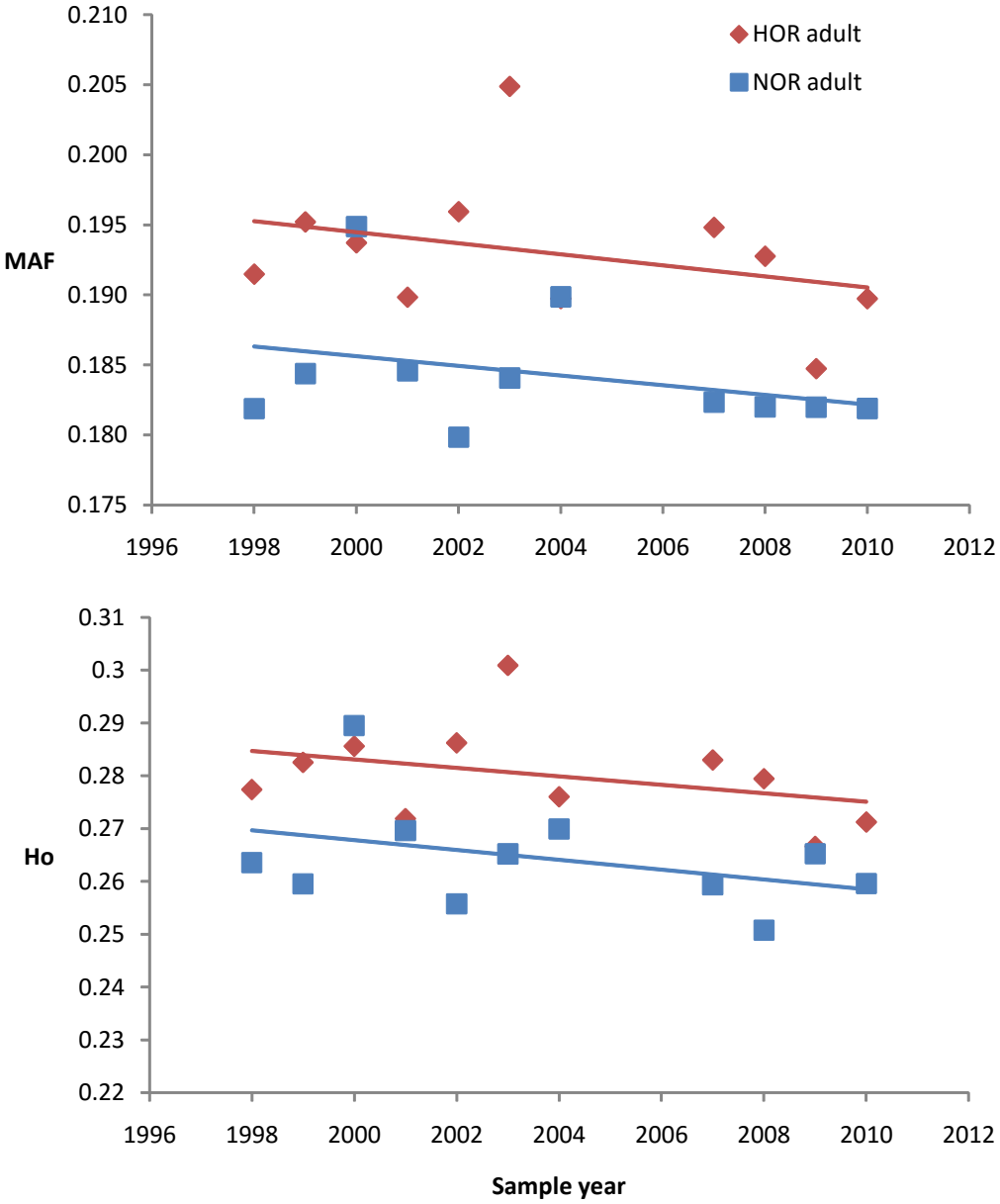


Figure 2. Observed average minor allele frequencies (MAF) and observed heterozygosities (Ho) of 119 SNP loci from 15 collections of natural origin juvenile steelhead from Wenatchee River tributaries, the lower Wenatchee River and the Entiat River. There were no consistent temporal trends in MAF or Ho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

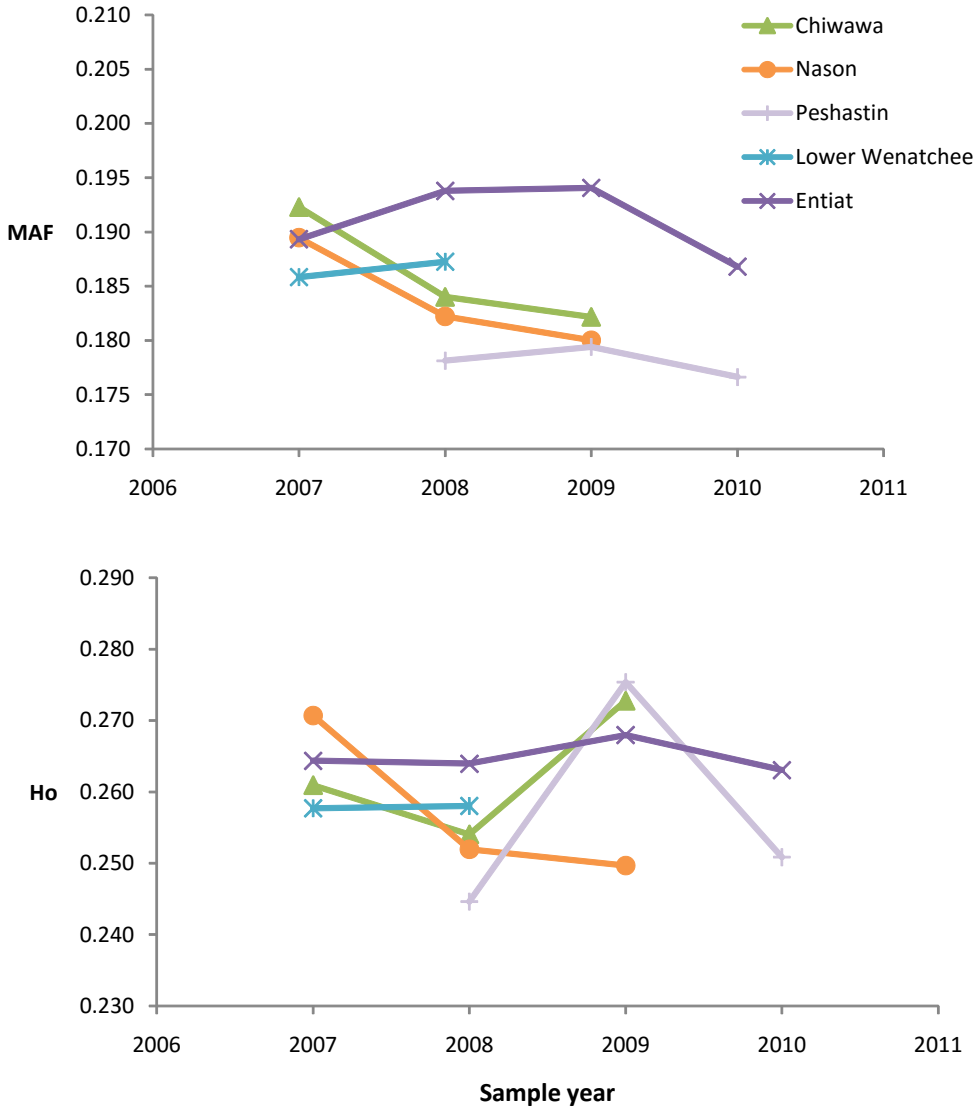


Figure 3. The relationship of time with pairwise F_{ST} estimates between hatchery-produced (adipose fin clipped) and natural origin (unclipped) adults of the same sample year. The line is the prediction based on beta regression.

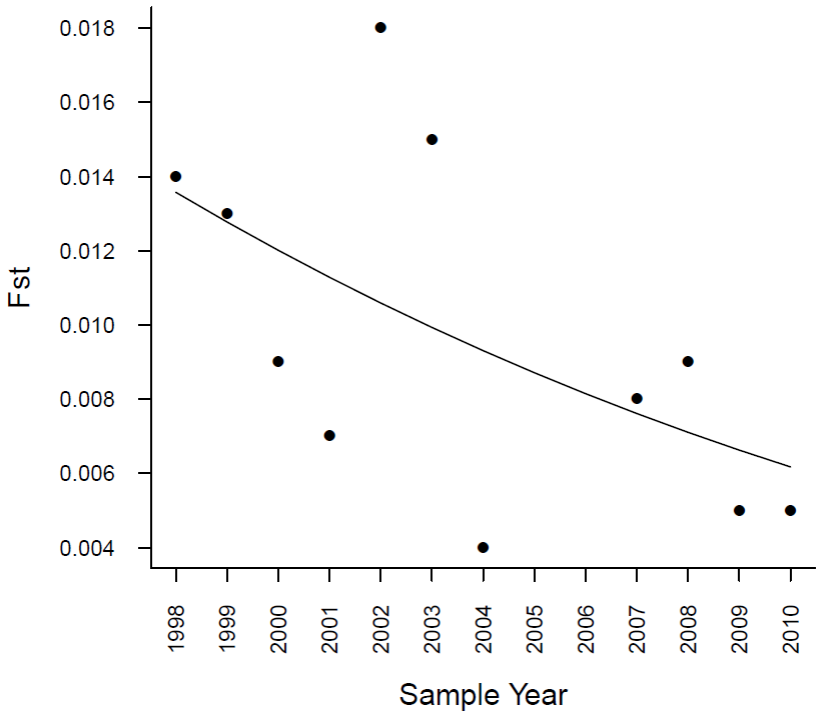


Figure 4. Principal component (PC) 1 versus 2 (top left), PC 1 versus 3 (bottom left), and PC 2 versus 3 (bottom right) based on an analysis using all adults aggregated into origin and spawn-year collections. Natural-origin spawn-years are shown in italicized typeface. The percentage within the label 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 $R^2 = 0.45$.

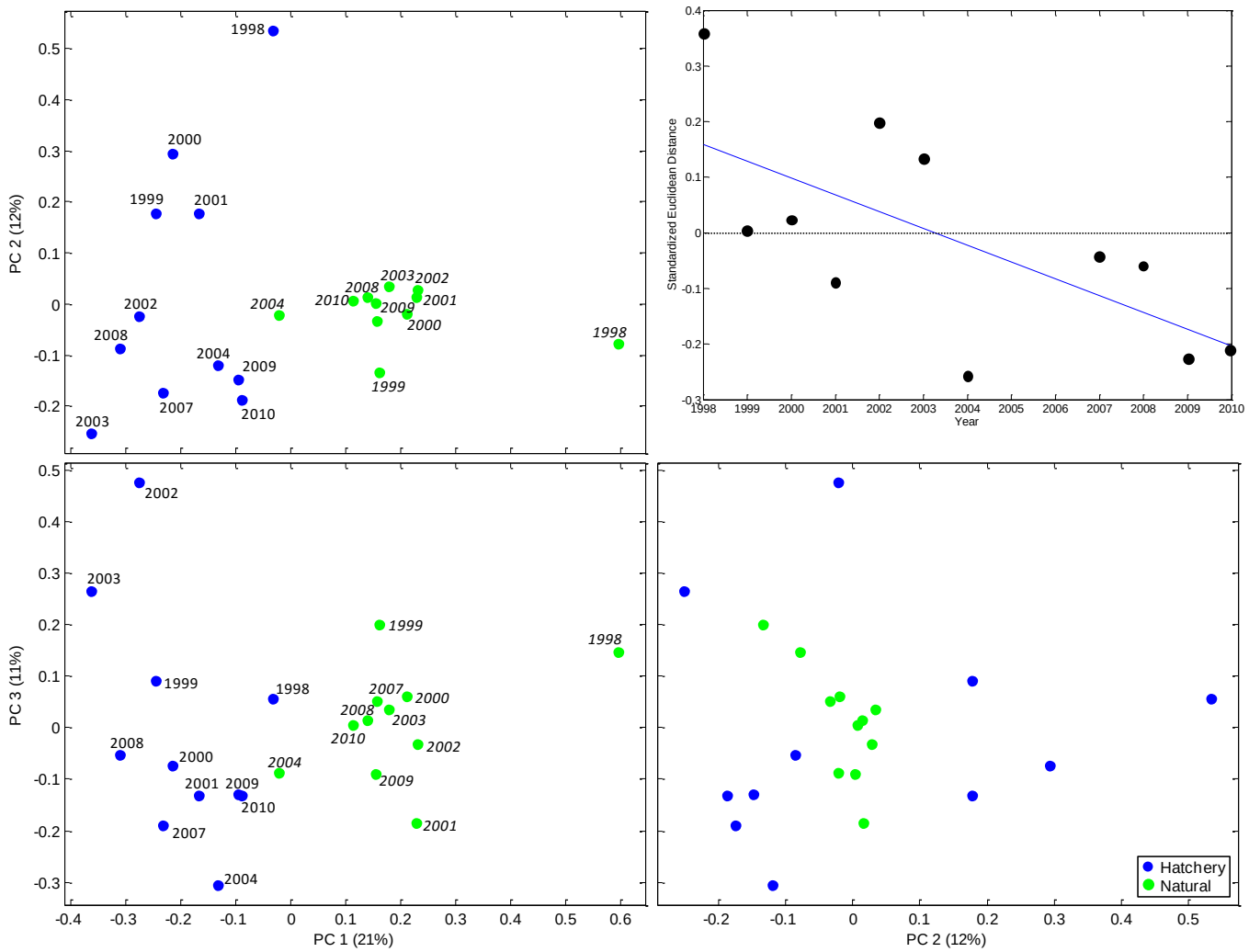


Figure 5. Principal components (PC) 1 versus 2 (top) and 3 (bottom) for adults aggregated into brood-year (BY; left) and spawn-year (SY; right). Spawn-year analysis is the same as in Figure x1, except fewer individuals per collection were included (see methods). Note that for the SY analysis here PC 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.

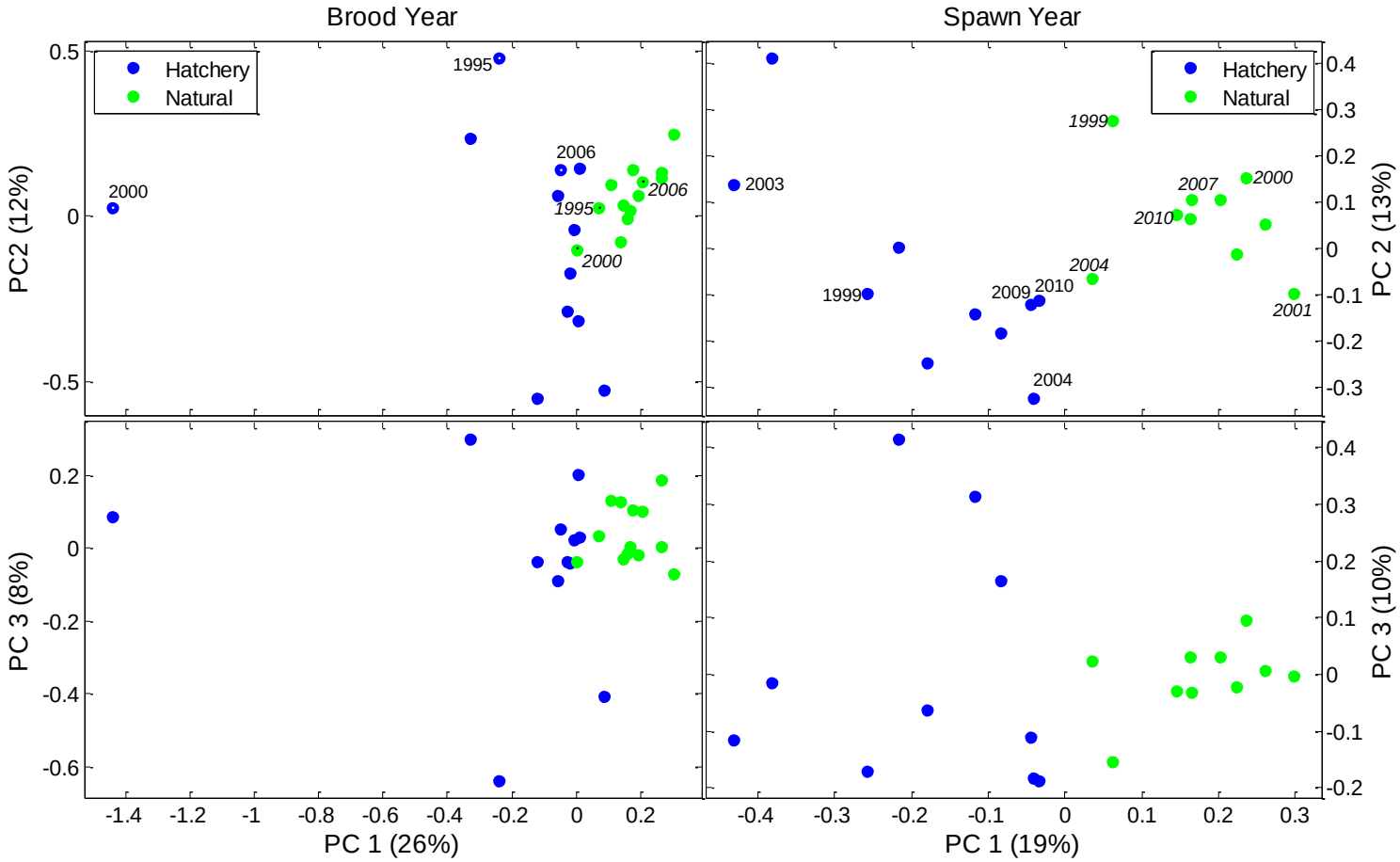


Figure 6. Principal component (PC) 1 versus 2 (top) and PC 1 versus 3 (bottom) based on an analysis using all adult and juvenile fish aggregated into age (juvenile versus adult), origin (hatchery versus adult) and spawn-year collections.

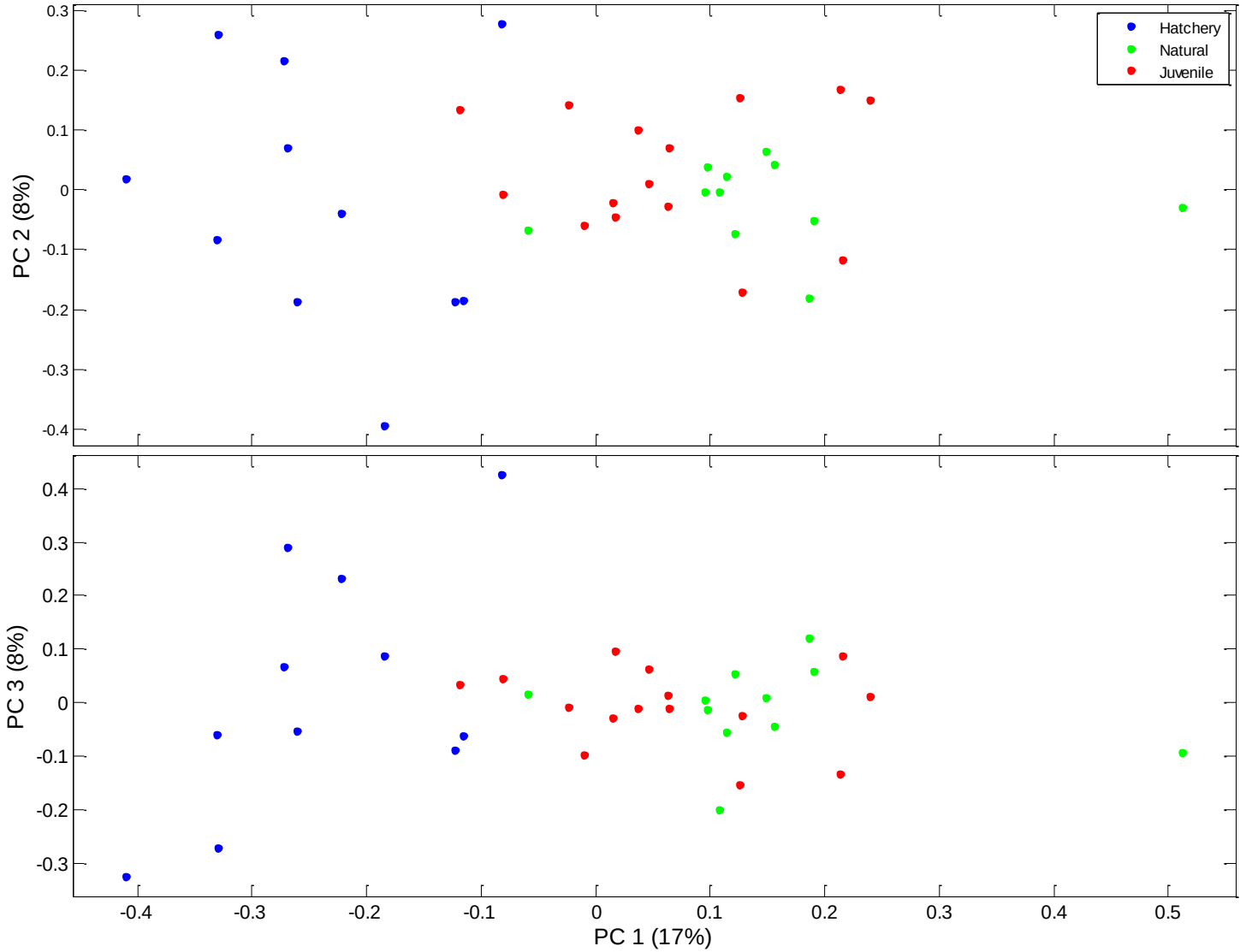


Figure 7. Pairwise Euclidian distances versus brood-year (top) and spawn-year (bottom), with zero distance equal to average distance across all pairwise distances. Blue lines are least-squares fits, which is not significant (slope = 0) for brood-year, but significant (slope > 0) for spawn-year.

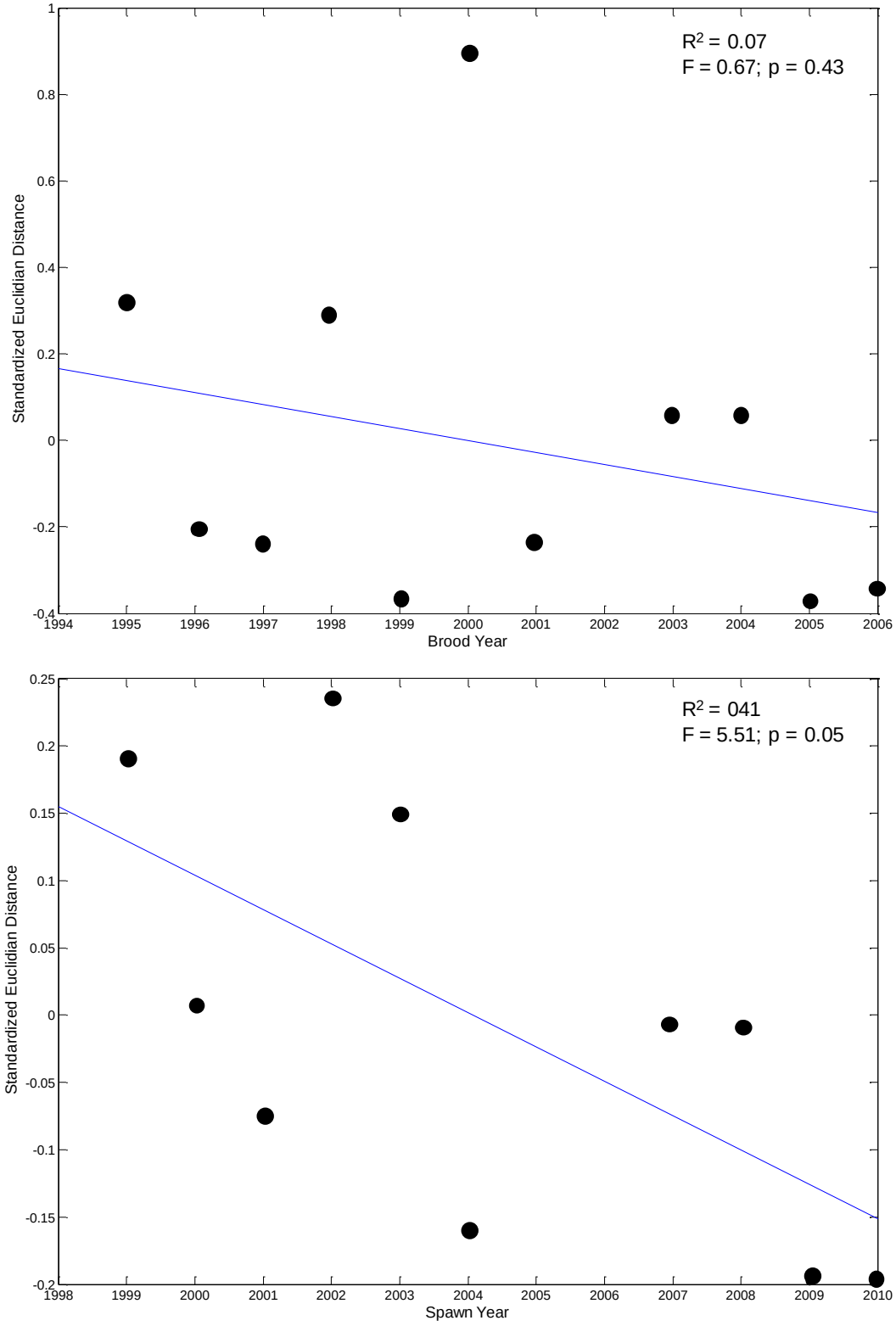


Figure 8. Effective population size estimates (N_b) from Wenatchee River adult hatchery-produced steelhead annual collections calculated using single sample methods implemented in the program LDNE (Waples and Do 2008). Each line connects annual estimates of N_b estimated with a different value of P_{crit}, 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.

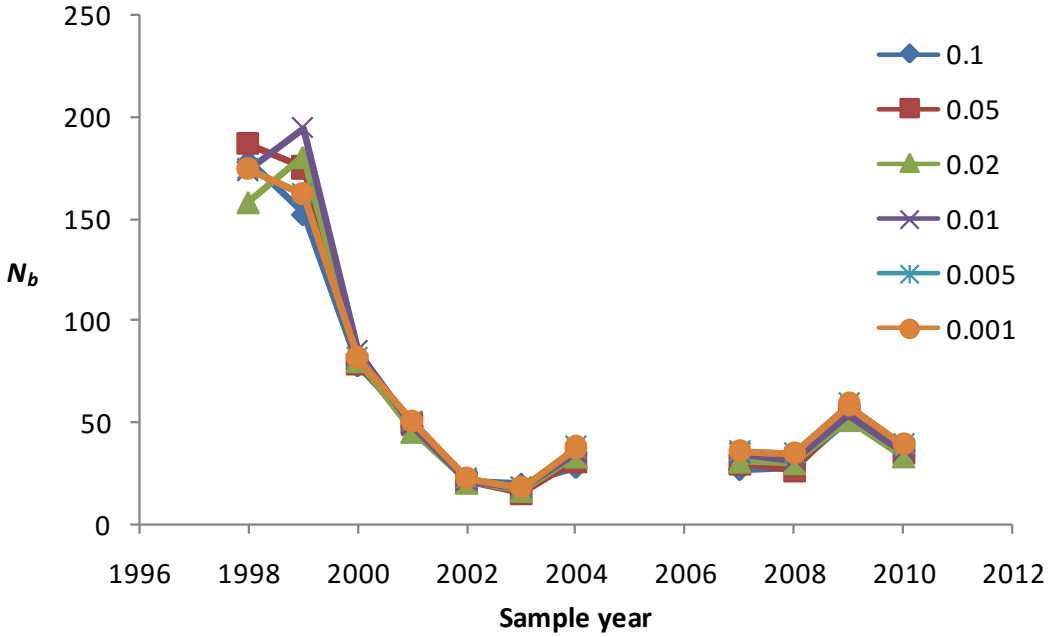


Figure 9. Estimates of Wenatchee River steelhead effective number of breeders (N_b) estimated using the single sample methods incorporated in the program LDNE (Waples and Do 2008). Estimates of N_b refer to parental (and even grantparental) generations. N_b data were plotted against their estimated parental 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).

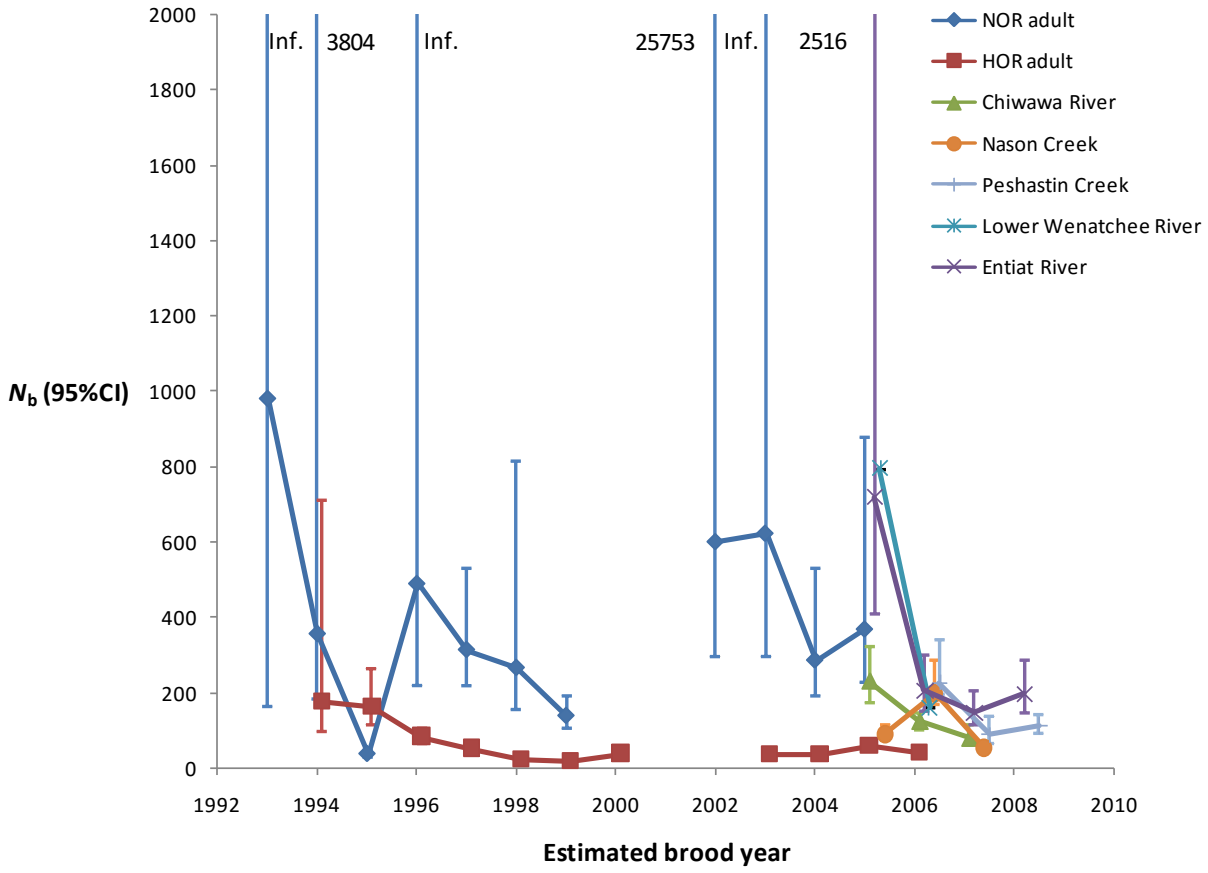


Figure 10. Estimates of N_b for collections of hatchery-produced (HOR) and natural origin (NOR) Wenatchee River summer steelhead grouped by brood year rather than spawn year. Brood year was estimated using scale-based age data. Error bars that extend past the top of the chart are all bounded by infinity.

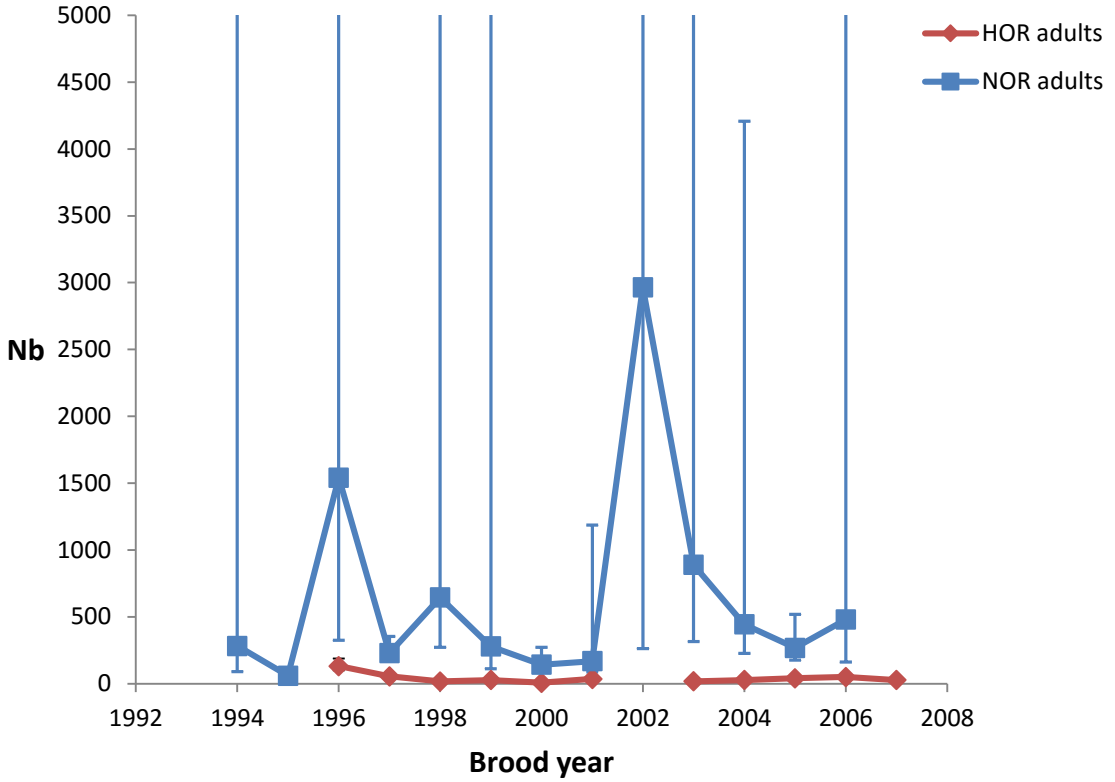


Figure 11. Estimates of N_b for combined annual adult hatchery-produced (HOR) and natural origin (NOR) steelhead and for HOR adults alone. The temporal patterns are similar, though estimates from combined collections are larger than those from HOR collections alone.

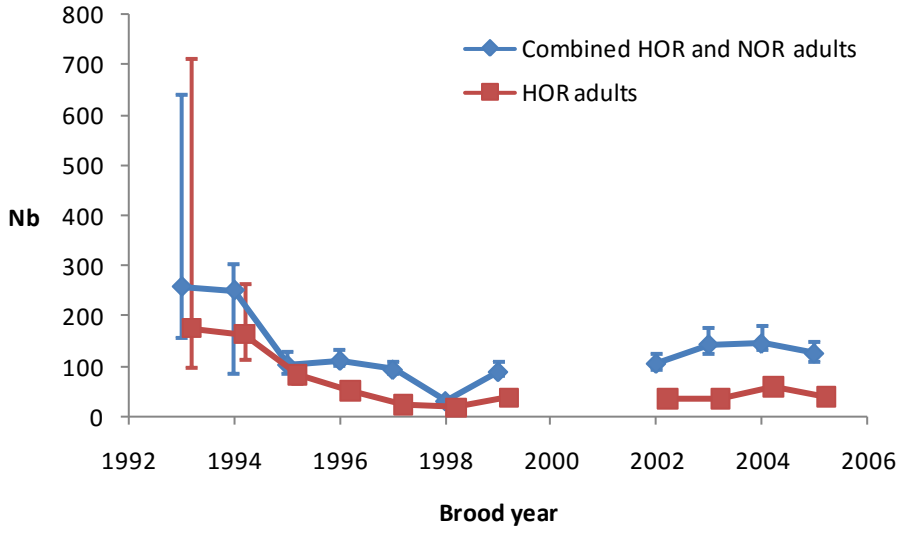


Figure 12. N_b/N ratios for hatchery-produced (HOR) and natural origin (NOR) adult Wenatchee River summer steelhead grouped by spawn year. The average N_b/N ratios are not different, though in later years NOR adults appear to have lower N_b/N ratios.

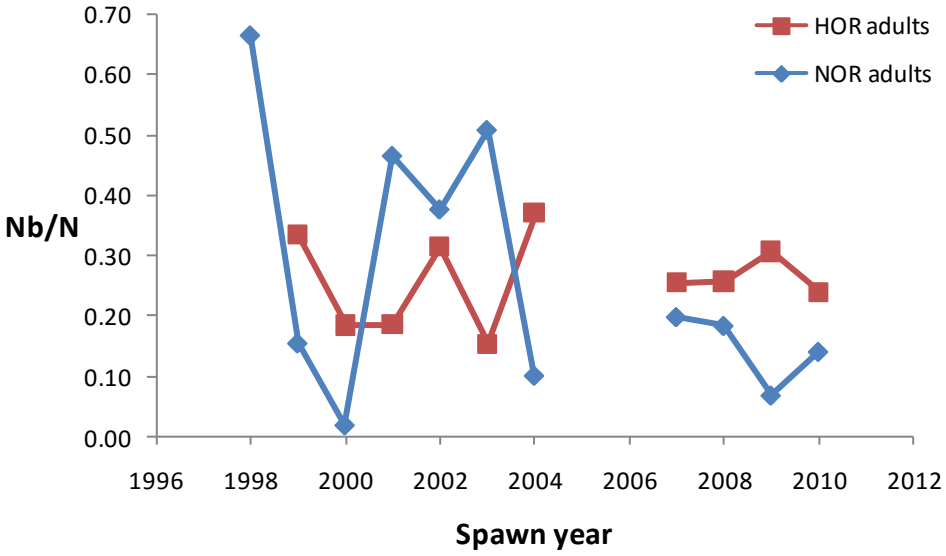
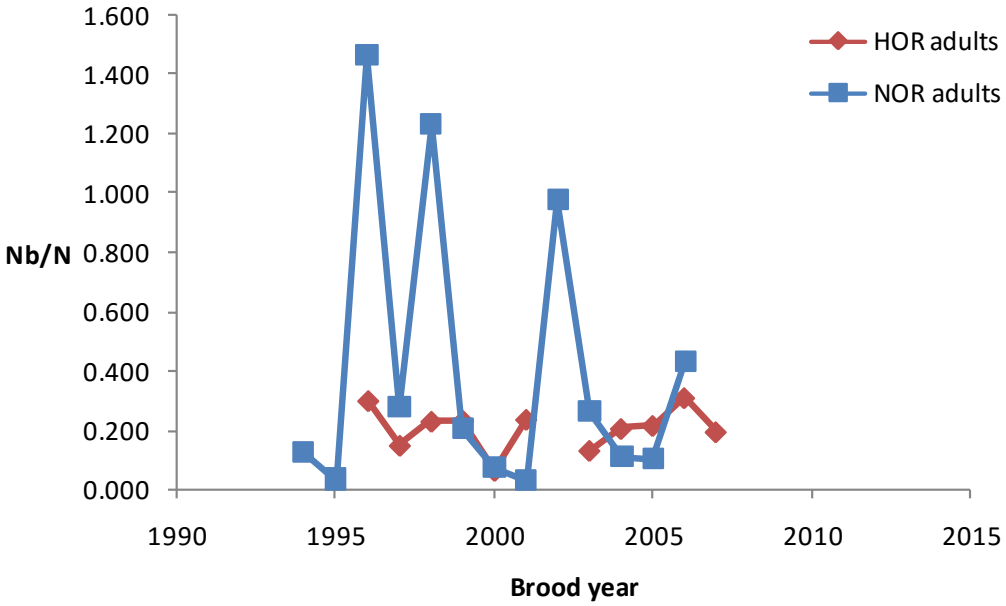


Figure 13. N_b/N ratios for hatchery-produced (HOR) and natural origin (NOR) adult Wenatchee River summer steelhead collections with individuals grouped in brood years rather than spawn years. Individual brood year was estimated using scale-based age data.



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, 2015

NPDES MONITORING FOR WDFW FACILITIES

The WDFW facilities requiring discharge reports are: Chelan Hatchery, Chelan Falls Hatchery, Eastbank Hatchery, Wells Hatchery, Chiwawa Ponds, Methow Hatchery, Similkameen Hatchery, Dryden Acclimation Pond, and Priest Rapids Hatchery. Carlton Acclimation Pond permit became inactive January 2014. An inactive permit is exempt from sampling and submitting discharge reports because production is below the permit requirements for monitoring discharges. NPDES permits are not required for the Twisp and Chewuch acclimation facilities, because they are below the levels that require a discharge permit.

The Wells Hatchery Pollution Abatement (PA) pond has no effluent data January through December. Priest Rapids Hatchery Pollution Abatement (PA) pond has no effluent data January through March, and September through December. The PA ponds for these facilities had no discharge throughout these months.

There were no violations reported at these NPDES permitted facilities during the period 1 January 2015 through 31 December 2015.

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 current permit was extended until 31 March 2016. The permit was renewed effective 1 April 2016 and will expire 31 March 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 guidelines 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 1 June 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 WA Dept 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
2015	JAN	28.43	0	0.4	0.4	7000	0.01		26.6		25412	6743
	FEB	28.43	0	0.4	0.4	8500	0.01		24.8		33757	4618
	MAR	20.68	0	0	0	10000	0.01		10		26814	5033
	APR	22.29	0	0	0	3000	0.01		21.4		19553	5573
	MAY	22.96	0	0	0	5000	0.01		14.2		27705	8855
	JUN	29.73	0	0.2	0.2	7500	0.01		15		37051	9782
	JUL	25.85	0	0.4	0.4	5000	0.01		10.6		35599	5821
	AUG	27.14	0	1	1.4	7500	0.01		20.8		17833	6587
	SEP	27.78	0	0.4	0.4	15000	0.01		39.8		24733	10184
	OCT	31.03	0	0.2	0.2	10000	0.01		2.6		35072	9143
	NOV	23.59	0	0	0	7500	0.01		17.6		24480	3504
	DEC	23.59	0	0.6	0.6	5000	0.01		15.6		19478	4759

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	SS DD	TSS DD
2015	JAN	16.85	0	0.2	0.2	**	**		**		69543	14511		
	FEB	19.41	0	0.2	0.2	**	**		**		79660	17750		
	MAR	18.96	0	0.2	0.2	**	**		**		101677	15519		
	APR	16.13	0	0.2	0.4	**	**		**		85708	9827	0.1	1.4
	MAY	11.54	0	0.6	0.6	**	**		**		30900	5296	0.17	3
	JUN	5.54	0	0.8	0.8	**	**		**		9177	1887		
	JUL	5.38	0	0.4	0.4	**	**		**		7459	2459		
	AUG	5.69	0.01	0.2	0.2	**	**		**		11132	6628		
	SEP	7.06	0.01	1	1	**	**		**		21400	7904		
	OCT	8.49	0.01	0.9	1	**	**		**		30343	8420		
	NOV	9.95	0	1.2	1.2	**	**		**		39509	13790		
	DEC	10.53	0.01	1.4	1.4	**	**		**		53633	14376		

** 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
2015	JAN	4.25	0	0.8	0.8	10040	300		
	FEB	3.62	0	1.4	1.4	15765	390		
	MAR	4.52	0	-0.4	-0.4	9775	260		
	APR	3.85	0	-0.2	-0.2	8194	132	0.04	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	4.22	0	1	1	6042	1012		
	NOV	3.65	0	-0.2	-0.2	11234	348		
	DEC	3.49	0	2	2	10026	341		

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
2015	JAN	6.18	0	2.2	2.2	16650	870		
	FEB	4.84	0	-0.8	-0.8	16280	1784		
	MAR	3.89	0	-1	-1	18300	3720		
	APR	No Monitoring				0	0		
	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	4.66	0	-0.8	-0.8	8800	1010		
	DEC	6.55	0	0.2	0.2	11817	1811		

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
2015	JAN	11.52	0	0.1	0.2	14400	0.1		0		9700	1300		
	FEB	11.52	0	0	0	14400	0.1		5.4		10500	1420		
	MAR	10.08	0	1.3	1.8	14400	0.1		0.2		9600	828		
	APR	10.08	0	-0.4	-0.4	14400	0.1		6.4		9700	900	0	0
	MAY	2.6	0	1.4	1.4	14400	0.1		5.2		1223	455	0.1	3.8
	JUN	3.77	0	0	0	14400	0.1		0		2036	757		
	JUL	4.32	0	0.6	0.6	14400	0.1		17.2		2600	600		
	AUG	4.32	0	0.2	0.2	14400	0.1		1		4000	1100		
	SEP	5.33	0	0	0	14400	0.1		0.8		6200	852		
	OCT	5.33	0	0	0	14400	0.1		0.8		10000	800		
	NOV	5.62	0	0	0	14400	0.1		3.2		10600	875		
	DEC	7.98	0	0.2	0.2	14400	0.1		0.2		11200	930		

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
2015	JAN	6.62	0	0.4	0.4				8461	44		
	FEB	6.62	0	-9.2	-9.2				8398	902		
	MAR	6.62	0	-1.4	-1.4				11325	2684		
	APR	6.62	0	-1	0.6				11313	2596	0	15.2
	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	6.34	0	0.6	0.6				11250	308		
	DEC	6.36	0	-0.4	-0.4				11116	132		

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
2015	JAN	4.2	0.05	-1	-1	68000	0.05		0.8		10780	3914
	FEB	5.2	0.05	0.8	0.8	68000	0.05		0.8		15461	5226
	MAR	7.4	0.05	-0.4	-0.4	68000	0.05		1		25346	10141
	APR	10	0.04	0	0	68000	0.05		1.4		9800	2697
	MAY	7.2	0.05	-0.2	-0.2	68000	0.05		1.4		5445	564
	JUN	7.2	0.05	0	0	68000	0.05		0.2		7470	2566
	JUL	9.5	0.04	-0.6	-0.6	68000	0.05		3.2		4687	4996
	AUG	7.5	0.05	0.8	0.8	68000	0.05		1.6		7211	9113
	SEP	7.5	0.05	0	0	68000	0.05		1.8		12347	9714
	OCT	6.7	0.05	0.2	0.4	68000	0.05		4.4		8357	4751
	NOV	7.2	0.05	0.4	0.4	68000	0.05		4.2		6604	3436
	DEC	7.2	0.05	0.4	0.4	68000	0.05		3		8472	3548

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
2015	JAN	12.9	0.05	-8.8	-8.8	857	0.05		0.8		31994	4568
	FEB	12.9	0.05	-1.9	-1.8	857	0.05		1.2		33820	1650
	MAR	12.8	0.05	-2.2	-2.2	857	0.05		1.4		34262	3766
	APR	12.6	0.05	0.2	0.2	857	0.05		1.6		35344	17751
	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	7	0.04	-9.4	-9.4	3000	0.05		0.2		17614	2227
	DEC	7	0.04	-1	-1	3000	0.05		0.8		19753	2481

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
2015	JAN	No Monitoring				0	0		
	FEB	No Monitoring				0	0		
	MAR	14.21	0	-0.8	-0.8	33366	2948		
	APR	15.26	-0.1	0.3	0.4	46973	5236	0.01	3.8
	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	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
2015	JAN	23.93	0	0.4	0.4	**	**	**	9211	202		
	FEB	26.98	0	0	0	**	**	**	10229	1180		
	MAR	28.24	0	0.4	0.4	**	**	**	14796	4440		
	APR	30.55	0	-0.4	-0.4		0		26695	13102		
	MAY	46.88	0	0	0		0		78430	30166		
	JUN	44.43	0	0.6	0.6		0		135899	34962	0	1.32
	JUL	No Monitoring							0	0		
	AUG	No Monitoring							0	0		
	SEP	60.35				**	**	**	0	0		
	OCT	65.95	0			**	**	**	0	0		
	NOV	65.95	0			**	**	**	0	0		
	DEC	24.25	0	-0.4	-0.4	**	**	**	8632	0		

**PA pond - No discharge this month

Appendix G

Steelhead Stock Assessment at Priest Rapids Dam, 2013-2014

Priest Rapids Dam 2013-2014 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 2013 steelhead sampling at Priest Rapids Dam began 8 July and concluded 14 November. Sampling consisted of operating the Priest Rapids Off Ladder Trap (OLAFT), located on the left bank Priest Rapids Dam, eight hours per day, up to three days per week, for a total of 57 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 2013 totaled 13.5%.

The Washington Department of Fish and Wildlife (WDFW) sampled 2,318 steelhead of the 2013/2014 run-cycle passing PRD, totaling 15,072 steelhead, for an overall sampling rate of 14.6%. Of the 2,196 steelhead sampled, 1,426 (64.9%) were hatchery origin and 770 (35.1%) were wild origin. The estimated 2013-2014 run- cycle total wild steelhead return was 4,657, representing 166.6% of the 1986-2012 average and about 88.6% of the most recent five-year average (Table 1).

Based on external marks and external and internal tags, 1,426 hatchery-origin steelhead were sampled at Priest Rapids Dam during the 2013 return cycle and included 19.5% Wenatchee hatchery-origin steelhead and 49.6% “above Wells Dam” hatchery-origin steelhead[1] (Table 2), while 12.0% of the hatchery-origin steelhead sampled could not be assigned to a specific hatchery program. Ringold FH origin steelhead represented about 12.5% of the hatchery sample (Table 2).

[1] Defined as “above Wells Dam” because hatchery origin, adipose-clipped steelhead release 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
1986-2012 average	**11,828**	**2,796**	**18.7**	**14,181**
2008-2012 average	**18,939**	**5,257**	**21.7**	**24,197**

[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, 8 July – 14 November 2013.

Steelhead origin																					
Wild			Hatchery																		
Wild			Wenatchee						Above Wells				Ringold FH			Unk. Hat.					
Criteria			VIE						Criteria				Criteria			Criteria			Total	Total	Total
NS	NM	Total	LTGR	RTGR	RTOR	RTPK	AD	Total	AD	LTYL	LV	Total	AD	RV	Total	SD	NM	Total	Wild	Hatchery	Total
x	x	770	x					9	x			692	x	x	178	x	x	263	770	1,426	2,196
				x				0		x		3									
					x			0			x	12									
						x		62													
							x	207													
Total		**770**						**278**				**707**			**178**			**263**	**770**	**1,426**	**2,196**
% Hatchery								**19.5**				**49.6**			**12.5**			**18.4**		**100.0**	
% Total		**35.1**						**12.7**				**32.2**			**8.0**			**12.0**	**35.1**	**64.9**	**100.0**

Reconciliation of salt water age of wild and hatchery steelhead sampled at Priest Rapids Dam during 2013 was accomplished through scale sample analysis. Salt-age analysis of the 2013 UCR steelhead run-cycle provides an estimated hatchery-origin return dominated by 1- salt and 2-salt age composition of 60.1% and 39.7%, respectively (Table 3). Natural-origin steelhead salt ages were 68.6% and 31.2% for salt ages 1 and 2, respectively. Three-salt age fish represented only 0.2% of the combined hatchery/wild sample (Table 3).

Table 3. Salt-water age composition of 2013 – 2014 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	845	60.1	521	68.6	1,366	63.1
2-salt	559	39.7	237	31.2	796	36.7
3-salt	3	0.2	1	0.1	4	0.2
4-salt	0	0.0	0	0.0	0	0.0
Total	**1,407**	**65.0**	**759**	**35.0**	**2,166**	

Freshwater residency of naturally produced Upper Columbia River steelhead present in the 2013-2014 run cycle were dominated by age-2 freshwater fish (70.8%), and was only slightly lower than the 1986-2012 average of 74.7% (Table 4).

Table 4. 2013 return year freshwater age of wild Upper Columbia River steelhead sampled at Priest Rapids Dam during steelhead stock assessment activities, compared to July – November 1986-2012 average.

Freshwater age	**2013-2014 run cycle**		**1986-2012 proportion**	
	N	**%**	***N***	**%**
1.x	31	4.4	458	8.4
2.x	495	70.8	4,086	74.7
3.x	161	23.0	885	16.2
4.x	12	1.7	39	0.7
5.x	0	0.0	3	>0.1
Total	**699**		**5,471**	

Wild and hatchery origin steelhead exhibited similar saltwater growth in the 2013 run-cycle. Wild 1- and 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 2013-2014 adult run-cycle return past PRD were comparable in size to the 1986-2012 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 2013 and the period between 1986-2012.

	Average fork length (cm)			
	2013-2014 run cycle		1986-2012 run cycle	
Salt age	Wild	Hatchery	Wild	Hatchery
x.1	57.7	57.2	60.3	59.0
x.2	70.5	69.7	72.7	71.8

Appendix H

Wenatchee Sockeye Salmon Spawning Escapement, 2015

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 20, 2015

To: HCP Hatchery Committee

From: Catherine Willard

Subject: 2015 Wenatchee Sockeye Mark/Recapture-Based Sockeye Escapement Estimates to Tributaries

Introduction

In 2015, 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 2015. 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 2015). Resulting tag files were queried in PTAGIS (2015), providing detection histories for each study fish.

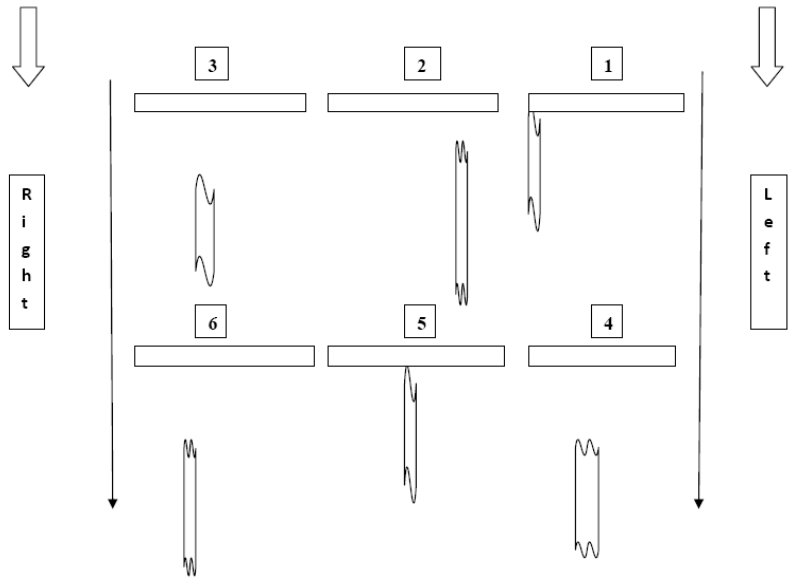


Figure 1. Schematic of a PIT array configuration.

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 51,410 adult sockeye salmon passed the facility during the 2015 migration, which was a sufficient return to open a recreational fishery in Lake Wenatchee for 2015. PIT tags were implanted in 943 of these fish at Tumwater and seven of these fish were PIT-tagged before passing Tumwater; 76 fish were subsequently detected at the Little Wenatchee PIT tag array and 371 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 4,113 adult sockeye and 20,087 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 2015, 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*	76	1.000	*76*	76	4

[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, 2009-2015.

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	51,410	7,916	4,113	20,087	24,200	0.470
Average	**45,333**	**6,989**	**2,933**	**22,181**	**25,115**	**0.684**

[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

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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:

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

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 $\tilde{S}_{i,j}$. The harmonic mean over all pairwise estimates of $\hat{N}_{b(i,j)}$ is $\tilde{N}_b$. SALMONNb (Waples et al. 2007) was used to calculate $\tilde{N}_b$.

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

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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

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 ($\tilde{N}_b$) 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 $\tilde{N}_b$ = 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 $\tilde{N}_b$ = 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 $\tilde{N}_b$ = 50.4. This estimate is not similar to our Chiwawa broodstock estimate. However, if we analyze the hatchery-origin Chinook only, our estimate is $\tilde{N}_b$ = 80.1 for collection years 1989 – 2006 (data not shown). Williamson et al. (submitted) report for Chiwawa naturally spawning Chinook a $\tilde{N}_b$ = 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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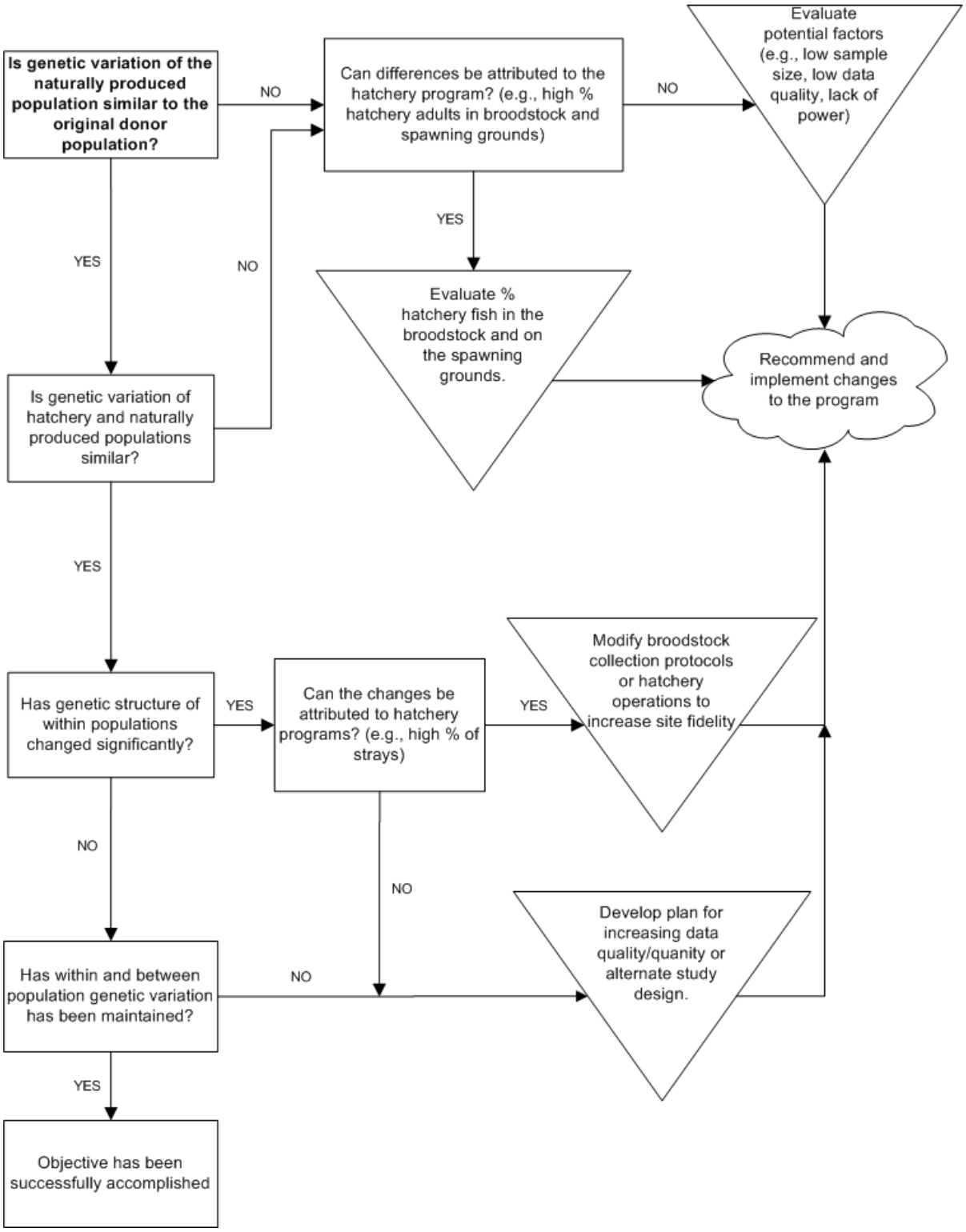

Figure 1. Conceptual process for evaluating potential changes in genetic variation in the Chiwawa naturally produced populations as a result of the supplementation hatchery programs (From Murdoch and Peven 2005).

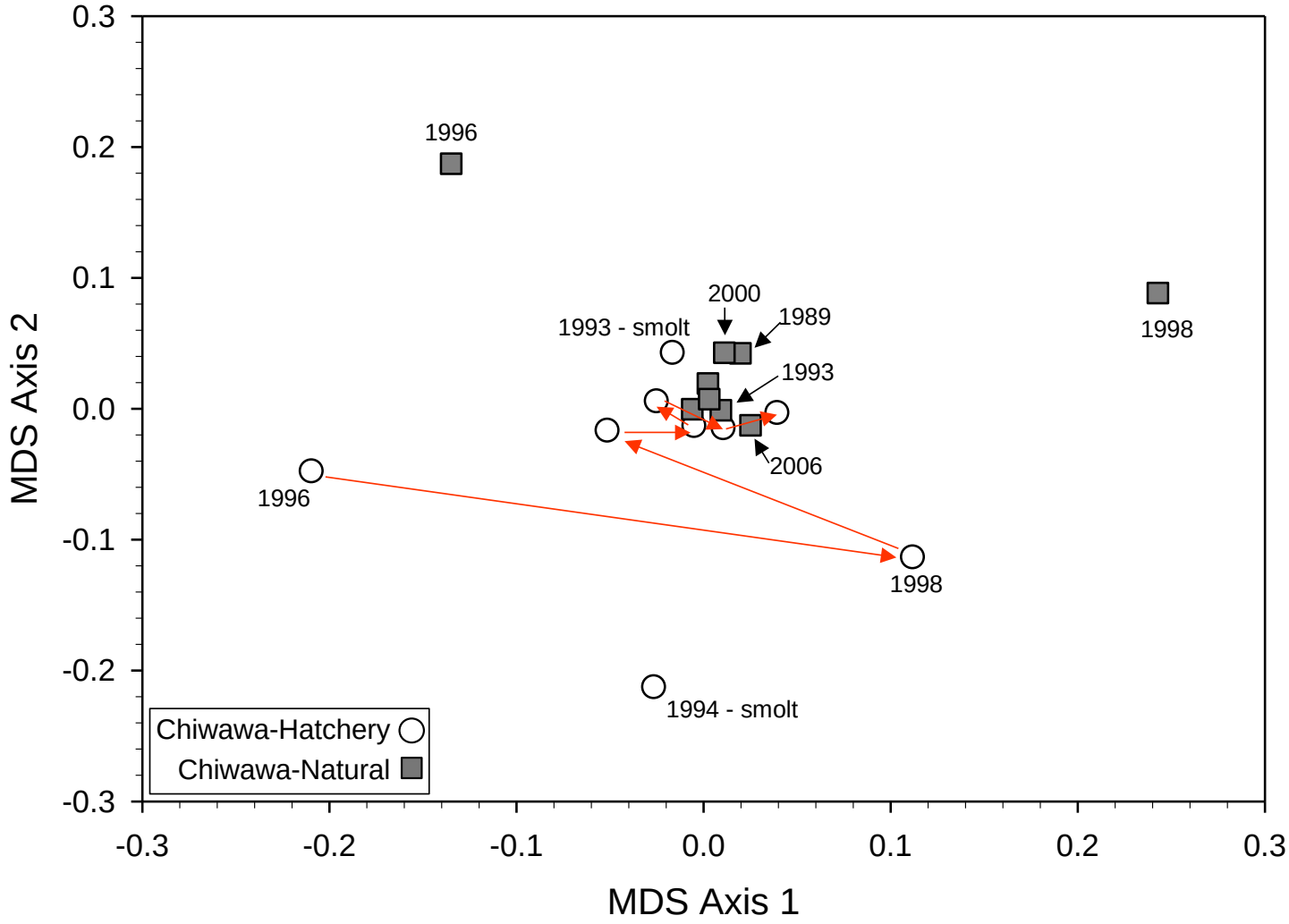

Figure 2. Multidimensional scaling plot from an allele-sharing distance matrix calculated from the Chiwawa data set organized by fish origin (i.e., hatchery versus natural). The red arrows connect consecutive hatchery-origin collections starting with the first adult collection (1996) and ending with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

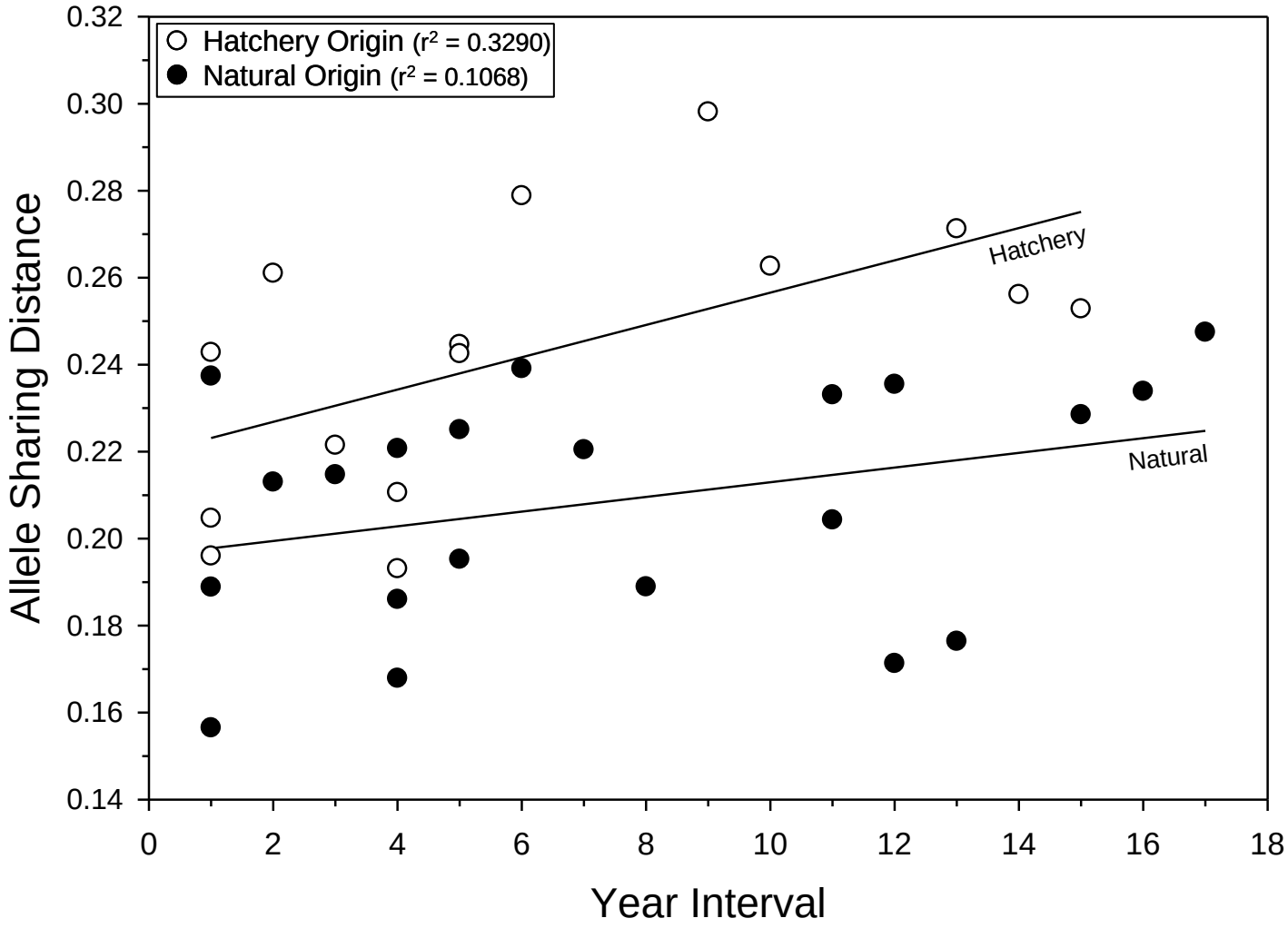

Figure 3. Relationships between the time interval in years and allele sharing distances, with each circle representing the pairwise relationship between two Chiwawa collections. Separate regression lines for the natural- and hatchery-origin collections. The slope for the natural-origin collection is 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.

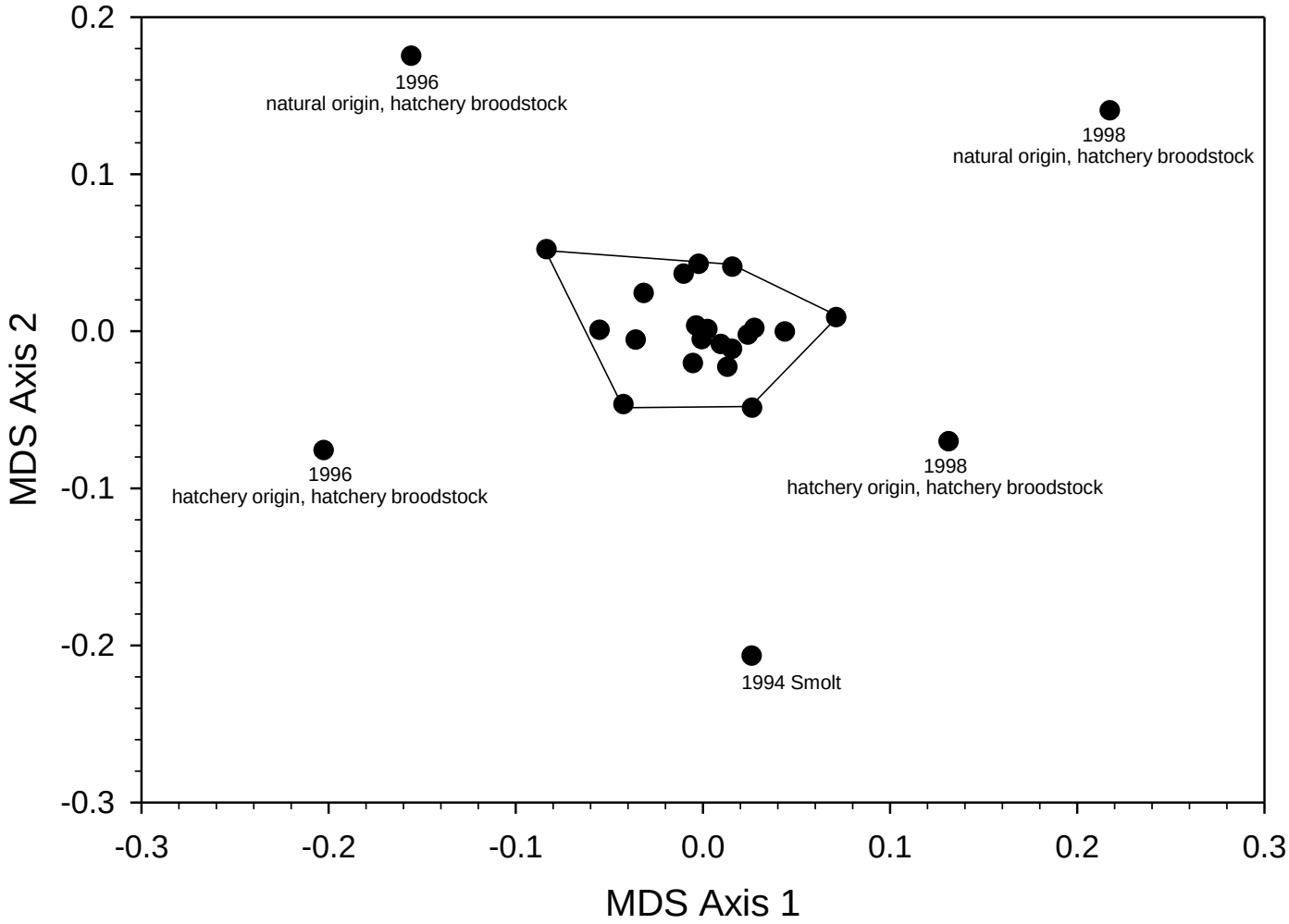

Figure 4. Multidimensional scaling plot from an allele-sharing distance matrix calculated from the Chiwawa data set organized by four treatment groups, as discussed in the text. Each circle represents a single collection within each of the four treatment groups, and the polygon encloses all groups that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

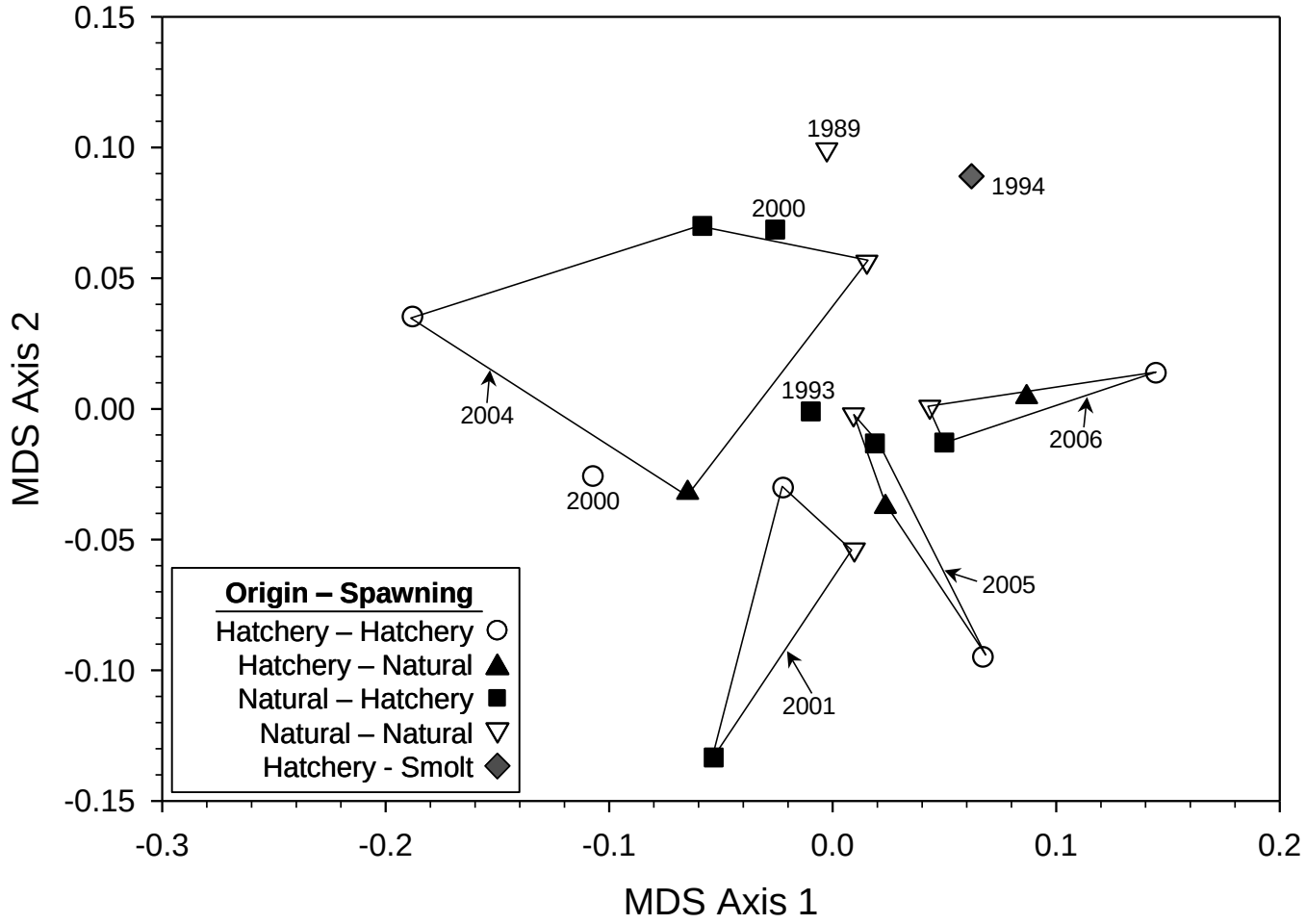

Figure 5. As in Figure 4, but allele-sharing distance matrix recalculated without the five outlier groups shown in Figure 4. Polygons group together treatment groups from the same collection year. Dates associated with symbols also refer to collection year. Collection years 2004-2006 included all four 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.

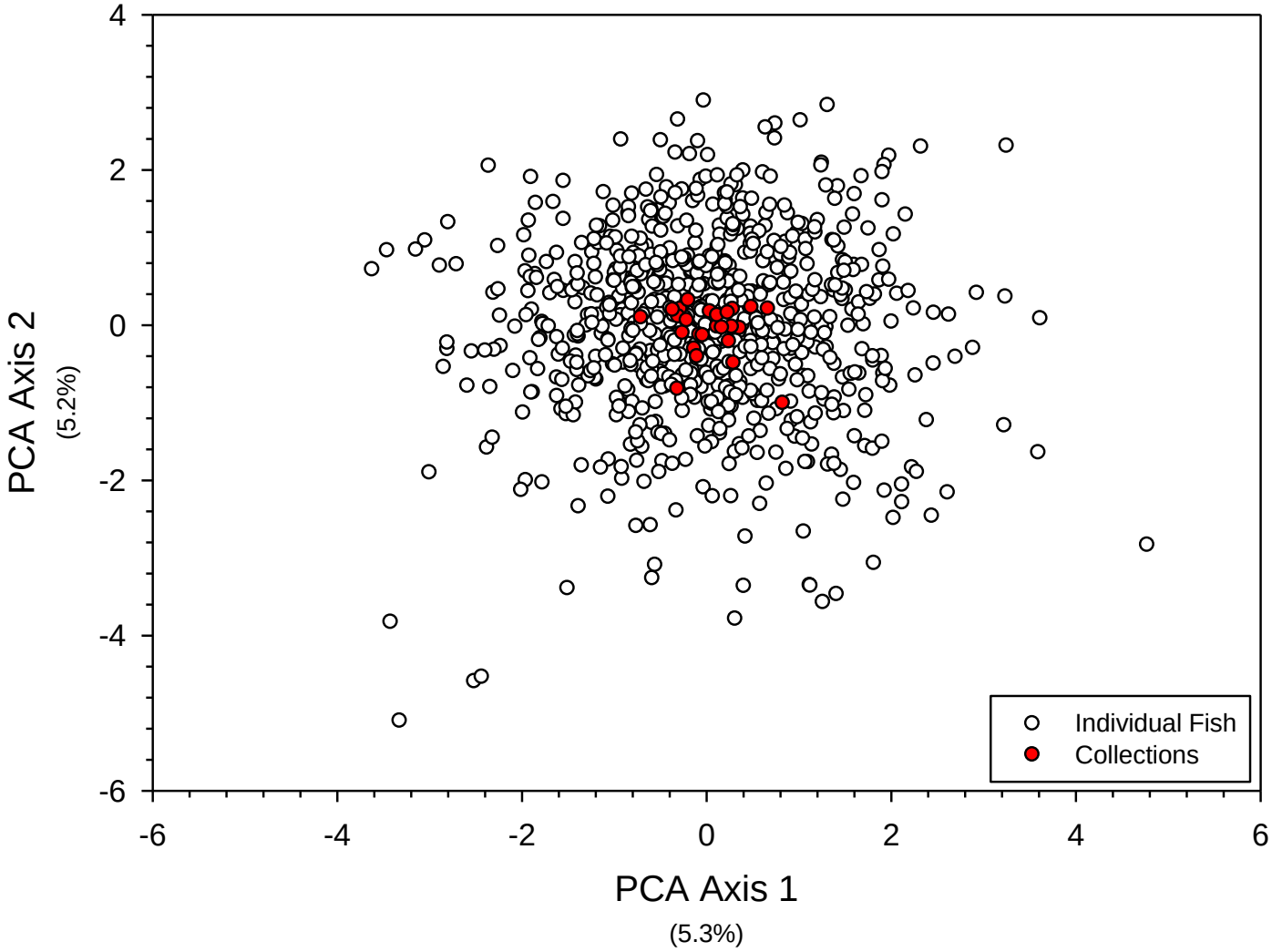

Figure 6. Principal component (PC) analysis of individual fish from the Chiwawa River. Only fish with complete microsatellite genotypes were included in the analysis (n = 757). Open circles are the PC scores for individual fish, and the filled circles are the centroids (bivariate means) for each of the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

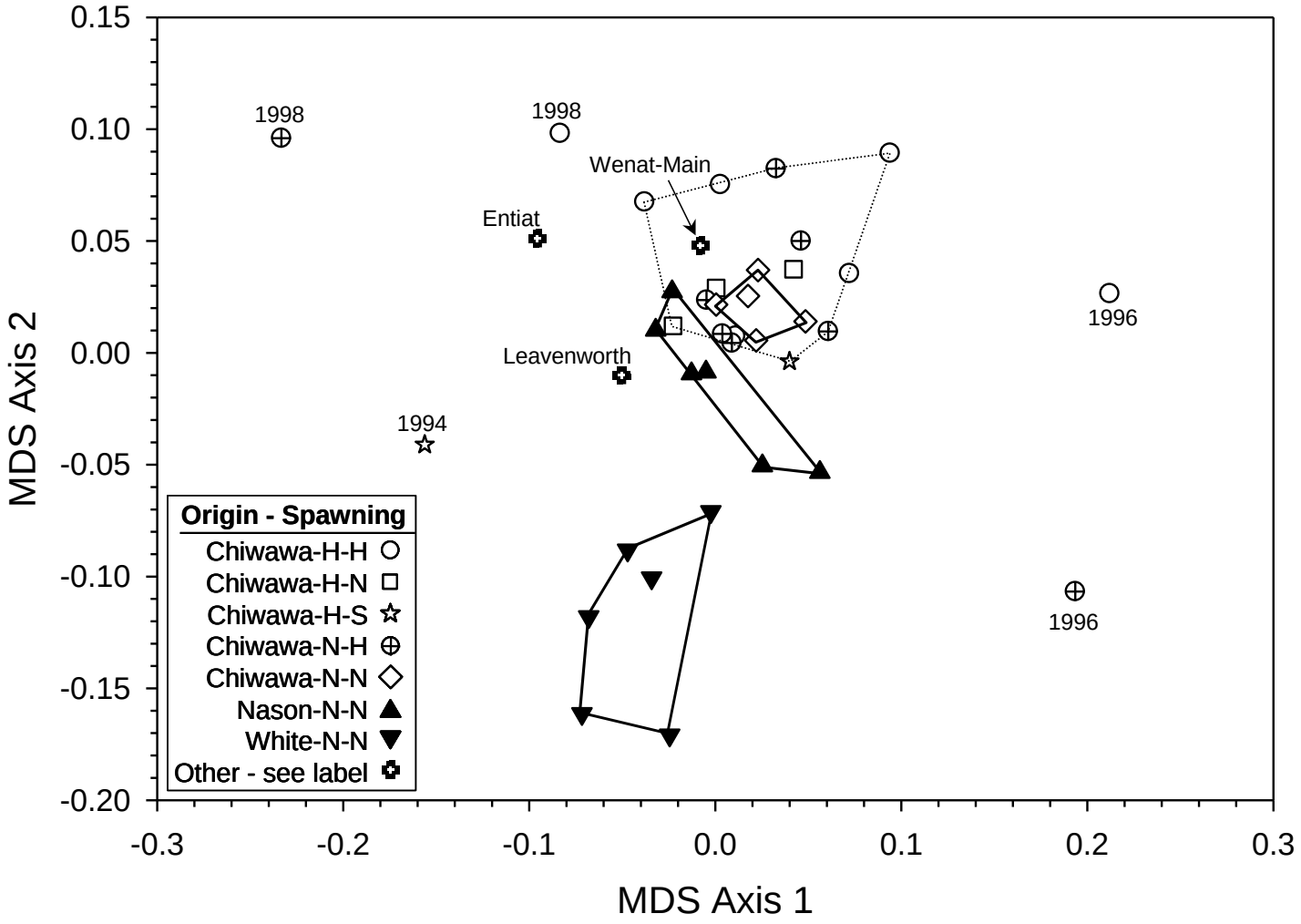

Figure 7. Multidimensional scaling plot from an allele-sharing distance matrix calculated from the Chiwawa origin data set and all other non-Chiwawa collections, except Little Wenatchee River. Legend is read with abbreviations beginning with origin and then spawning location. H=hatchery, N=natural, and 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*, $\tilde{S}$ 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	1993	1996	1998	2000	2001	2004	2005	2006
Pairwise $\tilde{S}$ (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	-

$\tilde{N}_b$ = 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*, $\tilde{S}$ 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	2004	2005	2006
Pairwise $\tilde{S}$ (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	-

$\tilde{N}_b$ = 386.8

Appendix K

Fish Trapping at the Nason Creek Smolt Trap 2015

Population Estimates for Juvenile Salmonids in Nason Creek, WA

2015 Annual Final Report

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ABSTRACT

In 2015, 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, 2015. We collected 745 spring Chinook salmon, 430 summer steelhead, 1 bull trout, and 5 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 BY2013 spring Chinook juveniles and coho juveniles of 57,525 (± 39,889) and 161 (± 714), respectively. We estimated the total BY2012 summer steelhead emigration at the trap to be 25,566 (± 6,020). Egg-to-emigrant survival rates for BY2013 spring Chinook and BY2012 summer steelhead were 5.8% and 3.0%, respectively. The egg-to-emigrant survival rate for BY2011 summer steelhead was 0.9%. Productivity, as measured by emigrants-per-redd, for spring Chinook and summer steelhead, was 271 and 162, respectively. With no coho redds on Nason Creek in 2013, egg-to-emigrant survival and productivity could not be estimated for the 2013 brood.

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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, Barry Hodges, 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, J.B. Walters, 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 65,600 acres 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 52,300 acres (79.7%) of the watershed while 12,800 acres (19.5%) are federal and 480 acres (0.1%) are state owned (USFS et al. 1996).

The channel morphology of the lower 25 kilometers 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 2015, mean daily discharge for Nason Creek was 285 cfs with mean daily stream temperatures ranging from 0.0°C to 21.3°C (Figure 2 & 3). Spring discharge was extremely limited due to deminished snowpack by the onset of the trapping season. Maximum daily mean discharge in the spring of 2015 was 733cfs; normal maximum mean (12-year) daily flows during spring freshets on Nason Creek are approximately 2,000cfs. The lack of snowpack prompted the early-onset of base-flow conditions (<100cfs) by the end of June. Base-flow conditions persisted into late October, at which time multiple rain-on-snow events pushed Nason Creek into flood conditions.

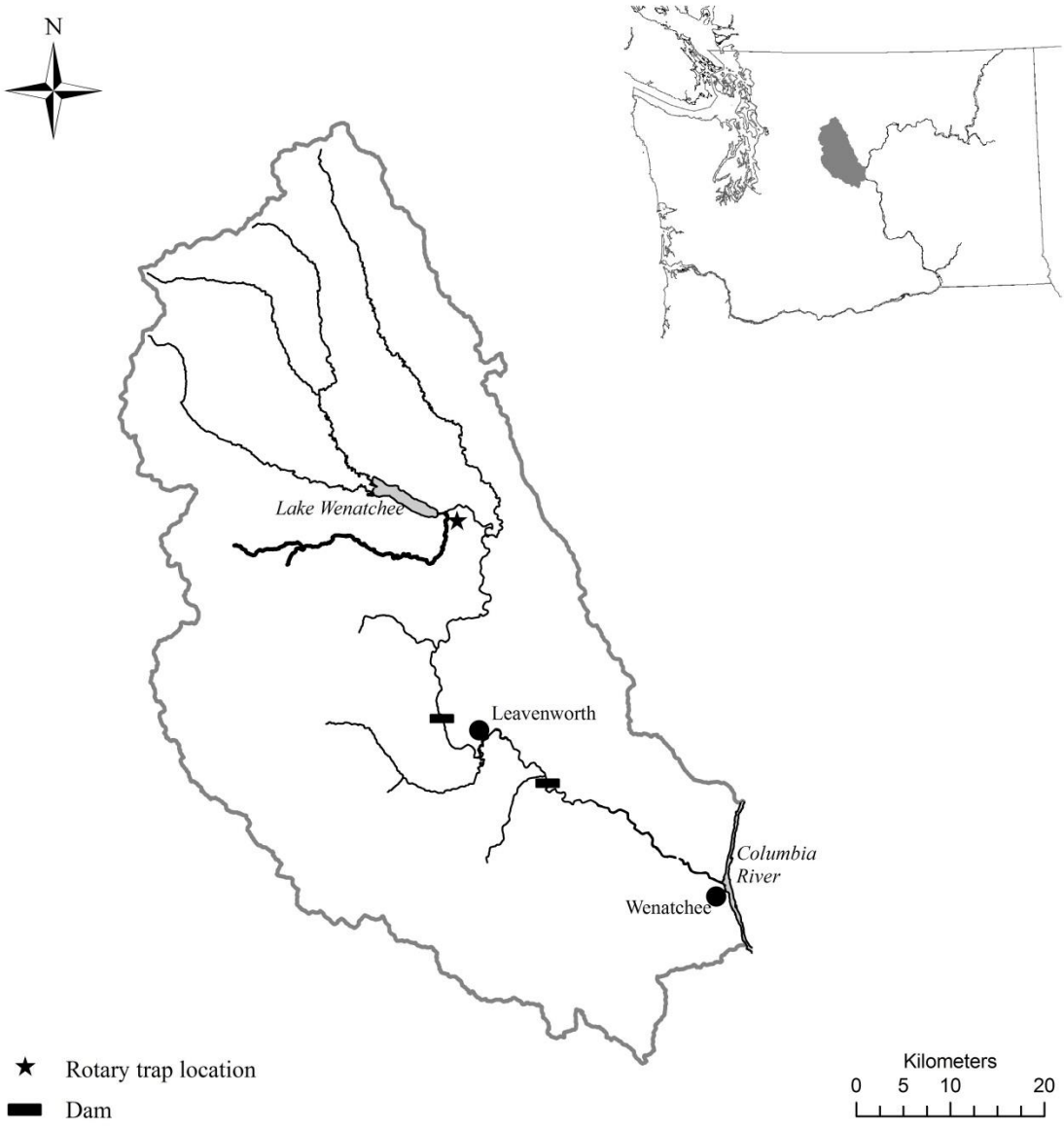


Figure 1. Map of Wenatchee River Subbasin with the Nason Creek rotary trap location.

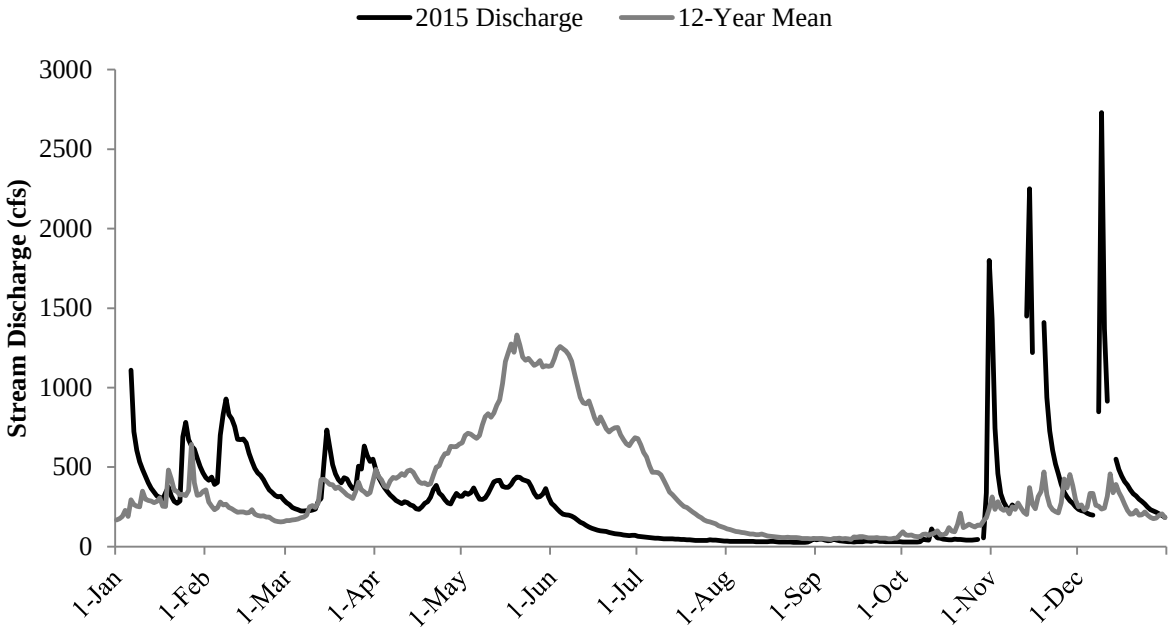


Figure 2. Mean daily stream discharge at the Nason Creek WDOE stream monitoring station in 2015.

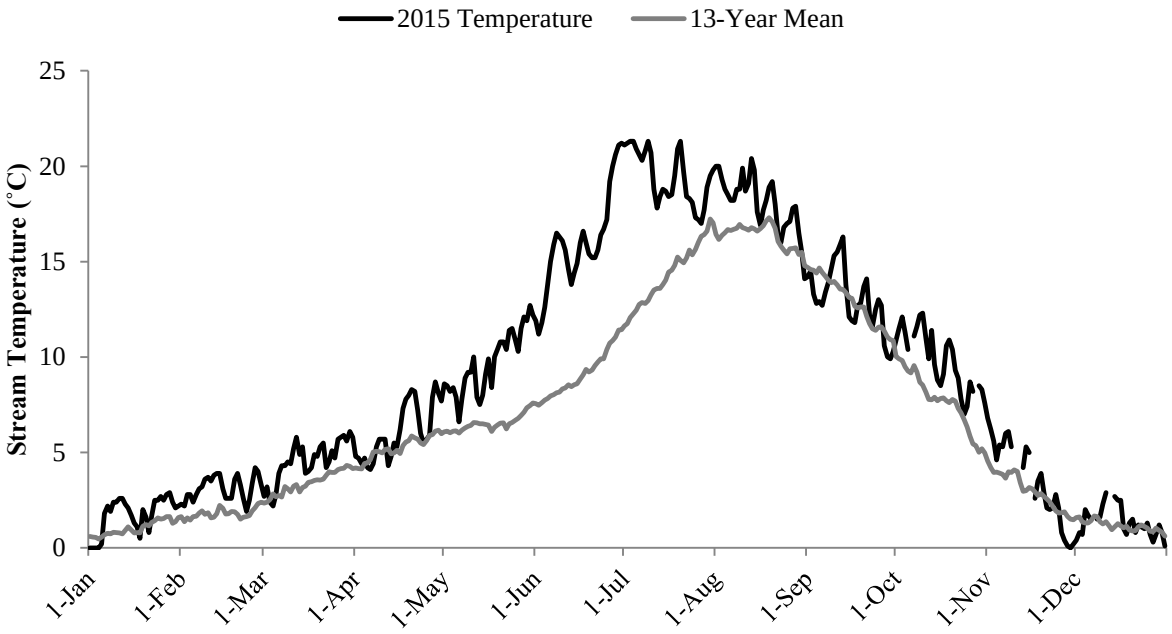


Figure 3. Mean daily water temperature at the Nason Creek DOE stream monitoring station in 2015.

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 (up to 72 hours; Section 7 permit 2011/05645). 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. During periods when the trap was not operating (e.g. high discharge, high debris or mechanical malfunction), the number of target species captured was estimated. The estimated number of fish captured was calculated using the average number of fish captured three days prior and three days after the break in operation. This estimate of daily capture was incorporated into the overall emigration estimate.

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 oxygen 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 ≥ 60mm 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 apposing 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 2015. 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:

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

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:

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

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,

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

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 $\hat{Var}(b_0+b_1\,flow_i)=M\hat{S}E\left(1+\frac{1}{n}+\frac{(flow_i-\overline{flow})^2}{(n-1)s^2_{flow}}\right)$, 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:

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

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, $\hat{\hat{e}}$ (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% 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 Abundance During The 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.

2.5.3 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 2015 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 2. We attempted to run the trap continuously 24 hours a day, 7 days per week. Intentional suspension of trapping activities occurred for a prolonged period in the summer-early fall due to extreme base flows (July 18 - October 20; Table 1). Pulling of the trap also occurred in the fall as a precaution during two major flood events. Trap stoppages were most frequent from July through November, as heavy debris loads and ice formation prevented continuous operation.

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	119
	Interrupted	Interrupted by debris	3
	Pulled	Intentionally pulled during periods of high flow, low flow, or significant ice formation	0
July 1 to November 30	Operating	Continuous data collection	34
	Interrupted	Interrupted by debris, ice and/or low flows	14
	Pulled	Intentionally pulled during periods of high flow, low flow, or significant ice formation	105

3.2 Daily Captures and Biological Sampling

3.2.1 Spring Chinook Yearlings (BY2013)

Between March 1 and June 30, a total of 152 wild Chinook yearlings were captured at the trap (Figure 4). The majority of these fish were collected following an intial spike in flow immediately following operation commencement. A peak catch of 10 yearling smolts coincided with a secondary spike in discharge occurring on March 27. Following the final freshets of March, catch dropped substantially with the last emigrating Chinook yearling captured on May 21. Although three trap stoppages occurred during this period, they likely did not adversely affect total Chinook smolts captured and therefore, estimates were forgone. Mean FL and weight for Chinook yearlings was 93mm (n = 152; SD = 7.0) and 8.4g (n = 152; SD = 2.2; Table 2), respectively. Tissue sample were collected from 138 fish for an ongoing, parental-based DNA analysis by WDFW. Five wild spring Chinook mortalities were incurred.

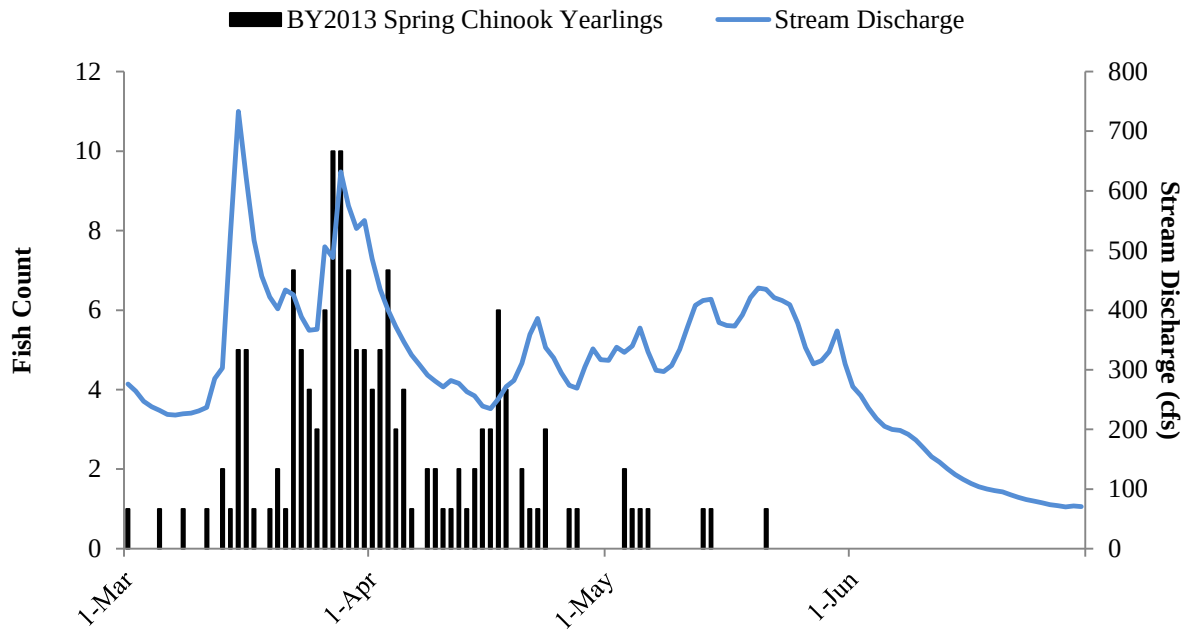


Figure 4. Daily catch of BY2013 spring Chinook yearlings with mean daily stream discharge at the Nason Creek rotary trap, March 1 to June 30, 2015.

Table 2. Summary of length and weight sampling of juvenile spring Chinook captured at the Nason Creek rotary trap in 2015.

Brood Year	Origin/Species/Stage	Fork Length (mm)			Weight (g)			K-Factor
		Mean	*n*	SD	Mean	*n*	SD	
2013	Wild Spring Chinook Yearling Smolt	93	152	7.0	8.4	152	2.2	1.03
2014	Wild Spring Chinook Subyearling Fry	45	338	9.9	1.0	338	0.9	0.87
2014	Wild Spring Chinook Subyearling Parr	84	210	8.0	6.5	209	1.7	1.08
2013	Hatchery Spring Chinook Yearling Smolt	136	284	12.3	29.5	284	8.8	1.13

3.2.2 Spring Chinook Subyearlings (BY2014)

A total of 210 wild spring Chinook subyearling parr were captured between July 1 and November 30, with an additional 338 subyearling fry captured prior to July 1 (Figure 5). A peak daily capture of 89 subyearling Chinook parr occurred on November 3, following the first fall high-water event of the year. Mean FL and weight among fall subyearling parr was 84mm (n = 210; SD = 8.0) and 6.5g (n = 209; SD = 1.7), respectively. We estimate that an additional 16 Chinook subyearling parr would have been captured during short stoppages (≤3 days) had the trap run without interruption. Estimates of daily abundance during the prolonged period of suspended trapping (July 14 – October 10) were not made due to a lack of documented pre- and post-suspension movement, as well as the duration of the suspenstion. Tissue samples were collected from 213 fish for an ongoing, parental-based DNA analysis by WDFW. A total of 10

subyearling Chinook (9 fry and 1 parr) mortalities occurred in 2015. Causes of death included trapping mortality, tagging/handing mortality, and pre-existing fungal infection/poor condition.

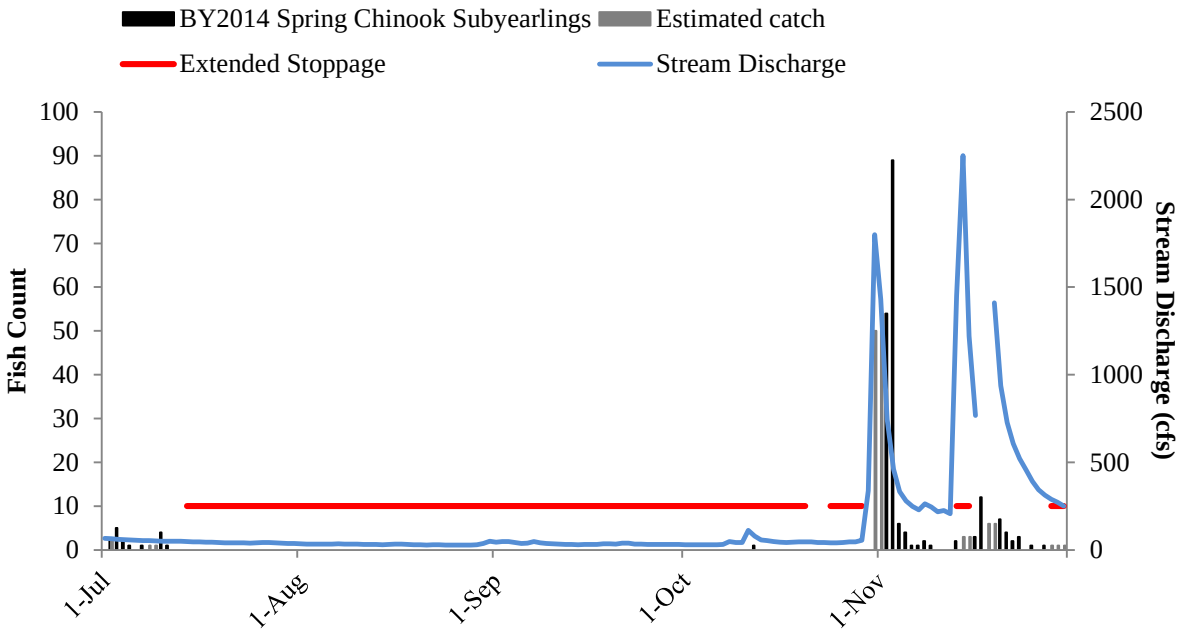


Figure 5. Daily catch of BY2014 spring Chinook subyearlings with mean daily stream discharge at the Nason Creek rotary trap, July 1 to November 30, 2015.

3.2.3 Hatchery Spring Chinook Smolts (BY2013)

During the months of April and May, a total of 43,082 hatchery spring Chinook smolts were released into Nason Creek (M. Babiar, personal communication, January 14, 2016). 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 714 smolts were captured with a mean FL and weight of 136mm (n =284; SD = 12.3) and 29.5g (n = 284; SD = 8.8), respectively (Figure 6). Hatchery spring Chinook were not captured at the smolt trap beyond May 10. There were no mortalities incurred.

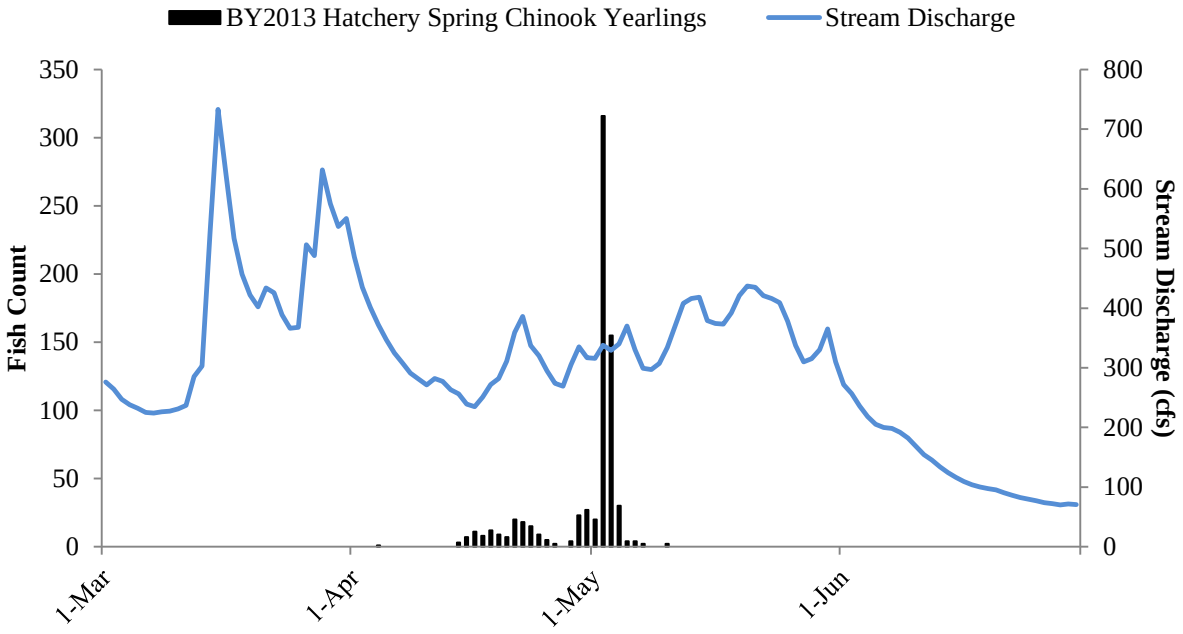


Figure 6. Daily catch of BY2013 hatchery spring Chinook smolts with mean daily stream discharge at the Nason Creek rotary trap, March 1 to June 30, 2015.

3.2.4 Summer Steelhead

A total of 430 wild summer steelhead juveniles were captured throughout the season from March 1 to November 30 with a peak catch of 89 juveniles on November 2 (Figure 6). We estimated that an additional 2 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 2015 using FL. We estimate that of the total steelhead captured, 182 were young-of-the-year, 233 were age-1, 14 were age-2, and 1 was age-3. Subyearling steelhead caught had a mean FL of 70mm (n = 182; SD = 15.5), and a mean weight of 4.3(n = 176; SD = 2.0). 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 88mm (n = 233; SD = 20.2; Table 3) and 8.3g (n = 233; SD = 6.7), respectively. Age-2 steelhead were caught primarily in the spring, with only one fish being captured after July 31. Mean FL and weight of age-2 fish was 149mm (n = 14; SD = 13.5) and 33.7g (n = 14; SD = 8.2), respectively. A single age-3 fish with a FL of 175mm and weight of 51.3g was also captured. Scales were taken from a sub-sample (n = 188) to be used for future age analyses. Two trapping mortalities were incurred (See **3.6 ESA Compliance**).

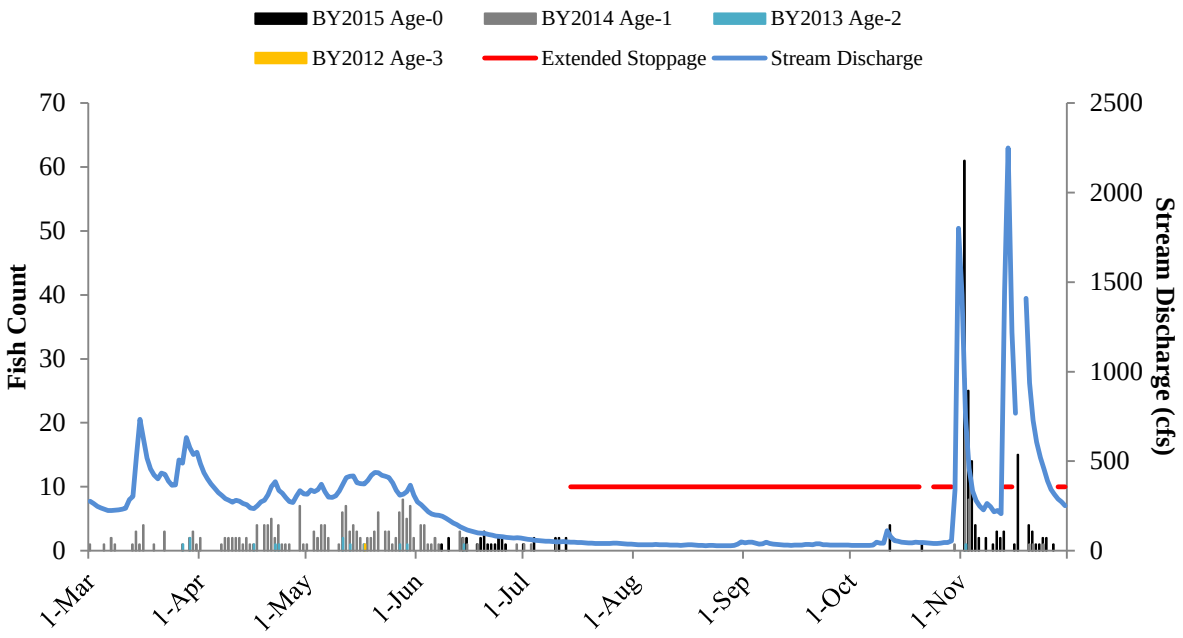


Figure 7. Daily catch of wild summer steelhead with mean daily stream discharge at the Nason Creek rotary trap, March 1 to November 30, 2015. Estimates of fish passage during trap interruptions are not depicted.

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	
2015	Wild Summer Steelhead (Age-0)	70	182	15.5	4.3	176	2.0	1.06
2014	Wild Summer Steelhead (Age-1)	88	233	20.2	8.3	233	6.7	1.04
2013	Wild Summer Steelhead (Age-2)	149	14	13.5	33.7	14	8.2	1.00
2012	Wild Summer Steelhead (Age-3)	191	1	—	73.8	1	—	1.06
2014	Hatch. Summer Steelhead Smolt	175	273	15.2	51.3	273	12.5	0.94

3.2.5 Hatchery Steelhead Smolts (BY2014)

During April and May, WDFW directly planted a total of 86,613 hatchery summer steelhead smolts into Nason Creek (M. Babiar, personal communication, January 14, 2016). Subsequently, a total of 448 hatchery steelhead were captured at the smolt trap with a mean FL and weight of 175mm (n =273; SD = 15.2) and 51.3g (n = 273; SD = 12.5), respectively (Figure 7). The presence of hatchery-origin steelhead at the trap was limited to 45 days after initial release, and did not continue into the summer. Hatchery origin was determined by the presence of coded wire tags (CWT). One mortality was incurred.

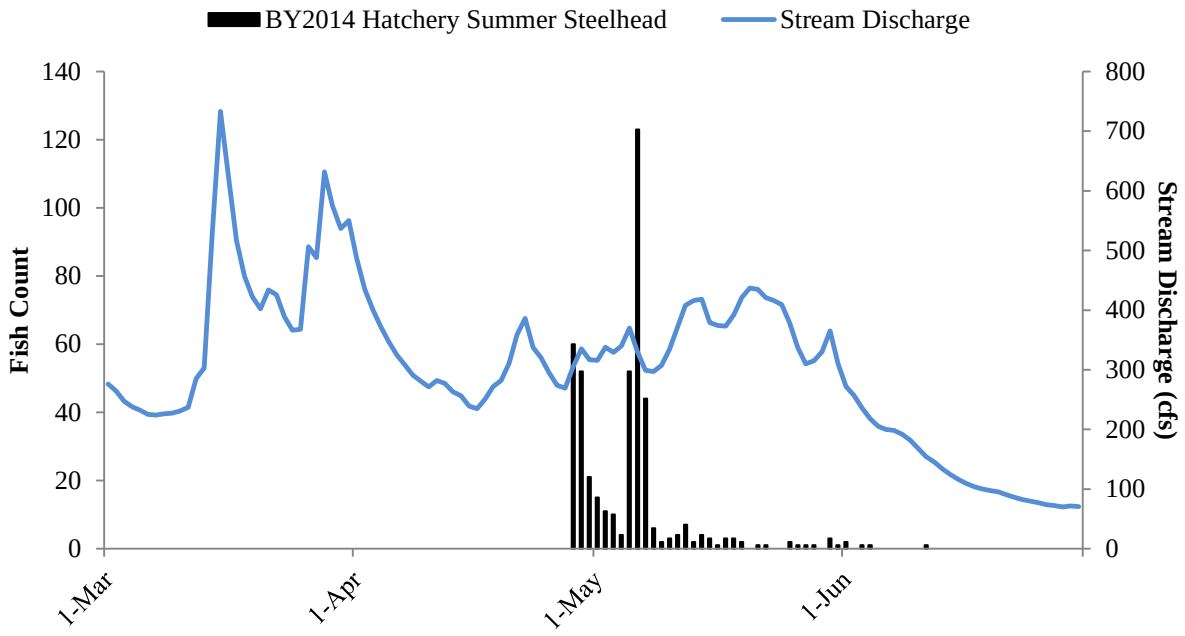


Figure 8. Daily catch of BY2014 hatchery steelhead smolt with mean daily stream discharge at the Nason Creek rotary trap, March 1 to June 30, 2015.

3.2.6 Bull Trout

Bull trout presence at the trap in 2015 was limited to a single fish with a FL of 180mm and weight of 50.1g. The bull trout was released immediately after morphometric measurements were taken. No other sampling/tagging activities were performed.

3.2.7 Coho Yearlings (BY2013)

Two naturally produced coho yearlings were captured during spring emigration between March 1 and June 30 (Figure 8). Mean FL and weight were 109mm (n = 2; SD = 4.9) and 12.0g (n = 2;

SD = 0.1), respectively (Table 5). Scale and tissue samples were not taken from naturally-produced coho smolts in 2015. There were no coho yearling mortalities.

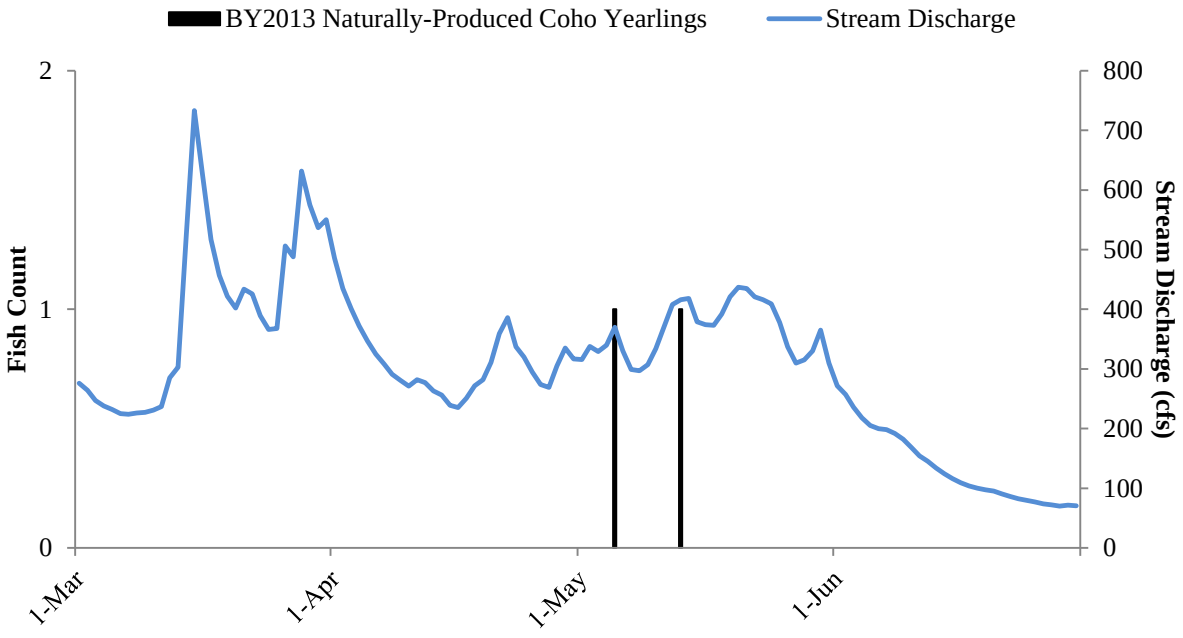


Figure 9. Daily catch of BY2013 naturally-produced coho yearlings with mean daily stream discharge at the Nason Creek rotary trap, March 1 to June 30, 2015.

Table 4. Summary of length and weight sampling of juvenile coho salmon captured at the Nason Creek rotary trap in 2015.

Brood Year	Origin/Species/Stage	Fork Length (mm)			Weight (g)			K-Factor
		Mean	*n*	SD	Mean	*n*	SD	
2013	Naturally Produced Coho Yearling Smolts	109	2	4.9	12.0	2	0.1	0.95
2014	Naturally Produced Coho Subyearling Fry	47	7	13.7	1.4	7	1.5	0.86
2014	Naturally Produced Coho Subyearling Parr	69	3	7.0	4.0	3	1.3	1.20
2013	Hatchery Coho Yearling Smolts	131	952	9.9	23.3	952	4.8	1.03

3.2.8 Coho Subyearlings (BY2014)

A total of three naturally produced coho subyearling parr were captured during between July 1 and November 30 (Figure 9). Mean FL and weight were 69mm (*n* = 3; *SD* = 7.0) and 4.0g (*n* = 3; *SD* = 1.3), respectively. An additional seven subyearling coho fry were also captured with a mean FL of 47mm. There were no coho subyearling mortalities.

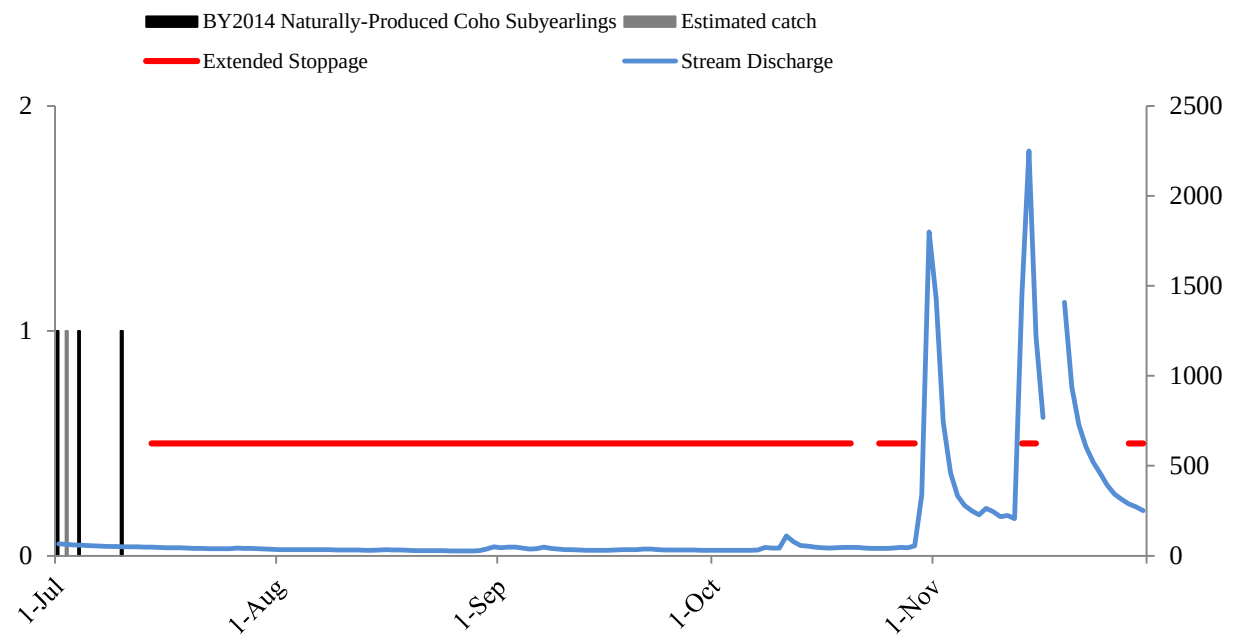


Figure 10. Daily catch of BY2014 naturally-produced coho subyearlings with mean daily stream discharge at the Nason Creek rotary trap, July 1 to November 30, 2015.

3.2.9 Hatchery Coho Smolts (BY2013)

A total of 253,242 hatchery coho were released into Nason Creek above the trap in spring of 2015. 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 1,798 hatchery coho were captured at the trap (Figure 10). Mean FL was 131mm (n = 952; SD = 9.9) and mean weight was 23.3g (n = 952; SD = 4.8; Table 2). A peak daily catch of 215 hatchery coho smolts occurred on May 5 following volitional release into Nason Creek. One trapping mortality was 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.

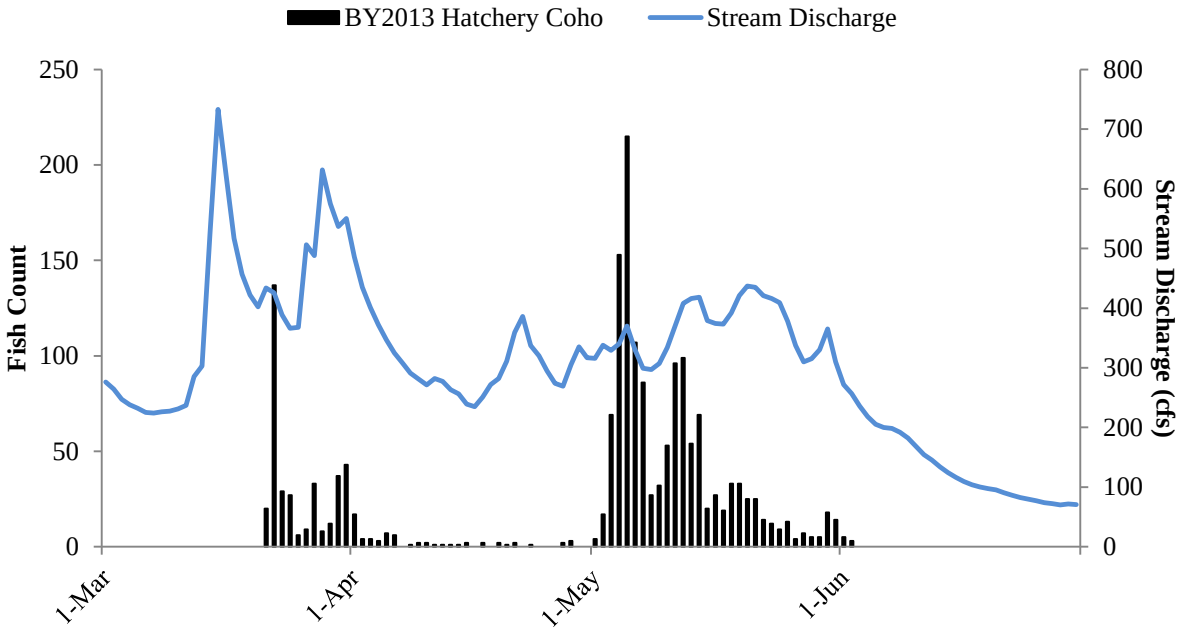


Figure 11. Daily catch of BY2013 hatchery coho smolt with mean daily stream discharge at the Nason Creek rotary trap, March 1 to June 30, 2015.

3.3 Remote Parr Tagging (BY2013 Spring Chinook)

YNFRM and WDFW personnel PIT tagged and released a total of 1,821 BY2013 spring Chinook parr between September 22 and October 24, 2014. 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 311 re-sights of the remote tagged Chinook were documented at the NAL array (Figure 12). Of these detections, only 13 were during the winter non-trapping period. PTAGIS event logs for the NAL array indicated that it operated continuously for the duration of this time with no alterations (PTAGIS 2015).

Subsequent to the remote tagging effort, 30 remote-tagged BY2013 spring Chinook were recaptured at the Nason Creek smolt trap. Total spring Chinook catch at the smolt trap was 798 emigrants during the same period. The pooled tag rate for remote-tagged spring Chinook captured at the Nason smolt trap was 3.8%.

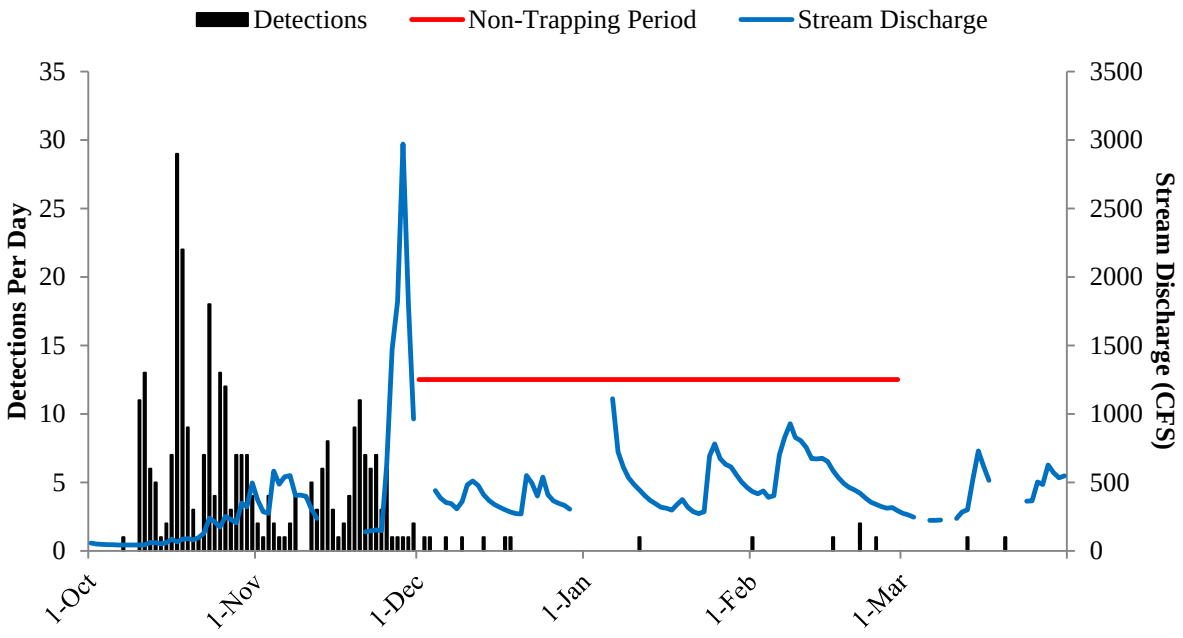


Figure 12. Daily detections of remote-tagged BY2013 spring Chinook at the lower Nason Creek PIT tag antenna array (NAL) between October 2014 and March 2015.

3.4 Trap Efficiency Calibration and Population Estimates

3.4.1 Spring Chinook Yearlings (BY2013)

Infrequent releases, low abundance, and a lack of recaptures did not allow a species-specific model to be used on BY2013 yearling emigrants. In order to produce an estimate, a pooled efficiency (2.07%) composed of spring Chinook yearling and hatchery-origin coho yearling surrogate trials 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 6,992 (± 32,823; 95% CI) BY2013 Chinook yearlings emigrated in spring of 2015 (Table 7). Parr emmigration during the non-trapping period was estimated using a flow-efficiency regression (r^2 = 0.61; p = 0.0002) based on detections at the NAL pit tag array. We estimated that 6,822 (± 9,035; 95% CI) BY2013 spring Chinook emigrated out of Nason Creek during the non-trapping period. Combined with a recalculated BY2013 subyearling estimate of 43,711 (± 20,788; 95% CI), we estimated that a total of 57,526 (± 39,889; 95% CI) BY2013 spring Chinook juveniles emigrated from Nason Creek.

Table 5. Trap efficiency trials conducted with BY2013 wild spring Chinook yearlings and hatchery-origin coho yearling surrogates.

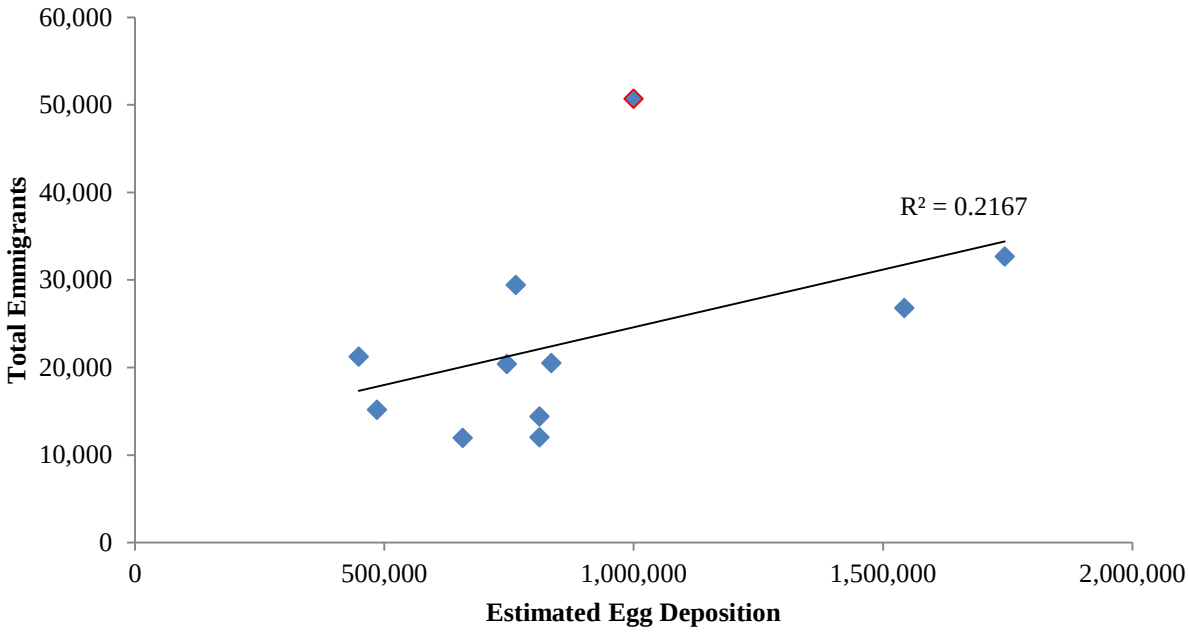
Origin/Species/Stage	Age	Date	Marked	Recaptured	Discharge (cfs)
Wild Chinook Yearlings	1+	4/23/2015	7	0	337
Wild Chinook Yearlings	1+	4/27/2015	2	0	269

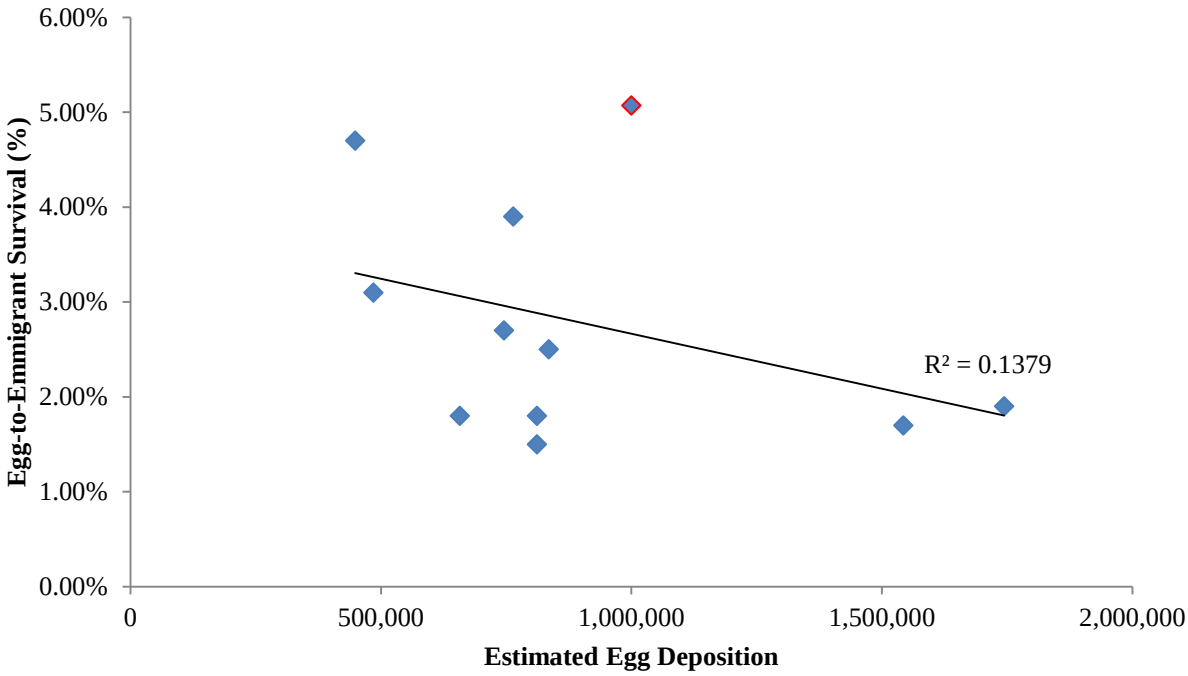
Wild Chinook Yearlings	1+	5/6/2015	5	0	330
Wild Chinook Yearlings	1+	5/10/2015	1	0	334
Wild Chinook Yearlings	1+	5/14/2015	22	0	418
Wild Chinook Yearlings	1+	5/22/2015	1	0	421
Hatchery-Origin Coho Yearlings	1+	5/5/2015	98	2	370
Hatchery-Origin Coho Yearlings	1+	5/12/2015	224	8	408
Hatchery-Origin Coho Yearlings	1+	5/14/2015	101	3	418
Hatchery-Origin Coho Yearlings	1+	5/19/2015	102	0	421
Hatchery-Origin Coho Yearlings	1+	5/23/2015	66	0	416
Total			**629**	**13**	

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

Brood Year	No. of 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	DNOT		4,683	—	—	—
2003	83	5,844	485,052	8,829		6,358	15,187 ± 1,605	3.1%	183
2004	169	4,799	811,031	11,822		2,597	14,419 ± 2,766	1.8%	85
2005	193	4,327	835,111	11,814		8,696	20,510 ± 5,018	2.5%	106
2006	152	4,324	657,248	4,144		7,798	11,942 ± 1,744	1.8%	79
2007	101	4,441	448,541	15,556		5,679	21,235 ± 2,864	4.7%	210
2008	336	4,592	1,542,912	23,182		3,611	26,793 ± 6,756	1.7%	80
2009	167	4,573	763,691	27,720		1,705	29,425 ± 12,777	3.9%	176
2010	188	4,314	811,032	8,491		3,535	12,026 ± 1,954	1.5%	64
2011	170	4,385	745,450	17,991		2,422	20,413 ± 3,889	2.7%	120
2012	413	4,223	1,744,099	28,110		4,561	32,671 ± 4,863	1.9%	79
2013	212	4,716	999,792	43,711	6,822	6,992	57,525 ± 39,889	5.8%	271
2014	115	4,467	513,705	13,903	—	—	—	—	—
Avg.c	199	4,594	894,905	18,306	—	4,905	23,831	2.9%	132

[a] Data provided by Hillman et al. 2015.
[b] Does not include subyearling fry prior to July 1.
[c] 11-year average of complete brood data, BY2003-2013.
[d] Estimated emigration during the winter non-trapping period (December 1 – February 28).


60,000
50,000
40,000
30,000
20,000
10,000
0
Total Emmigrants
R² = 0.2167
0
500,000
1,000,000
1,500,000
2,000,000
Estimated Egg Deposition


6.00%
5.00%
4.00%
3.00%
2.00%
1.00%
0.00%
Egg-to-Emmigrant Survival (%)
R² = 0.1379
0
500,000
1,000,000
1,500,000
2,000,000
Estimated Egg Deposition

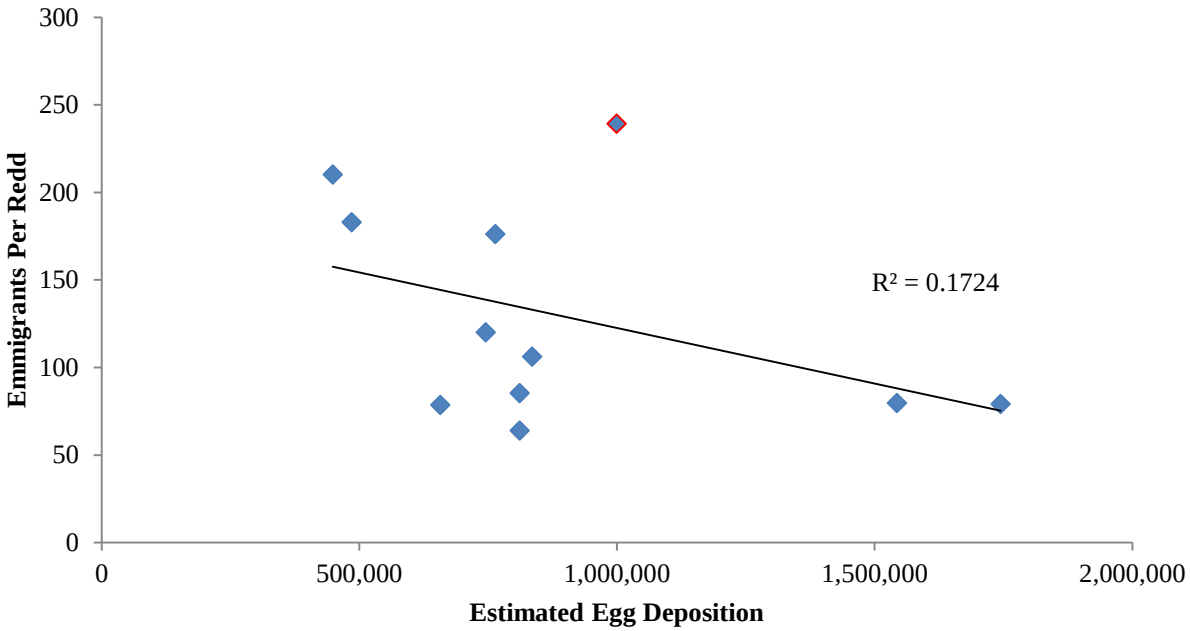


Figure 13. Relationships between estimated egg deposition and total emigrants produced, egg-to-emigrant survival, and emigrants per redd for Nason Creek spring Chinook, BY 2003 to 2013. *2013 brood (denoted by red border) does not include non-trapping estimate.

3.4.2 Spring Chinook Subyearlings (BY2014)

A linear regression model was developed using subyearling mark groups released in the fall of 2014 and 2015. This weighted regression was not significant ($r^2 = 0.36$; $p = 0.09$) at our accepted limit ($\alpha = 0.05$). However, previous comparisons to pooled estimates suggest that linear regression analysis would be a more viable means of estimation despite less than optimal significance. Also, extreme high flows, low yearling Chinook abundance, and sporadic trap operation in the month of November would have greatly hindered the development of a pooled estimate. As a multi-year regression, this initial flow-efficiency relationship represents the starting point from which we will build further estimates. Using this model, we estimated that a total of 13,903 (± 11,963; 95% CI) BY2014 spring Chinook emigrated past the trap in the Fall of 2013 (Table 6).

Table 7. Trap efficiency trials conducted with BY2014 wild spring Chinook subyearlings.

Origin/Species/Stage	Age	Date	Marked	Recaptured	Discharge (cfs)
Wild Chinook Subyearlings	0	11/3/2015	138	0	460
Wild Chinook Subyearlings	0	11/23/2015	9	0	520

3.4.3 Summer Steelhead

Low abundance of summer steelhead emigrants in the spring of 2015 required a pooled estimate be used in light of the inability to meet minimum mark-group sizes (n = 50) for regression analysis (Table 8). Releases of PIT-tagged steelhead were subsequently released every four days upstream at the established release location (Table 9). In a total of 13 separate trials, 116 wild summer steelhead were released upstream with only 1 recapture (0.86%). 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 22,504 (± 3,175; 95% CI) BY2014 age-1, 1,508 (± 897; 95% CI) BY2013 age-2, and 116 (± 436; 95% CI) BY2012 age-3 steelhead emigrated past the trap in 2015 (Table 10). We estimate that total (age 1-3) BY2012 emigration to be 25,566 (± 6,020; 95% CI).

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

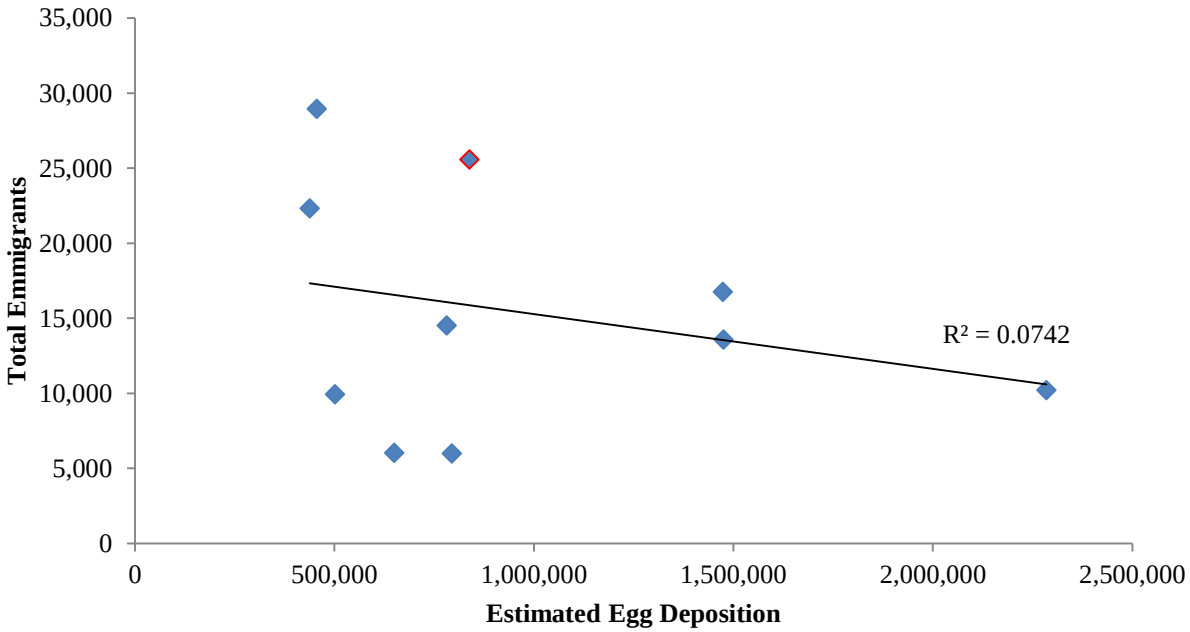
Origin/Species/Stage	Date	Marked	Recaptured	Discharge (cfs)
Wild Steelhead Parr/Smolt	4/23/2015	17	1	337
Wild Steelhead Parr/Smolt	4/27/2015	3	0	269
Wild Steelhead Parr/Smolt	5/2/2015	8	0	338
Wild Steelhead Parr/Smolt	5/6/2015	13	0	330
Wild Steelhead Parr/Smolt	5/10/2015	3	0	334
Wild Steelhead Parr/Smolt	5/14/2015	1	0	418
Wild Steelhead Parr/Smolt	5/18/2015	6	0	392
Wild Steelhead Parr/Smolt	5/22/2015	10	0	421
Wild Steelhead Parr/Smolt	5/26/2015	9	0	337
Wild Steelhead Parr/Smolt	5/30/2015	26	0	365
Wild Steelhead Parr/Smolt	6/4/2015	9	0	218
Wild Steelhead Parr/Smolt	6/8/2015	4	0	192
Wild Steelhead Parr/Smolt	6/16/2015	7	0	109
Total		**116**	**1**	

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	0.8%	49
2004	127	5,118	649,986	5,107	906	22	6,035 ± 885	0.9%	48
2005	412	5,545	2,284,540	7,416	2,502	298	10,216 ± 2,147	0.4%	25
2006	77	5,688	437,976	19,609	2,673	37	22,319 ± 5,722	5.1%	290
2007	78	5,840	455,520	26,518	2,325	117	28,960 ± 7,739	6.4%	371

2008	88	5,693	500,984	8,782	1,164	0	9,946 ± 2,382	2.0%	113
2009	126	6,199	781,074	13,606	608	312	14,526 ± 2,868	1.9%	115
2010	270	5,458	1,473,660	12,767	3,999	0	16,776 ± 3,885	1.1%	62
2011	235	6,276	1,474,860	13,109	482	0	13,591 ± 3,525	0.9%	58
2012	158	5,309	838,822	24,637	813	116[c]	25,566 ± 6,020	3.0%	162
2013	135	5,749	777,735	11,837	1,508[c]	—	—	—	—
2014	198	5,831	1,154,538	22,504[c]	—	—	—	—	—
Avg[b]	169	5,769	969,130	13,646	1,653	90	15,380	2.3%	129

[a] Data provided by Hillman et al. 2015
[b] 10-year average of complete brood estimates, BY2003-2012
[c] Pooled estimate



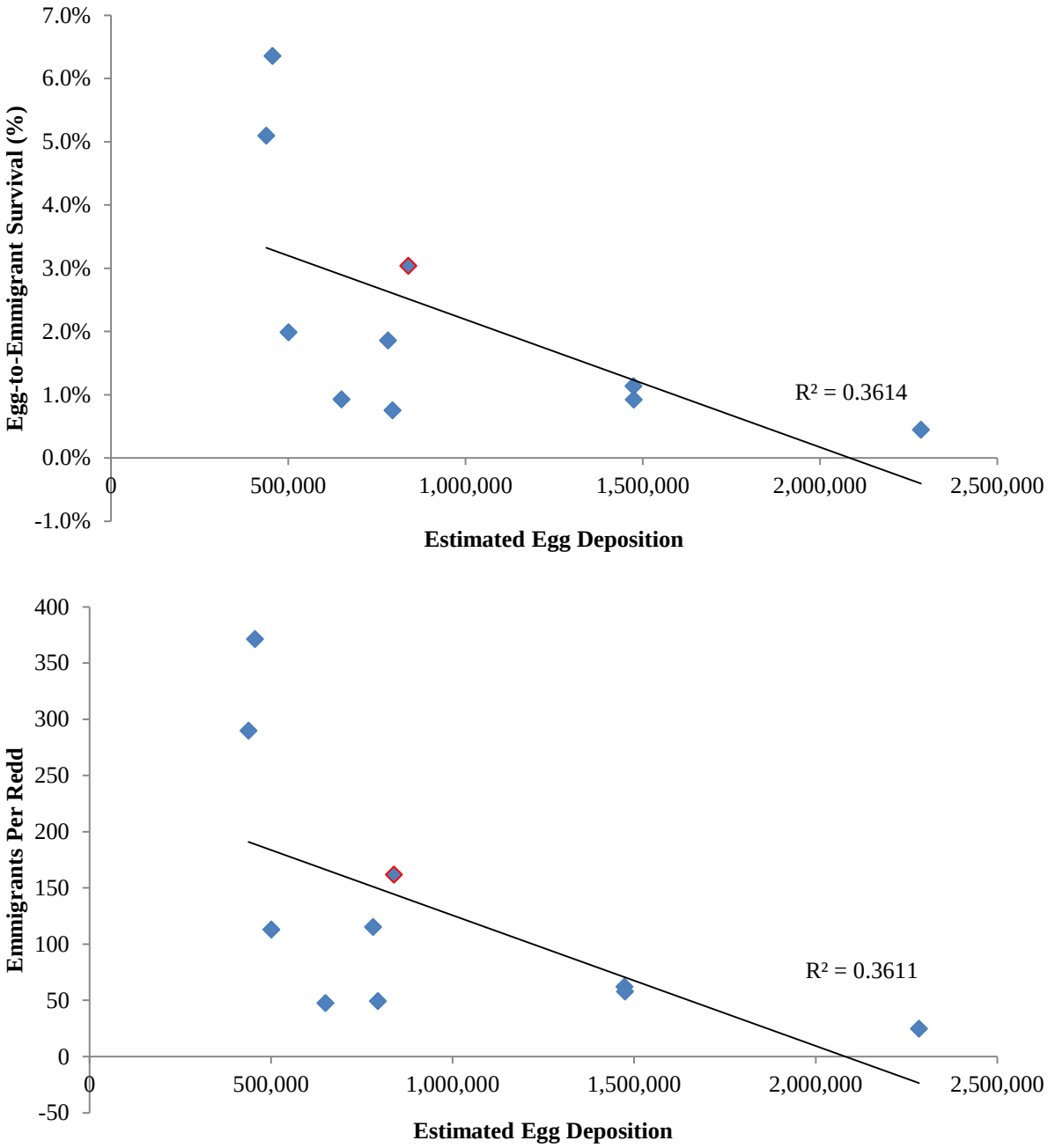


Figure 14. Relationships between estimated egg deposition and total emigrants produced, egg-to-emigrant survival, and emigrants per redd for Nason Creek summer Steelhead, BY 2003 to 2012. *2012 brood denoted by red border.

3.4.4 Coho Yearlings (BY2013)

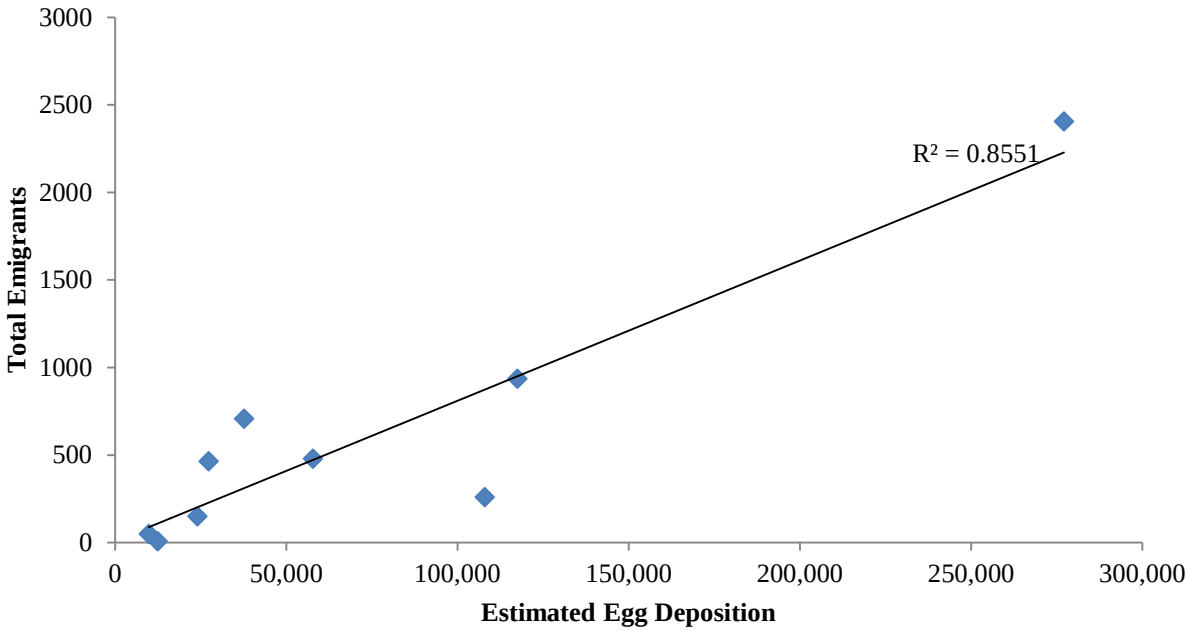
Limited abundance of BY2013 coho yearlings did not provide any opportunities to perform any efficiency trials in the spring of 2015. In lieu of a species-specific model, a pooled estimate using releases of marked hatchery-origin coho smolts was applied to wild coho smolts. In the

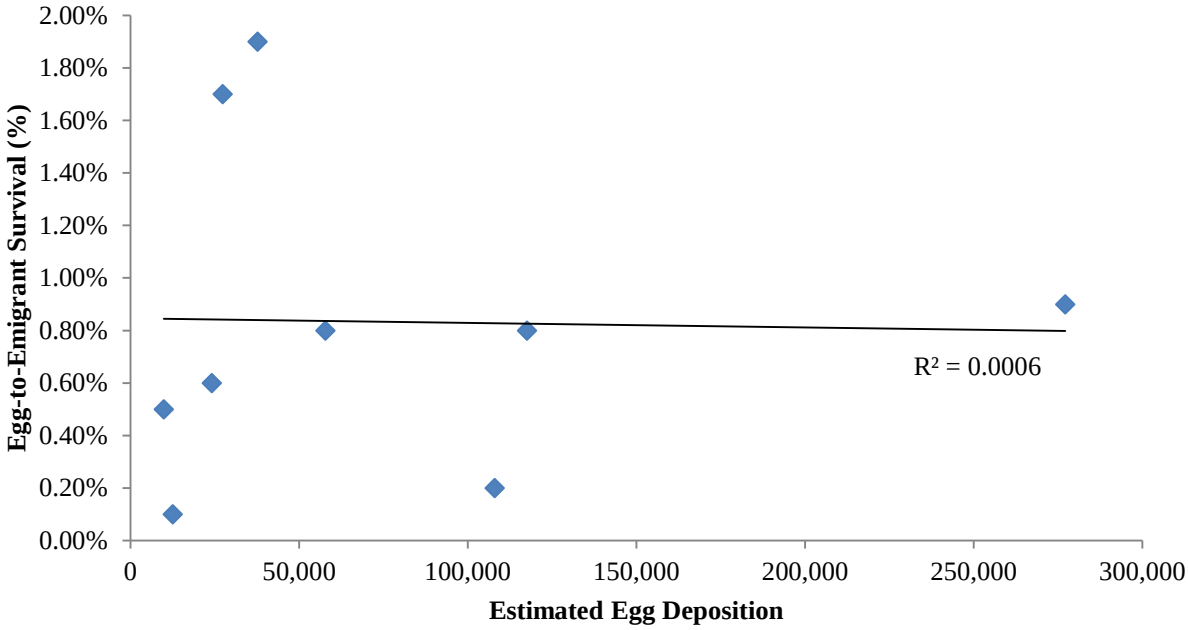
spring of 2015, we estimated that 91 (± 711; 95% CI) emigrated past the trap (Table 11). This gave us a total BY2013 emigrant estimate of 161 (± 714; 95% CI).

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	0.2%	7
2005	41	2,866	117,506	27	910	937 ± 347	0.8%	23
2006	4	3,126	12,504	7	0	7 ± 10	0.1%	2
2007	10	2,406	24,060	14	136	150 ± 104	0.6%	15
2008	3	3,275	9,825	50	0	50 ± 57	0.5%	17
2009	14	2,691	37,674	471	237	708 ± 478	1.9%	51
2010	8	3,411	27,288	27	437	464 ± 231	1.7%	58
2011	89	3,114	277,146	1,018	1,387	2,405 ± 612	0.9%	27
2012	21	2,752	57,792	46	434	480 ± 237	0.8%	23
2013	0	2,973	0	70	91	161 ± 714	NA	NA
2014	16	2,992	47,872	84	—	—	—	—
Avg.[b]	23	2,970	67,174	193	369	562	0.8%	25

[a] Does not include subyearling fry prior to July 1.
[b] 10-year average of complete brood data, BY2004-2013.


3000
2500
2000
1500
1000
500
0
Total Emigrants
R² = 0.8551
0
50,000
100,000
150,000
200,000
250,000
300,000
Estimated Egg Deposition


2.00%
1.80%
1.60%
1.40%
1.20%
1.00%
0.80%
0.60%
0.40%
0.20%
0.00%
Egg-to-Emigrant Survival (%)
R² = 0.0006
0
50,000
100,000
150,000
200,000
250,000
300,000
Estimated Egg Deposition

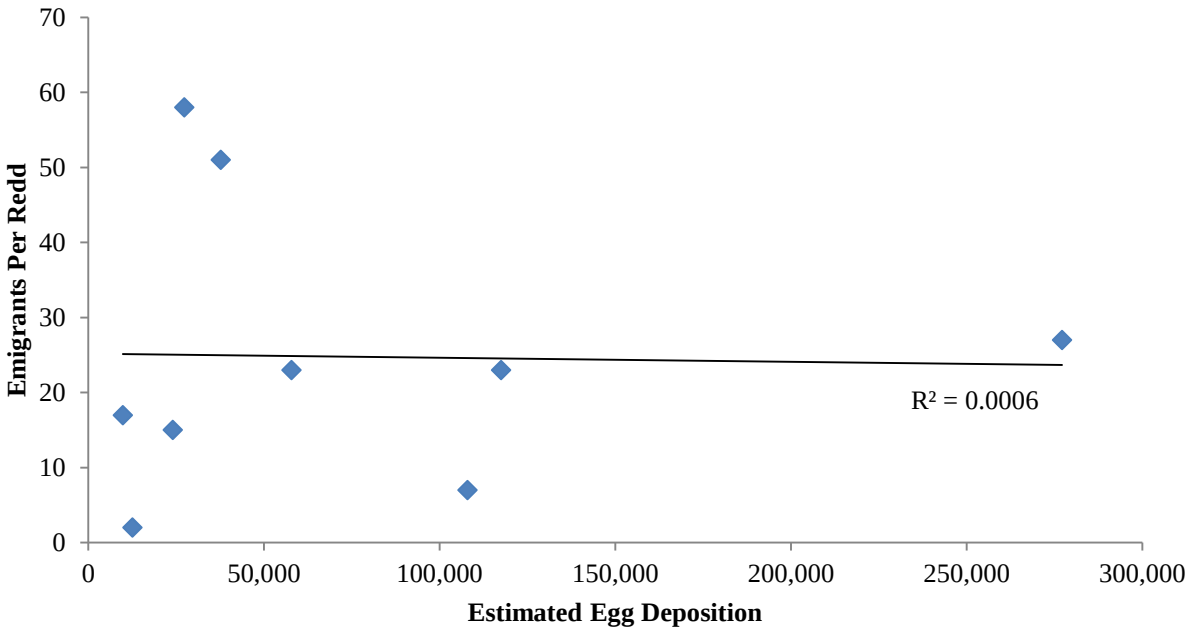


Figure 15. Relationships between estimated egg deposition and total emigrants produced, egg-to-emigrant survival, and emigrants per redd for Nason Creek naturally-produced coho, BY 2004 to 2012.

3.4.5 Coho Subyearlings (BY2014)

A total of only three coho subyearling parr did not allow us to make any attempts to build a species/age specific a regression model at the new trap location. The subyearling spring chinook flow-efficiency regression model was used to estimate subyearling coho parr emigrants. We estimated that 84 (± 70 ; 95% CI) emigrated past the trap in the fall of 2015 (Table 11).

3.5 PIT Tagging

During the 2015 trapping season, we PIT tagged 361 wild spring Chinook, 383 steelhead, and 2 naturally produced coho (Table 12). All tagging files were submitted to the PTAGIS database. One shed PIT tag (implanted in steelhead parr) was recovered in holding boxes where fish had been held for 24-72 hours after tagging.

Table 11. Number of PIT tagged coho, Chinook, and steelhead with shed rates at the Nason Creek rotary trap in 2015.

Species/Stage	Year-to-date Catch	Year-to-date PIT Tagged	No. of Shed Tags	Percent Shed Tags
Chinook Yearling Smolt	152	142	0	0.00%
Chinook Subyearling Parr (Mar 1 to June 30)	111	28	0	0.00%
Chinook Subyearling Parr (July 1 to Nov 30)	201	191	0	0.00%
Steelhead Parr	388	371	1	0.27%
Steelhead Smolt	12	12	0	0.00%

Coho Yearling Smolt	2	2	0	0.00%
Coho Subyearling Parr	5	0	—	—

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

During remote tagging efforts in the fall of 2014, 1,893 spring Chinook were PIT tagged by YNFRM and WDFW personnel. Of the total tagged, 78% were held overnight to determine tag retention. Shed rate for this tagging effort was 0.07%.

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 13 are: bull trout *Salvelinus confluentus*, cutthroat trout *Oncorhynchus clarki*, fathead minnow *Pimephales promelas*, longnose dace *Rhinichthys cataractae*, northern pikeminnow *Ptychocheilus oregonensis*, redside shiner *Richardsonius balteatus*, sculpin *Cottus sp.*, sucker *Catostomus sp.*, summer sockeye salmon fry *Oncorhynchus nerka*, and mountain whitefish *Prosopium williamsoni*.

Table 12. Summary of length and weight sampling of incidental species captured at the Nason Creek rotary trap in 2015.

Species	Total Count	Length (mm)			Weight (g)		
		Mean	N	SD	Mean	N	SD
Bull Trout	1	180	1	—	50.1	1	—
Cutthroat Trout	1	168	1	—	45.3	1	—
Fathead Minnow	2	46	2	12.0	1.1	2	0.9
Longnose Dace	117	92	117	24.8	11.7	116	6.6
Northern Pikeminnow	11	142	11	78.9	58.4	11	78.8
Redside Shiner	8	58	8	13.8	2.8	7	1.1
Sculpin	81	78	81	38.7	12.3	78	17.3
Sucker	39	120	39	91.4	20.7	34	58.5
Summer Sockeye Fry	2	32	2	8.5	0.5	1	—
Whitefish Fry	4	40	4	9.3	0.8	3	0.1
Whitefish	21	97	21	68.8	25.0	20	65.5

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 wild summer steelhead, hatchery summer steelhead, and bull trout (Table 14). Final spring Chinook lethal take for 2015 was at the 2% maximum. Exceedance of this maximum in early March was addressed in a memo sent to NMFS (See Appendix D). 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 (BY2013)	152	5*	3.29%
Spring Chinook Subyearling (BY 2014)	548	9*	1.64%
Total Wild Spring Chinook	**700**	**14**	**2.00%**
Total Hatchery Spring Chinook	**714**	**0**	**0.00%**
Steelhead Age-0 (BY2015)	182	1	0.55%
Steelhead Age-1 (BY2014)	233	1	0.43%
Steelhead Age-2 (BY2013)	28	0	0.00%
Steelhead Age-3 (BY2012)	1	0	0.00%
Total Wild Summer Steelhead	**444**	**2**	**0.45%**
Total Hatchery Summer Steelhead	**448**	**1**	**0.22%**
Total Bull Trout	**1**	**0**	**0.00%**
Coho Yearling (BY2013)	2	0	0.00%
Coho Subyearling (BY2014)	5	1	20.00%
Total Naturally-Produced Coho	**7**	**1**	**14.29%**

*Majority occurring during incident detailed in Appendix D.

4.0 DISCUSSION

Operations in 2015 marked the first full season of continuous trapping at the Bolser site. Preliminary trapping this new site has achieved the goal of minimizing interactions with the public; we have yet to encounter any act of vandalism or tampering with the trap since the move. Aside from the benefit of added safety to the public and captured fish, relocation of the Nason Creek trap was intended to improve the quality of data collected via simplified trapping regime and favorable channel morphology. Initial subyearling Chinook releases in the fall of 2014 suggested that the flow-efficiency relationship was statistically significant at the flows tested (r^2 =0.63, p = 0.007). However, in three of the contributing trials, a stoppage or inconsistent operation during the recapture period dictated that they be omitted from any expansions performed (non-continuous operation of the trap in the 3-day recapture period is a violation of our estimation protocol). Although the flow-efficiency regression was ultimately rendered unusable, subyearling Chinook efficiency trials in 2014 were an indication that a consistent flow-efficiency relationship is present at the new site.

Attempts to further develop our flow-efficiency models in 2015 were largely prevented by extreme low spring/summer and high fall flow conditions, as well as low fish abundance. Steelhead and Chinook mark-group releases were generally small ($n \leq 26$), providing little chance for recaptures given potentially low trap efficiency. A single large release of 138 subyearling spring Chinook on November 3 failed to produce any recaptures, initially suggesting a trap efficiency of less than 1.0%. Later examination of daily subyearling spring Chinook catch showed that the release was performed concurrently with a significant drop in abundance, from 89 to 6 fish captured. The release also coincided with a rapidly decreasing hydrograph following a significant peak in discharge. The precipitous drop in catch may have resulted in a lack of active migration, with the spring Chinook subyearlings becoming less prone to downstream displacement as flows subsided. The suspected non-migratory behavior of spring Chinook subyearlings in Nason Creek during that period likely contributed to a lack of recaptures despite the large mark-group size. However, given that the trial occurred during the recognized subyearling spring Chinook migratory period and lacked any violations of release or trapping protocols, it was deemed valid.

With viable regression models unavailable for all species/stages, pooled estimates were predominantly used. These estimates were used as a means to produce some form of emigrant estimate, albeit with a higher degree of bias. All pooled estimates reported are considered provisional, and will be recalculated as viable flow-efficiency regressions are developed.

Spring Chinook

Nason Creek spring Chinook egg-to-emigrant survival rates are generally lower than those of the Chiwawa River and White River populations (Figure 16). However, the 2013 Nason Creek spring Chinook brood deviated from this trend markedly, with an survival rate exceeding those of the other two tributaries. Whereas the Chiwawa River and White River populations saw egg-to-emigrant survival rates typical of their corresponding estimated egg depositions in 2013, Nason Creek produced an outlier value (Figures 13 & 17). The total BY2013 spring Chinook estimate (excluding the non-trapping period) of 50,703 (± 38,852; 95% CI) emigrants greatly exceeded the corresponding 11-year average (n = 23,211).

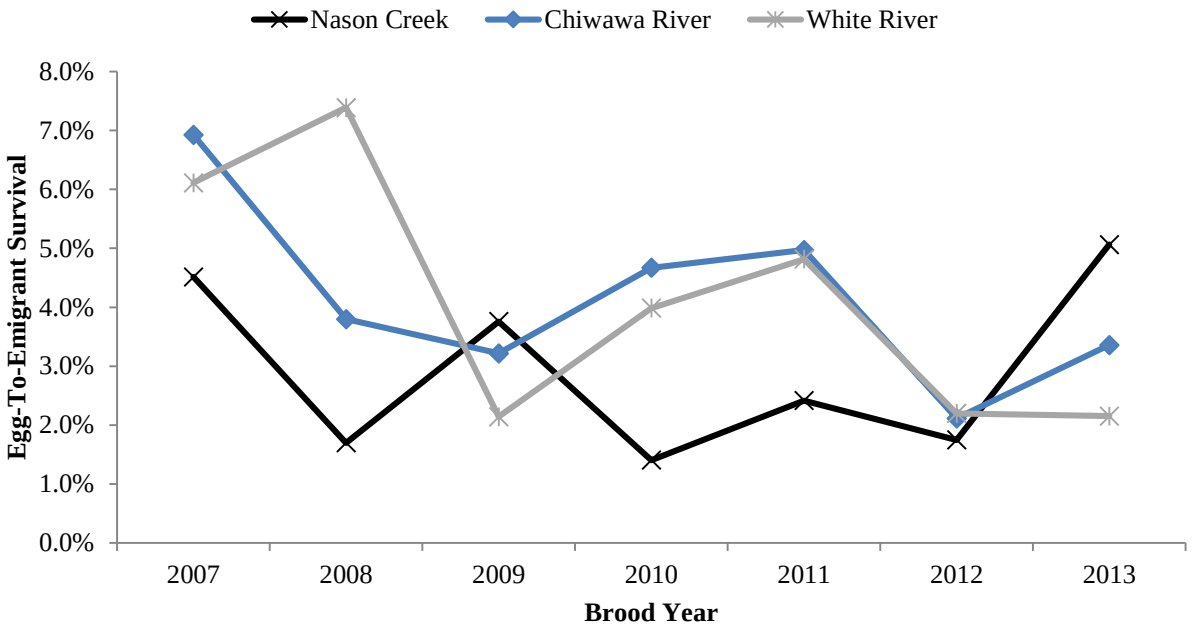


Figure 16. Comparison of wild spring Chinook abundance estimates (BY2007-2013) made at the White River, Nason Creek, and Chiwawa River smolt traps. *Non-trapping estimates not included.

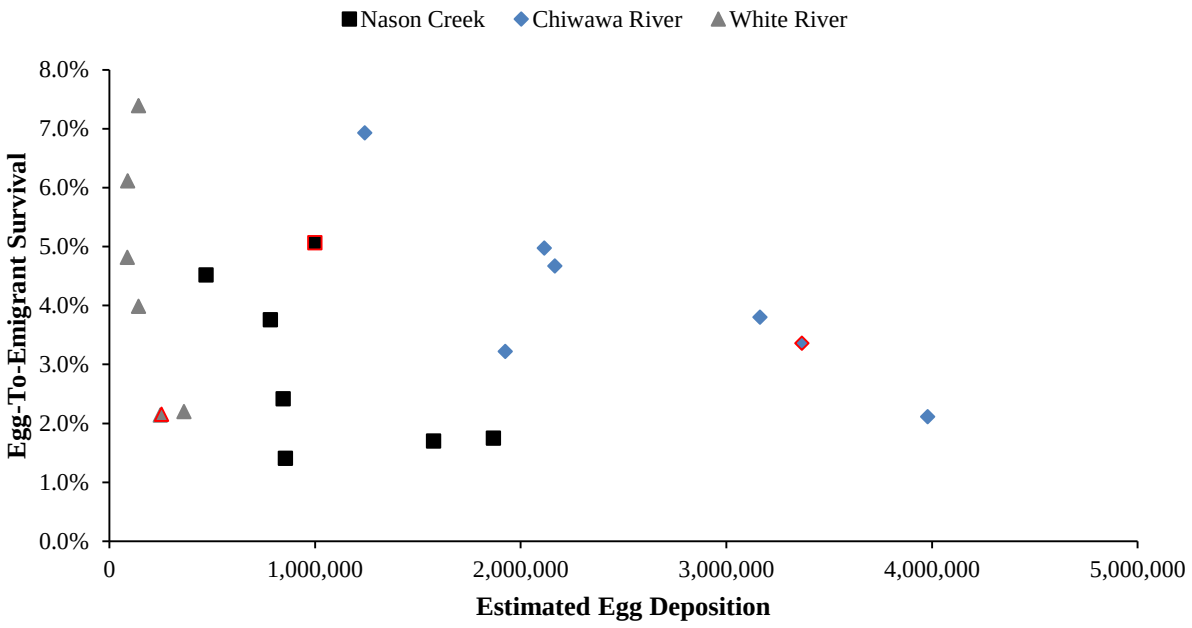


Figure 17. Comparison of egg-to-emigrant survival (BY 2007-2013) and egg deposition for Nason Creek, Chiwawa River, and White River spring Chinook. *Non-trapping estimates not included.

Though possible that the Nason Creek population alone saw above-average survival, it is likely that some degree of overestimation by our modeled and pooled estimates occurred. Composed

primarily of smaller ($n \leq 96$) trials, the weighted (mark-group size) model was heavily influenced by the aforementiomned large ($n = 138$) release in 2015 that did not produce any recaptures. Because the unsuccessful trial was performed at the high end of the discharge range tested, it decreased the slope of the regression, and therefore the trap efficiencies used to expand catch at elevated flows. Additional trials at higher flows will mitigate the effect of this subyearling release outlier and likely produce a lower emigrant estimate when recalculated. Overestimation of the yearling pooled estimate was also likely influenced by a lack of consistent releases throughout the migratory period. We expect that eventual recalculation of BY2013 yearlings will also contribute to a lowering of the overall emigrant estimate.

The non-trapping period estimate of 6,822 (± 9,035; 95% CI) BY2013 migrants suggests that movement out of the system was present in the winter, but at a much lower rate in comparison to the fall. Winter emigration for the 2013 spring Chinook brood accounted for 11.9% of the total estimate, whereas fall subyearling migrants made up a total of 76.0%. Yearling spring emigrants composed a slightly larger proportion than non-trapping period, with 12.1% of the total run. Upon eventual recalculation of the BY2013 trapping estimates, proportion of non-trapping period to total run will likely increase as the smolt trap-derived estimates decrease. Although detections during the winter confirm movement, they are too few and infrequent to determine fine-scale temporal trends in emigration and/or relation to environmental conditions.

Summer Steelhead

The pooled estimate used to expand 2015 steelhead migrants was based on 13 mark-groups; a total of 116 fish released, and 1 recapture. Consequently, the model tended to overestimate emigrant abundance as an efficiency of 0.86% was used to expand all daily catch. With no prior mark-group releases at this location, we are unsure if the low efficiency observed is accurate, or the product of the abnormally low water-year and its potential effects on steelhead migratory behavior. Comparisons of yearling Chinook and hatchery coho efficiencies at the new trap site to those of the old show they are comparatively lower, but not to the degree seen in 2015 summer steelhead migrants.

The total estimate of 25,566 (± 6,020; 95% CI) BY2012 steelhead exceeded the 10-year mean of 15,380 emigrants, and was the second highest estimate in the past 10 broods. Although the model used to expand age-3 fish was admittedly skewed toward overestimation, their contribution to the overall estimate was small ($n = 116$), and therefore did not impact it greatly. Both models used to calculate the bulk of the estimate (age-1 and age-2) were satistically robust ($\alpha \leq 0.05$); the product of trapping at the former site. The above-average emigrant survival and emigrants per redd of the 2012 brood despite relatively low egg deposition is characteristic of Nason Creek. In previous years, the highest rates of survival have corresponded to the lowest levels of spawner success, suggesting denstiy-dependence.

The migratory timing of summer steelhead captured in 2015 was typical of what we have previously seen in Nason Creek. Of the steelhead caught in the spring migratory period, 81.5% were were age-1, with age-2 (5.4%) and age-3 (0.4%) classes constituting a small portion of the total. The majority of the summer/fall non-migratory period was not trapped as a consequence of low flows. This period is normally dominated by young-of-the-year fry and parr.

Coho

A poor return of adult coho in 2013 required exhaustive measures to collect program broodstock, including increased retention at Tumwater Dam (Kamphaus et al. 2016). As a result, a limited number of adult coho (n =32) were allowed to pass into the upper-basin. Spawner escapement into Nason Creek was estimated at zero fish, with no redds documented during surveys in the fall of 2013. We attribute the capture of natural-origin coho to surveyor error, which may have lead to one or more redds to go unseen.

The BY2013 naturally-produced coho estimate of 161 (± 714; 95% CI) was likely overestimated to some degree by the under-developed models used for expansion. Despite the likely overestimation, the BY2013 estimate was less than the 10-year mean emigrant abundace (n = 562), and the third lowest estimate thus far at Nason Creek. We assume that the comparatively low estimate is a reflection of the poor spawner escapement of 2013. Recalculation of BY2013 emigrants will likely produce and even lower emigrant abudance.

2016 Trap Operations at Nason Creek

Pooled estimates have been used here, and in previous reports as an alternative when regression analysis is not feasible. However, this has proven problematic as each method requires a different efficiency-testing strategy. While flow-efficiency modeling can be built by gauging efficiency at specific flows over multiple years, a pooled estimate is based on regular releases over discrete strata. Pooled estimates based on few, unevenly-spaced releases will utimately be skewed toward the efficiencies of the discrete periods tested, not the entire migratory period. Recognizing the necessity to produce viable models depite potentially low emigrant abundaces in 2016, we have revised our system of efficiency trials to accommodate both pooled, and regression models. Along with the accustomed targeting of specific flows, regular releases at even intervals will occur throughout the year. Regardless of mark group size, or flows tested, migratory juveniles will be transported every three to four days upstream to be released. In doing so, we will ensure that estimates made with either methodology are as sound as possible.

Additionally, we will verify that the location of our upstream release point upholds smolt trapping assumption 3: that marked fish are randomly dispersed in the population prior to recapture. Currently, marked fish are released evenly on both sides of the creek to eliminate the potential bias of a single release point on one bank. In 2016, pre-release scans of both right, and left-bank release-groups will test if recapture probability differs depending on the side of the channel. In the event that recapture rates are markedly different between the two sites, we will pursue a different release point.

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APPENDIX A. Daily Stream Discharge and Stream Temperature

Date	Stream Discharge (CFS)	Water Temperature (˚C)
1/1/2015		0.0
1/2/2015		0.0
1/3/2015		0.0
1/4/2015		0.0
1/5/2015		0.2
1/6/2015	1110	1.8
1/7/2015	723	2.2
1/8/2015	607	1.9
1/9/2015	534	2.4
1/10/2015	485	2.4
1/11/2015	444	2.6
1/12/2015	402	2.6
1/13/2015	368	2.3
1/14/2015	343	2.1
1/15/2015	319	1.7
1/16/2015	311	1.3
1/17/2015	296	1.1
1/18/2015	338	0.5
1/19/2015	375	2.0
1/20/2015	318	1.6
1/21/2015	285	0.8
1/22/2015	272	1.7
1/23/2015	286	2.5
1/24/2015	691	2.5
1/25/2015	781	2.7
1/26/2015	673	2.5
1/27/2015	632	2.8
1/28/2015	613	2.9
1/29/2015	556	2.4
1/30/2015	503	2.1
1/31/2015	463	2.2
2/1/2015	433	2.3
2/2/2015	417	2.2
2/3/2015	438	2.8
2/4/2015	392	2.8
2/5/2015	404	2.4
2/6/2015	701	2.8
2/7/2015	832	3.1
2/8/2015	929	3.2
2/9/2015	829	3.6
2/10/2015	804	3.7
2/11/2015	756	3.5
2/12/2015	675	3.8
2/13/2015	674	3.9
2/14/2015	677	3.9
2/15/2015	653	3.1
2/16/2015	587	2.6
2/17/2015	536	2.6
2/18/2015	492	2.6
2/19/2015	463	3.6
2/20/2015	447	3.9
2/21/2015	422	3.3
2/22/2015	387	2.6
2/23/2015	357	1.9
2/24/2015	341	2.5
2/25/2015	323	3.4
2/26/2015	312	4.2
2/27/2015	317	4.0
2/28/2015	295	3.3
3/1/2015	276	2.7
3/2/2015	264	3.2
3/3/2015	247	2.4
3/4/2015	238	2.2
3/5/2015	232	2.8
3/6/2015	225	3.9
3/7/2015	224	4.3
3/8/2015	226	4.3
3/9/2015	227	4.5
3/10/2015	231	4.4
3/11/2015	237	5.2
3/12/2015	285	5.8
3/13/2015	303	4.9
3/14/2015	526	5.3
3/15/2015	733	3.9
3/16/2015	624	4.0
3/17/2015	517	4.2
3/18/2015	457	4.9
3/19/2015	422	4.8
3/20/2015	402	5.3
3/21/2015	434	5.5
3/22/2015	426	4.2
3/23/2015	389	4.5

3/24/2015	366	5.1	5/8/2015	297	8.9
3/25/2015	368	4.7	5/9/2015	307	9.2
3/26/2015	506	5.7	5/10/2015	334	9.2
3/27/2015	488	5.8	5/11/2015	371	10.0
3/28/2015	632	5.9	5/12/2015	408	7.9
3/29/2015	575	5.6	5/13/2015	416	7.5
3/30/2015	537	6.1	5/14/2015	418	8.0
3/31/2015	550	5.8	5/15/2015	379	9.1
4/1/2015	486	4.8	5/16/2015	374	9.9
4/2/2015	435	4.7	5/17/2015	373	8.4
4/3/2015	401	4.4	5/18/2015	392	10.0
4/4/2015	372	4.7	5/19/2015	421	10.4
4/5/2015	347	4.2	5/20/2015	437	10.8
4/6/2015	325	4.1	5/21/2015	435	10.8
4/7/2015	308	4.4	5/22/2015	421	10.4
4/8/2015	291	5.3	5/23/2015	416	11.4
4/9/2015	281	5.7	5/24/2015	409	11.5
4/10/2015	271	5.7	5/25/2015	378	10.9
4/11/2015	282	5.7	5/26/2015	337	10.3
4/12/2015	277	4.3	5/27/2015	310	11.5
4/13/2015	263	4.8	5/28/2015	315	12.1
4/14/2015	256	5.5	5/29/2015	330	11.9
4/15/2015	239	5.3	5/30/2015	365	12.7
4/16/2015	235	6.2	5/31/2015	310	12.2
4/17/2015	251	7.3	6/1/2015	272	11.9
4/18/2015	272	7.8	6/2/2015	257	11.2
4/19/2015	282	8.0	6/3/2015	236	11.8
4/20/2015	311	8.3	6/4/2015	218	12.6
4/21/2015	359	8.2	6/5/2015	205	13.8
4/22/2015	386	7.2	6/6/2015	200	15.0
4/23/2015	337	6.0	6/7/2015	198	15.9
4/24/2015	320	5.5	6/8/2015	192	16.5
4/25/2015	295	5.7	6/9/2015	182	16.3
4/26/2015	274	5.9	6/10/2015	168	16.1
4/27/2015	269	7.9	6/11/2015	154	15.6
4/28/2015	305	8.7	6/12/2015	145	14.6
4/29/2015	335	8.1	6/13/2015	134	13.8
4/30/2015	317	7.7	6/14/2015	124	14.4
5/1/2015	316	8.6	6/15/2015	116	14.9
5/2/2015	338	8.5	6/16/2015	109	16.0
5/3/2015	329	8.2	6/17/2015	104	16.6
5/4/2015	340	8.4	6/18/2015	100	16.0
5/5/2015	370	7.9	6/19/2015	97.2	15.4
5/6/2015	330	6.6	6/20/2015	95.1	15.2
5/7/2015	299	7.8	6/21/2015	90.3	15.2

6/22/2015	85.9	15.6	8/6/2015	33.7	18.2
6/23/2015	82.1	16.4	8/7/2015	34.1	18.2
6/24/2015	79.6	16.7	8/8/2015	33.1	18.8
6/25/2015	76.9	17.2	8/9/2015	32.3	18.8
6/26/2015	74	19.2	8/10/2015	32.2	19.9
6/27/2015	72.1	20.0	8/11/2015	31.8	18.7
6/28/2015	70.2	20.6	8/12/2015	31.7	19.1
6/29/2015	71.8	21.1	8/13/2015	30.4	20.4
6/30/2015	70.5	21.2	8/14/2015	30.3	19.8
7/1/2015	66.2	21.1	8/15/2015	31.9	17.6
7/2/2015	63.8	21.2	8/16/2015	33.4	16.9
7/3/2015	61.1	21.3	8/17/2015	32.2	17.7
7/4/2015	58.8	21.3	8/18/2015	31.2	18.2
7/5/2015	56.8	20.9	8/19/2015	30	18.9
7/6/2015	55.2	20.6	8/20/2015	28.9	19.2
7/7/2015	53.5	20.3	8/21/2015	28.5	18.0
7/8/2015	52.5	20.8	8/22/2015	28.7	16.4
7/9/2015	50.9	21.3	8/23/2015	28.6	16.0
7/10/2015	49.7	20.7	8/24/2015	28	16.8
7/11/2015	49.5	18.8	8/25/2015	27.5	17.0
7/12/2015	50.2	17.8	8/26/2015	27.5	17.1
7/13/2015	48.9	18.4	8/27/2015	27	17.8
7/14/2015	47.8	18.8	8/28/2015	27.1	17.9
7/15/2015	46.5	18.7	8/29/2015	29	16.5
7/16/2015	45.3	18.4	8/30/2015	37.1	15.5
7/17/2015	44.8	18.5	8/31/2015	49.4	14.1
7/18/2015	43.9	19.5	9/1/2015	43.9	14.2
7/19/2015	42.7	20.9	9/2/2015	47.7	14.5
7/20/2015	41.1	21.3	9/3/2015	48.1	13.3
7/21/2015	40.1	19.7	9/4/2015	42.2	12.8
7/22/2015	39.7	18.4	9/5/2015	37.1	12.9
7/23/2015	39.6	18.3	9/6/2015	38.6	12.7
7/24/2015	39.3	18.1	9/7/2015	48	13.4
7/25/2015	40	17.3	9/8/2015	40.4	13.9
7/26/2015	42.7	17.2	9/9/2015	37.2	14.6
7/27/2015	41.5	17.0	9/10/2015	34.7	15.3
7/28/2015	40.2	17.7	9/11/2015	33	15.5
7/29/2015	38.8	18.9	9/12/2015	32	15.9
7/30/2015	37.2	19.5	9/13/2015	30.6	16.3
7/31/2015	35.9	19.8	9/14/2015	30.1	13.8
8/1/2015	34.7	20.0	9/15/2015	30.5	12.1
8/2/2015	33.9	20.0	9/16/2015	30.8	11.9
8/3/2015	33.1	19.3	9/17/2015	31.4	11.8
8/4/2015	33.7	18.8	9/18/2015	34.3	12.7
8/5/2015	33.2	18.5	9/19/2015	34.2	12.8

9/20/2015	33.4	13.7	11/4/2015	333	4.6
9/21/2015	38.1	14.1	11/5/2015	280	5.4
9/22/2015	38	12.2	11/6/2015	249	5.3
9/23/2015	33.8	11.6	11/7/2015	228	6.0
9/24/2015	32.5	12.5	11/8/2015	263	6.1
9/25/2015	32	13.0	11/9/2015	245	5.3
9/26/2015	32	12.7	11/10/2015		
9/27/2015	31.7	10.6	11/11/2015		
9/28/2015	31.3	10.0	11/12/2015		
9/29/2015	30.8	9.9	11/13/2015	1450	4.2
9/30/2015	30.5	10.3	11/14/2015	2250	5.3
10/1/2015	30.1	10.9	11/15/2015	1220	5.0
10/2/2015	29.7	11.5	11/16/2015		
10/3/2015	29.5	12.1	11/17/2015		2.6
10/4/2015	30	11.3	11/18/2015		3.5
10/5/2015	30.1	10.4	11/19/2015	1410	3.9
10/6/2015	29.9		11/20/2015	938	2.9
10/7/2015	31.9	11.1	11/21/2015	728	2.1
10/8/2015	46.9	11.6	11/22/2015	607	2.0
10/9/2015	42.8	12.2	11/23/2015	520	2.1
10/10/2015	42.2	12.3	11/24/2015	457	2.8
10/11/2015	111	11.0	11/25/2015	391	2.1
10/12/2015	78.3	9.9	11/26/2015	343	0.8
10/13/2015	56.2	11.4	11/27/2015	313	0.4
10/14/2015	53.4	9.6	11/28/2015	288	0.1
10/15/2015	47.9	8.8	11/29/2015	273	0.0
10/16/2015	44.8	8.5	11/30/2015	251	0.2
10/17/2015	43	9.1	12/1/2015	234	0.4
10/18/2015	43.8	10.6	12/2/2015	226	0.8
10/19/2015	46.8	10.9	12/3/2015	222	0.7
10/20/2015	46	10.4	12/4/2015	210	2.0
10/21/2015	45.5	9.3	12/5/2015	203	1.7
10/22/2015	43	8.9	12/6/2015	198	1.5
10/23/2015	41.7	7.8	12/7/2015		
10/24/2015	40.7	7.0	12/8/2015	848	1.5
10/25/2015	40.9	7.4	12/9/2015	2730	1.6
10/26/2015	42.8	8.7	12/10/2015	1370	2.3
10/27/2015	45.7	8.2	12/11/2015	915	2.9
10/28/2015			12/12/2015		
10/29/2015	54.9	8.5	12/13/2015		
10/30/2015	338	8.3	12/14/2015	551	2.7
10/31/2015	1800	7.6	12/15/2015	486	2.5
11/1/2015	1430	6.8	12/16/2015	444	2.5
11/2/2015	745	6.2	12/17/2015	409	1.0
11/3/2015	460	5.6	12/18/2015	387	0.7

12/19/2015	357	1.3
12/20/2015	332	1.5
12/21/2015	318	0.8
12/22/2015	298	1.2
12/23/2015	285	1.1
12/24/2015	269	1.0
12/25/2015	248	1.3
12/26/2015	232	0.7
12/27/2015	225	0.3
12/28/2015	217	0.7
12/29/2015	207	1.2
12/30/2015	197	0.8
12/31/2015	184	0.1

APPENDIX B. Daily Trap Operation

Date	Trap Status	Comments
3/1/2015	Op.	
3/2/2015	Op.	
3/3/2015	Op.	
3/4/2015	Op.	
3/5/2015	Op.	
3/6/2015	Op.	
3/7/2015	Op.	
3/8/2015	Op.	
3/9/2015	Op.	
3/10/2015	Op.	
3/11/2015	Op.	
3/12/2015	Op.	
3/13/2015	Op.	
3/14/2015	No Op.	Stopped - debris
3/15/2015	No Op.	Stopped - debris
3/16/2015	Op.	
3/17/2015	Op.	
3/18/2015	Op.	
3/19/2015	Op.	
3/20/2015	Op.	
3/21/2015	Op.	
3/22/2015	Op.	
3/23/2015	Op.	
3/24/2015	Op.	
3/25/2015	Op.	
3/26/2015	Op.	
3/27/2015	Op.	
3/28/2015	Op.	
3/29/2015	Op.	
3/30/2015	Op.	
3/31/2015	Op.	
4/1/2015	Op.	
4/2/2015	Op.	
4/3/2015	Op.	
4/4/2015	Op.	
4/5/2015	Op.	
4/6/2015	Op.	
4/7/2015	Op.	
4/8/2015	Op.	
4/9/2015	Op.	
4/10/2015	Op.	
4/11/2015	Op.	
4/12/2015	Op.	
4/13/2015	Op.	
4/14/2015	Op.	
4/15/2015	Op.	
4/16/2015	Op.	
4/17/2015	Op.	
4/18/2015	Op.	
4/19/2015	Op.	
4/20/2015	Op.	
4/21/2015	Op.	
4/22/2015	Op.	
4/23/2015	Op.	
4/24/2015	Op.	
4/25/2015	Op.	
4/26/2015	Op.	
4/27/2015	Op.	
4/28/2015	Op.	
4/29/2015	Op.	
4/30/2015	Op.	
5/1/2015	Op.	
5/2/2015	Op.	
5/3/2015	Op.	
5/4/2015	Op.	
5/5/2015	Op.	
5/6/2015	Op.	
5/7/2015	Op.	
5/8/2015	Op.	
5/9/2015	Op.	
5/10/2015	Op.	
5/11/2015	Op.	
5/12/2015	Op.	
5/13/2015	Op.	
5/14/2015	Op.	
5/15/2015	Op.	
5/16/2015	Op.	
5/17/2015	Op.	
5/18/2015	Op.	
5/19/2015	Op.	
5/20/2015	Op.	

5/21/2015	Op.	
5/22/2015	Op.	
5/23/2015	Op.	
5/24/2015	Op.	
5/25/2015	Op.	
5/26/2015	Op.	
5/27/2015	Op.	
5/28/2015	Op.	
5/29/2015	Op.	
5/30/2015	Op.	
5/31/2015	Op.	
6/1/2015	Op.	
6/2/2015	Op.	
6/3/2015	Op.	
6/4/2015	Op.	
6/5/2015	Op.	
6/6/2015	Op.	
6/7/2015	Op.	
6/8/2015	Op.	
6/9/2015	Op.	
6/10/2015	Op.	
6/11/2015	Op.	
6/12/2015	Op.	
6/13/2015	Op.	
6/14/2015	Op.	
6/15/2015	Op.	
6/16/2015	Op.	
6/17/2015	No Op.	Stopped - debris
6/18/2015	Op.	
6/19/2015	Op.	
6/20/2015	Op.	
6/21/2015	Op.	
6/22/2015	Op.	
6/23/2015	Op.	
6/24/2015	Op.	
6/25/2015	Op.	
6/26/2015	Op.	
6/27/2015	Op.	
6/28/2015	Op.	
6/29/2015	Op.	
6/30/2015	Op.	
7/1/2015	Op.	
7/2/2015	No Op.	Stopped - bed contact
7/3/2015	Op.	
7/4/2015	Op.	
7/5/2015	Op.	
7/6/2015	Op.	
7/7/2015	Op.	
7/8/2015	No Op.	Stopped - bed contact
7/9/2015	No Op.	Stopped - bed contact
7/10/2015	Op.	
7/11/2015	Op.	
7/12/2015	Op.	
7/13/2015	Op.	
7/14/2015	No Op.	Stopped - bed contact
7/15/2015	No Op.	Stopped - bed contact
7/16/2015	No Op.	Stopped - low flow
7/17/2015	No Op.	Stopped - low flow
7/18/2015	No Op.	Pulled - low water
7/19/2015	No Op.	Pulled - low water
7/20/2015	No Op.	Pulled - low water
7/21/2015	No Op.	Pulled - low water
7/22/2015	No Op.	Pulled - low water
7/23/2015	No Op.	Pulled - low water
7/24/2015	No Op.	Pulled - low water
7/25/2015	No Op.	Pulled - low water
7/26/2015	No Op.	Pulled - low water
7/27/2015	No Op.	Pulled - low water
7/28/2015	No Op.	Pulled - low water
7/29/2015	No Op.	Pulled - low water
7/30/2015	No Op.	Pulled - low water
7/31/2015	No Op.	Pulled - low water
8/1/2015	No Op.	Pulled - low water
8/2/2015	No Op.	Pulled - low water
8/3/2015	No Op.	Pulled - low water
8/4/2015	No Op.	Pulled - low water
8/5/2015	No Op.	Pulled - low water
8/6/2015	No Op.	Pulled - low water
8/7/2015	No Op.	Pulled - low water
8/8/2015	No Op.	Pulled - low water
8/9/2015	No Op.	Pulled - low water
8/10/2015	No Op.	Pulled - low water
8/11/2015	No Op.	Pulled - low water
8/12/2015	No Op.	Pulled - low water
8/13/2015	No Op.	Pulled - low water
8/14/2015	No Op.	Pulled - low water

8/15/2015	No Op.	Pulled - low water	9/29/2015	No Op.	Pulled - low water
8/16/2015	No Op.	Pulled - low water	9/30/2015	No Op.	Pulled - low water
8/17/2015	No Op.	Pulled - low water	10/1/2015	No Op.	Pulled - low water
8/18/2015	No Op.	Pulled - low water	10/2/2015	No Op.	Pulled - low water
8/19/2015	No Op.	Pulled - low water	10/3/2015	No Op.	Pulled - low water
8/20/2015	No Op.	Pulled - low water	10/4/2015	No Op.	Pulled - low water
8/21/2015	No Op.	Pulled - low water	10/5/2015	No Op.	Pulled - low water
8/22/2015	No Op.	Pulled - low water	10/6/2015	No Op.	Pulled - low water
8/23/2015	No Op.	Pulled - low water	10/7/2015	No Op.	Pulled - low water
8/24/2015	No Op.	Pulled - low water	10/8/2015	No Op.	Pulled - low water
8/25/2015	No Op.	Pulled - low water	10/9/2015	No Op.	Pulled - low water
8/26/2015	No Op.	Pulled - low water	10/10/2015	No Op.	Pulled - low water
8/27/2015	No Op.	Pulled - low water	10/11/2015	No Op.	Pulled - low water
8/28/2015	No Op.	Pulled - low water	10/12/2015	No Op.	Stopped - low flow
8/29/2015	No Op.	Pulled - low water	10/13/2015	No Op.	Pulled - low water
8/30/2015	No Op.	Pulled - low water	10/14/2015	No Op.	Pulled - low water
8/31/2015	No Op.	Pulled - low water	10/15/2015	No Op.	Pulled - low water
9/1/2015	No Op.	Pulled - low water	10/16/2015	No Op.	Pulled - low water
9/2/2015	No Op.	Pulled - low water	10/17/2015	No Op.	Pulled - low water
9/3/2015	No Op.	Stopped - low flow	10/18/2015	No Op.	Pulled - low water
9/4/2015	No Op.	Pulled - low water	10/19/2015	No Op.	Pulled - low water
9/5/2015	No Op.	Pulled - low water	10/20/2015	No Op.	Pulled - low water
9/6/2015	No Op.	Pulled - low water	10/21/2015	Op.	
9/7/2015	No Op.	Pulled - low water	10/22/2015	Op.	
9/8/2015	No Op.	Pulled - low water	10/23/2015	Op.	
9/9/2015	No Op.	Pulled - low water	10/24/2015	No Op.	Stopped - low flow
9/10/2015	No Op.	Pulled - low water	10/25/2015	No Op.	Pulled - low water
9/11/2015	No Op.	Pulled - low water	10/26/2015	No Op.	Pulled - low water
9/12/2015	No Op.	Pulled - low water	10/27/2015	No Op.	Pulled - low water
9/13/2015	No Op.	Pulled - low water	10/28/2015	No Op.	Pulled - low water
9/14/2015	No Op.	Pulled - low water	10/29/2015	No Op.	Pulled - low water
9/15/2015	No Op.	Pulled - low water	10/30/2015	No Op.	Stopped - low flow
9/16/2015	No Op.	Pulled - low water	10/31/2015	No Op.	Pulled - low water
9/17/2015	No Op.	Pulled - low water	11/1/2015	No Op.	Pulled - low water
9/18/2015	No Op.	Pulled - low water	11/2/2015	Op.	
9/19/2015	No Op.	Pulled - low water	11/3/2015	Op.	
9/20/2015	No Op.	Pulled - low water	11/4/2015	Op.	
9/21/2015	No Op.	Pulled - low water	11/5/2015	Op.	
9/22/2015	No Op.	Pulled - low water	11/6/2015	Op.	
9/23/2015	No Op.	Pulled - low water	11/7/2015	Op.	
9/24/2015	No Op.	Pulled - low water	11/8/2015	Op.	
9/25/2015	No Op.	Pulled - low water	11/9/2015	Op.	
9/26/2015	No Op.	Pulled - low water	11/10/2015	Op.	
9/27/2015	No Op.	Pulled - low water	11/11/2015	Op.	
9/28/2015	No Op.	Pulled - low water	11/12/2015	Op.	

11/13/2015	No Op.	Pulled - high water
11/14/2015	No Op.	Pulled - high water
11/15/2015	No Op.	Pulled - high water
11/16/2015	Op.	
11/17/2015	Op.	
11/18/2015	No Op.	Pulled - high water
11/19/2015	No Op.	Pulled - high water
11/20/2015	Op.	
11/21/2015	Op.	
11/22/2015	Op.	
11/23/2015	Op.	
11/24/2015	Op.	
11/25/2015	Op.	
11/26/2015	Op.	
11/27/2015	Op.	
11/28/2015	No Op.	Stopped - ice
11/29/2015	No Op.	Stopped - ice
11/30/2015	No Op.	Stopped - ice

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
Wild Chinook Smolt	1+	3/31/2007	Back	40	2	0.08	0.28	869
Wild Chinook Smolt	1+	4/6/2006	Back	42	9	0.24	0.51	264
Wild Chinook Smolt	1+	4/14/2010	Back	42	4	0.12	0.35	173
Wild Chinook Smolt	1+	3/31/2012	Back	43	5	0.14	0.38	250
Wild Chinook Smolt	1+	4/3/2007	Back	46	1	0.04	0.21	656
Wild Chinook Smolt	1+	4/19/2012	Back	48	7	0.17	0.42	434
Wild Chinook Smolt	1+	4/10/2007	Back	53	4	0.09	0.31	966
Wild Chinook Smolt	1+	4/21/2009	Back	53	0	0.02	0.14	732
Wild Chinook Smolt	1+	4/13/2012	Back	53	4	0.09	0.31	358
Wild Chinook Smolt	1+	4/16/2012	Back	53	7	0.15	0.40	443
Wild Chinook Smolt	1+	4/24/2008	Back	57	8	0.158	0.409	210
Wild Chinook Smolt	1+	4/23/2012	Back	58	1	0.034	0.187	1380
Wild Chinook Smolt	1+	4/24/2006	Back	59	3	0.068	0.263	368
Wild Chinook Smolt	1+	3/23/2007	Back	59	7	0.136	0.377	876
Wild Chinook Smolt	1+	3/17/2007	Back	64	7	0.125	0.361	936
Wild Chinook Smolt	1+	4/18/2010	Back	67	2	0.045	0.213	330
Wild Chinook Smolt	1+	4/17/2008	Back	72	13	0.194	0.457	274
Wild Chinook Smolt	1+	4/3/2006	Back	81	10	0.136	0.377	188
Wild Chinook Smolt	1+	3/20/2007	Back	91	13	0.154	0.403	1230
Wild Chinook Smolt	1+	5/1/2008	Back	102	16	0.167	0.421	315
Wild Chinook Smolt	1+	4/28/2008	Back	127	19	0.157	0.408	271
Wild Chinook Smolt	1+	4/14/2008	Back	195	40	0.21	0.476	327
Wild Chinook Smolt	1+	3/9/2014	Back	65	4	0.077	0.281	958
Wild Chinook Smolt	1+	3/13/2014	Back	67	9	0.149	0.397	566

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
Wild Chinook Parr	0	10/26/2006	Back	183	50	0.28	0.56	51
Wild Chinook Parr	0	10/30/2006	Back	168	52	0.32	0.60	63
Wild Chinook Parr	0	11/1/2010	Back	254	42	0.17	0.42	198
Wild Chinook Parr	0	11/4/2010	Back	287	49	0.17	0.43	215
Wild Chinook Parr	0	11/7/2010	Back	168	32	0.20	0.46	241
Wild Chinook Parr	0	11/13/2010	Back	185	35	0.19	0.46	131

Wild Chinook Parr	0	11/3/2012	Back	201	25	0.13	0.37	402
Wild Chinook Parr	0	11/7/2012	Back	233	27	0.12	0.35	394
Wild Chinook Parr	0	11/11/2012	Back	328	87	0.27	0.54	217
Wild Chinook Parr	0	11/15/2012	Back	195	34	0.18	0.44	213
Wild Chinook Parr	0	9/30/2013	Back	171	12	0.08	0.28	542
Wild Chinook Parr	0	10/2/2013	Back	213	43	0.21	0.47	328
Wild Chinook Parr	0	10/3/2013	Back	181	41	0.23	0.50	296
Wild Chinook Parr	0	10/7/2013	Back	242	31	0.13	0.37	233
Wild Chinook Parr	0	10/9/2013	Back	203	40	0.20	0.47	303
Wild Chinook Parr	0	11/27/2013	Back	241	55	0.23	0.50	182

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
Wild Chinook Parr	0	7/13/2006	Back	52	8	0.17	0.43	171
Wild Chinook Parr	0	7/17/2006	Back	138	15	0.12	0.35	129
Wild Chinook Parr	0	7/20/2006	Back	74	5	0.08	0.29	113
Wild Chinook Parr	0	7/28/2006	Back	54	5	0.11	0.34	91
Wild Chinook Parr	0	7/31/2006	Back	99	7	0.08	0.29	79
Wild Chinook Parr	0	9/18/2006	Back	55	10	0.20	0.46	46
Wild Chinook Parr	0	7/31/2008	Back	60	15	0.27	0.54	121
Wild Chinook Parr	0	8/12/2008	Back	103	2	0.03	0.17	85.6
Wild Chinook Parr	0	8/22/2008	Back	75	11	0.16	0.41	97
Wild Chinook Parr	0	8/28/2008	Back	72	7	0.11	0.34	81.9
Wild Chinook Parr	0	10/9/2008	Back	110	22	0.21	0.48	63.5
Wild Chinook Parr	0	10/27/2008	Back	51	12	0.26	0.53	56.1
Wild Chinook Parr	0	10/30/2008	Back	84	15	0.19	0.45	53
Wild Chinook Parr	0	11/6/2008	Back	78	8	0.12	0.35	77.7
Wild Chinook Parr	0	11/10/2008	Back	88	0	0.01	0.11	309
Wild Chinook Parr	0	7/14/2009	Back	86	2	0.04	0.19	193
Wild Chinook Parr	0	7/15/2009	Back	105	4	0.05	0.22	179
Wild Chinook Parr	0	7/17/2009	Back	122	8	0.07	0.28	157
Wild Chinook Parr	0	7/20/2009	Back	89	2	0.03	0.19	135
Wild Chinook Parr	0	8/17/2009	Back	73	1	0.03	0.17	58
Wild Chinook Parr	0	9/10/2009	Back	56	7	0.14	0.39	60
Wild Chinook Parr	0	8/8/2010	Back	58	1	0.03	0.19	85
Wild Chinook Parr	0	8/11/2010	Back	114	8	0.08	0.29	77
Wild Chinook Parr	0	9/11/2010	Back	68	9	0.15	0.39	75
Wild Chinook Parr	0	10/12/2010	Back	216	42	0.20	0.46	126
Wild Chinook Parr	0	10/15/2010	Back	192	37	0.20	0.46	95
Wild Chinook Parr	0	10/18/2010	Back	193	36	0.19	0.45	81
Wild Chinook Parr	0	10/22/2010	Back	92	18	0.21	0.47	69
Wild Chinook Parr	0	10/25/2010	Back	60	7	0.13	0.37	78
Wild Chinook Parr	0	10/29/2010	Back	127	0	0.01	0.09	95.1
Wild Chinook Parr	0	8/19/2011	Back	106	5	0.06	0.24	123

Model: Chinook Subyearling (Fall '14-'15) Bolser Site (r^2 = 0.36; p = 0.09)

Origin/Species/Stage	Age	Date	Trap Position	Mark	Recap	Trap Efficiency (R+1)/M	ASIN Transform	Discharge
Wild Chinook Parr	0	7/14/2014	Back	89	7	0.09	0.30	171
Wild Chinook Parr	0	7/21/2014	Back	74	4	0.07	0.26	129
Wild Chinook Parr	0	7/27/2014	Back	72	4	0.07	0.27	113
Wild Chinook Parr	0	10/27/2014	Back	71	3	0.06	0.24	91
Wild Chinook Parr	0	10/30/2014	Back	70	5	0.09	0.30	79
Wild Chinook Parr	0	11/1/2014	Back	96	6	0.07	0.27	46
Wild Chinook Parr	0	11/3/2015	Back	138	0	0.01	0.09	121

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
Wild Steelhead Parr/Smolt	1+	3/20/2007	Back	55	1	0.04	0.19	1230
Wild Steelhead Parr/Smolt	1+	3/31/2007	Back	56	4	0.09	0.30	869
Wild Steelhead Parr/Smolt	1+	4/10/2007	Back	60	8	0.15	0.40	966
Wild Steelhead Parr/Smolt	1+	5/1/2007	Back	52	2	0.06	0.24	783
Wild Steelhead Parr/Smolt	1+	6/9/2007	Back	71	9	0.14	0.38	842
Wild Steelhead Parr/Smolt	1+	6/12/2007	Back	65	8	0.14	0.38	704
Wild Steelhead Parr/Smolt	1+	6/14/2007	Back	61	5	0.10	0.32	687
Wild Steelhead Parr/Smolt	1+	6/21/2007	Back	67	4	0.07	0.28	751
Wild Steelhead Parr/Smolt	1+	4/14/2008	Back	149	46	0.32	0.60	327
Wild Steelhead Parr/Smolt	1+	4/17/2008	Back	75	3	0.05	0.23	274
Wild Steelhead Parr/Smolt	1+	4/28/2008	Back	74	11	0.16	0.41	271
Wild Steelhead Parr/Smolt	1+	5/1/2008	Back	176	29	0.17	0.43	315
Wild Steelhead Parr/Smolt	1+	5/12/2008	Back	55	8	0.16	0.42	663
Wild Steelhead Parr/Smolt	1+	5/15/2008	Back	57	1	0.04	0.19	1390
Wild Steelhead Parr/Smolt	1+	6/9/2008	Back	142	20	0.15	0.39	938
Wild Steelhead Parr/Smolt	1+	6/12/2008	Back	83	10	0.13	0.37	823
Wild Steelhead Parr/Smolt	1+	6/16/2008	Back	81	8	0.11	0.34	1140
Wild Steelhead Parr/Smolt	1+	4/20/2010	Back	121	11	0.10	0.32	675
Wild Steelhead Parr/Smolt	1+	4/22/2010	Back	121	10	0.09	0.31	726
Wild Steelhead Parr/Smolt	1+	6/20/2010	Back	128	11	0.09	0.31	926
Wild Steelhead Parr/Smolt	1+	4/5/2011	Back	52	1	0.04	0.20	761
Wild Steelhead Parr/Smolt	1+	5/22/2011	Back	84	3	0.05	0.22	1540
Wild Steelhead Parr/Smolt	1+	6/12/2012	Back	69	5	0.09	0.30	1170
Wild Steelhead Parr/Smolt	1+	7/26/2012	Back	63	4	0.08	0.29	278
Wild Steelhead Parr/Smolt	1+	4/22/2013	Back	66	6	0.11	0.33	520

Wild Steelhead Parr/Smolt	1+	4/26/2013	Back	50	2	0.06	0.25	642
Wild Steelhead Parr/Smolt	1+	4/30/2013	Back	54	2	0.06	0.24	778
Wild Steelhead Parr/Smolt	1+	5/8/2013	Back	62	0	0.02	0.13	2170
Wild Steelhead Parr/Smolt	1+	5/19/2013	Back	122	15	0.13	0.37	1130
Wild Steelhead Parr/Smolt	1+	5/22/2013	Back	58	4	0.09	0.30	1080
Wild Steelhead Parr/Smolt	1+	5/26/2013	Back	79	3	0.05	0.23	724
Wild Steelhead Parr/Smolt	1+	5/30/2013	Back	92	7	0.09	0.30	849
Wild Steelhead Parr/Smolt	1+	6/3/2013	Back	71	6	0.10	0.32	962
Wild Steelhead Parr/Smolt	1+	6/7/2013	Back	94	4	0.05	0.23	1420
Wild Steelhead Parr/Smolt	1+	6/13/2013	Back	64	2	0.05	0.22	745
Wild Steelhead Parr/Smolt	1+	6/17/2013	Back	115	5	0.05	0.23	883
Wild Steelhead Parr/Smolt	1+	6/29/2013	Back	60	12	0.22	0.48	730
Wild Steelhead Parr/Smolt	1+	7/7/2013	Back	75	9	0.13	0.37	325
Wild Steelhead Parr/Smolt	1+	5/5/2014	Back	55	3	0.07	0.27	1260
Wild Steelhead Parr/Smolt	1+	5/20/2014	Back	57	0	0.02	0.13	1490
Wild Steelhead Parr/Smolt	1+	6/3/2014	Back	75	1	0.03	0.16	1610

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
Wild Chinook Smolt	1+	3/31/2007	Back	40	2	0.08	0.28	869
Wild Chinook Smolt	1+	4/6/2006	Back	42	9	0.24	0.51	264
Wild Chinook Smolt	1+	4/14/2010	Back	42	4	0.12	0.35	173
Wild Chinook Smolt	1+	3/31/2012	Back	43	5	0.14	0.38	250
Wild Chinook Smolt	1+	4/3/2007	Back	46	1	0.04	0.21	656
Wild Chinook Smolt	1+	4/19/2012	Back	48	7	0.17	0.42	434
Wild Chinook Smolt	1+	4/10/2007	Back	53	4	0.09	0.31	966
Wild Chinook Smolt	1+	4/21/2009	Back	53	0	0.02	0.14	732
Wild Chinook Smolt	1+	4/13/2012	Back	53	4	0.09	0.31	358
Wild Chinook Smolt	1+	4/16/2012	Back	53	7	0.15	0.40	443
Wild Chinook Smolt	1+	4/24/2008	Back	57	8	0.158	0.409	210
Wild Chinook Smolt	1+	4/23/2012	Back	58	1	0.034	0.187	1380
Wild Chinook Smolt	1+	4/24/2006	Back	59	3	0.068	0.263	368
Wild Chinook Smolt	1+	3/23/2007	Back	59	7	0.136	0.377	876
Wild Chinook Smolt	1+	3/17/2007	Back	64	7	0.125	0.361	936
Wild Chinook Smolt	1+	4/18/2010	Back	67	2	0.045	0.213	330
Wild Chinook Smolt	1+	4/17/2008	Back	72	13	0.194	0.457	274
Wild Chinook Smolt	1+	4/3/2006	Back	81	10	0.136	0.377	188
Wild Chinook Smolt	1+	3/20/2007	Back	91	13	0.154	0.403	1230
Wild Chinook Smolt	1+	5/1/2008	Back	102	16	0.167	0.421	315
Wild Chinook Smolt	1+	4/28/2008	Back	127	19	0.157	0.408	271

Wild Chinook Smolt	1+	4/14/2008	Back	195	40	0.21	0.476	327
Wild Chinook Smolt	1+	3/9/2014	Back	65	4	0.077	0.281	958
Wild Chinook Smolt	1+	3/13/2014	Back	67	9	0.149	0.397	566

Model: Spring Chinook 2010-2014 Non-Trapping Period Array (NAL) Efficiency, (r^2 = 0.61; p = 0.0002)

Origin/Species/Stage	Age	Date	Mark	Detections	Trap Efficiency (R+1) / M	ASIN Transform	Discharge
Wild Chinook Parr	0	11/4/2010	254	95	0.38	0.66	224
Wild Chinook Parr	0	11/7/2010	287	70	0.25	0.52	248
Wild Chinook Parr	0	11/10/2010	168	74	0.45	0.73	169
Wild Chinook Parr	0	11/13/2010	74	41	0.57	0.85	140
Wild Chinook Parr	0	11/18/2010	185	22	0.12	0.36	278
Wild Chinook Parr	0	11/3/2012	201	21	0.11	0.34	384
Wild Chinook Parr	0	11/7/2012	233	31	0.14	0.38	378
Wild Chinook Parr	0	11/11/2012	328	66	0.20	0.47	223
Wild Chinook Parr	0	11/15/2012	195	68	0.35	0.64	219
Wild Chinook Parr	0	11/4/2013	130	51	0.40	0.68	130
Wild Chinook Parr	0	11/8/2013	106	39	0.38	0.66	148
Wild Chinook Parr	0	3/9/2014	65	4	0.08	0.28	880
Wild Chinook Parr	0	3/13/2014	67	5	0.09	0.30	541
Wild Chinook Parr	0	11/4/2014	114	5	0.05	0.23	370
Wild Chinook Parr	0	11/1/2014	96	5	0.06	0.25	583
Wild Chinook Parr	0	11/10/2014	78	8	0.12	0.35	398

APPENDIX D. Historical Morphometric Data

Spring Chinook (2004-2015)

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	1.0
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	7.0	8.4	152	2.2	1.0
2015	2014	Wild Chinook Subyearling Fry	45	338	9.9	1.0	338	0.9	0.9
2015	2014	Wild Chinook Subyearling Parr	84	210	8.0	6.5	209	1.7	1.1

2015	2013	Hatchery Chinook Yearling Smolt	136	284	12.3	29.5	284	8.8	1.1

Summer Steelhead (2004-2015)

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	1.0
2015	2015	0	Wild Summer Steelhead	70	182	15.5	4.3	176	2.0	1.1
2015	2014	1	Wild Summer Steelhead	88	233	20.2	8.3	233	6.7	1.0
2015	2013	2	Wild Summer Steelhead	149	14	13.5	33.7	14	8.2	1.0
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

Coho (2007-2015)

Trap Year	Brood Year	Origin/Species/Stage	Fork Length (mm)			Weight (g)			K-factor
			Mean	n	SD	Mean	n	SD	
2004	2002	Nat. Orig. Coho Yearling Smolt	—	—	—	—	—	—	—
2004	2003	Nat. Orig. Coho Subyearling Fry	—	—	—	—	—	—	—
2004	2003	Nat. Orig. Coho Subyearling Parr	—	—	—	—	—	—	—
2004	2002	Hatchery Coho Yearling Smolt	136.6	847	12.8	27.4	820	7.5	1.1
2005	2003	Nat. Orig. Coho Yearling Smolt	114.4	17	8.8	16.2	17	3.6	1.1
2005	2004	Nat. Orig. Coho Subyearling Fry	49.1	9	10.4	1.3	9	0.8	1.1
2005	2004	Nat. Orig. 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. Orig. Coho Yearling Smolt	—	—	—	—	—	—	—
2006	2005	Nat. Orig. Coho Subyearling Fry	—	—	—	—	—	—	—
2006	2005	Nat. Orig. Coho Subyearling Parr	71	4	13.6	3.8	4	2.9	1.1

2006	2004	Hatchery Coho Yearling Smolt	—	—	—	—	—	—	—
2007	2005	Nat. Orig. Coho Yearling Smolt	92.9	36	12.5	8.7	36	4	1.1
2007	2006	Nat. Orig. Coho Subyearling Fry	—	—	—	—	—	—	—
2007	2006	Nat. Orig. 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. Orig. Coho Yearling Smolt	—	—	—	—	—	—	—
2008	2007	Nat. Orig. Coho Subyearling Fry	—	—	—	—	—	—	—
2008	2007	Nat. Orig. 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. Orig. Coho Yearling Smolt	103	4	9.7	11.7	4	3.4	1.1
2009	2008	Nat. Orig. Coho Subyearling Fry	—	—	—	—	—	—	—
2009	2008	Nat. Orig. 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. Orig. Coho Yearling Smolt	—	—	—	—	—	—	—
2010	2009	Nat. Orig. Coho Subyearling Fry	48	2	—	1.3	2	—	1.2
2010	2009	Nat. Orig. 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. Orig. Coho Yearling Smolt	100.2	14	12.7	11.3	14	3.9	1.1
2011	2010	Nat. Orig. Coho Subyearling Fry	—	—	—	—	—	—	—
2011	2010	Nat. Orig. 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. Orig. Coho Yearling Smolt	102.1	17	9.1	11.9	17	3	1.1
2012	2011	Nat. Orig. Coho Subyearling Fry	36	1	—	—	—	—	—
2012	2011	Nat. Orig. 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. Orig. Coho Yearling Smolt	97	81	10	10	81	3.1	1.1
2013	2012	Nat. Orig. Coho Subyearling Fry	47.3	3	1	1	3	1	0.9
2013	2012	Nat. Orig. 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. Orig. Coho Yearling Smolt	96.3	20	9.8	9.9	20	3	1.1
2014	2013	Nat. Orig. Coho Subyearling Fry	36	1	—	—	—	—	—
2014	2013	Nat. Orig. 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	1.1
2015	2013	Nat. Orig. Coho Yearling Smolt	109	2	4.9	12.0	2	0.1402	0.9
2015	2014	Nat. Orig. Coho Subyearling Fry	47	7	13.7	1.4	7	1.4511	0.9
2015	2014	Nat. Orig. Coho Subyearling Parr	69	3	7.0	4.0	3	1.2583	1.2
2015	2013	Hatchery Coho Yearling Smolt	131	952	9.9	23.3	952	4.7946	1.0

Appendix D: Memo to NMFS Re: Exceedance of Allowed Lethal Take

MEMORANDUM

Yakama Nation Fisheries

Columbia River
Honor. Protect. Restore.

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To: Craig Busack

CC: Michelle Guay, Tom Scribner, Keely Murdoch, Bryan Ishida

From: Bryan Ishida

Date: March 15, 2015

RE: Nason Creek Smolt Trap Mortalities - 3/15/15

Dear Mr. Busack,

On March 15, 2015 YN FRM personnel arrived at the Nason rotary smolt trap at 9:00am to find it stopped by a 5'x6"x6" pressure-treated beam that had become wedged between the cone and the starboard pontoon. The halted rotation subsequently caused a small-diameter debris blockage at the rear of the cone preventing movement of fish and additional debris into the livebox. As a result, six wild spring Chinook subyearling fry and four wild spring Chinook yearling smolt mortalities were incurred. With a total of only 37 wild spring Chinook captured since trapping began on March 1, our mortality rate for the species is currently at 27%. The increase in debris load is attributed to a rapid spike in discharge level brought on by heavy rains. At the time of the stoppage, spring night operations (personnel on-site during hours of operation) had not yet commenced.

This event occurred during initial spring operations at the new Nason Creek smolt trap site (rkm 0.3). Due to its location on the outside of a channel bend, this new location appears to be more susceptible to debris stoppages than the previously-used site (rkm 0.9). In order to prevent further such instances, we will increase the duration of our night operations schedule to include high-water events prior to the scheduled May 1 start as needed. Upon initial onset of elevated spring flows, we will begin night operations and continue until discharge levels have subsided. Discharge data from the upstream Department of Ecology gauge and snowpack data from nearby snow telemetry (SNOTEL) sites will be used to will be used to predict trends in flow and guide trap operations. Additionally, the Nason Creek smolt trap will also be manned during fall freshets to mitigate the increased stoppage potential at the new site. We will increase our vigilance in the monitoring of high-water events and take the necessary precautions to prevent any further loss of ESA-listed species. Please feel free to contact me with any questions regarding this event.

Sincerely,

Bryan Ishida

Appendix L

Fish Trapping at the White River Smolt Trap during 2015

Population Estimates for Juvenile Spring Chinook Salmon in White River, WA

2015 Annual Final Report

Prepared by:
Bryan Ishida
Cory Kamphaus
Keely Murdoch

YAKAMA NATION
FISHERIES RESOURCE MANAGEMENT
Toppenish, WA 98948

Prepared for:

Public Utility District No. 2 of Grant County
Ephrata, Washington 98823

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, 2015. We used a 1.5 m rotary screw trap to collect 196 juvenile spring Chinook; 2 precocial parr, 11 fry, 151 subyearling parr, and 32 yearling smolts. Daily counts at the trap were expanded via regression analysis derived from mark and recapture trials. We estimated that 3,023 (± 2,728; 95% CI) BY2013 wild spring Chinook smolts and 1,449 (± 421; 95% CI) BY2014 wild spring Chinook parr emigrated past the White River trap. Combined with data collected in 2014, this gives us a total estimate of 5,484 (± 2,836; 95% CI) BY2012 emigrants. Using spring Chinook spawning ground data collected by Washington Department of Fish and Wildlife (WDFW) in 2013, we estimated egg-to-emigrant survival of BY2013 spring Chinook to be 2.2% (102 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, Barry Hodges, Tim Jeffris and Kevin Swager who maintained and operated the trap during all hours including nights/weekends through challenging weather conditions. Also thank you to Peter Graf (Grant County PUD) for administering contracting and funding as well as Mike Hughes, Mclain Johnson, John Walters, 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 2007, Public Utility District No. 2 of Grant County (GCPUD) contracted the Yakama Nation (YN) to operate a rotary trap in the White River. 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.

Data presented will be directly used to address Objective 2 in the Monitoring and Evaluation Plan for PUD Hatchery Programs (Hillman et al. 2013) 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).

In the fall of 2005, Washington State Department of Fish and Wildlife (WDFW) began smolt trapping in the lower reach of the 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, YN resumed trap operations on the White River for nine months of the year. This document reports data collected between March 1 and November 30, 2015 and provides emigration estimates for spring Chinook salmon yearlings (BY2013) and subyearlings (BY2014) during that time period. Data generated from this project was used to calculate annual egg-to-emigrant survival.

1.1 Watershed Description

The White River drainage encompasses 99,956 acres and originates in alpine glaciers and perennial snow fields (Figure 1; USFS 2004). Elevations in the drainage vary from 1,868 ft. at the Lake Wenatchee surface to 8,575 ft. 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 river kilometers (RK) 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

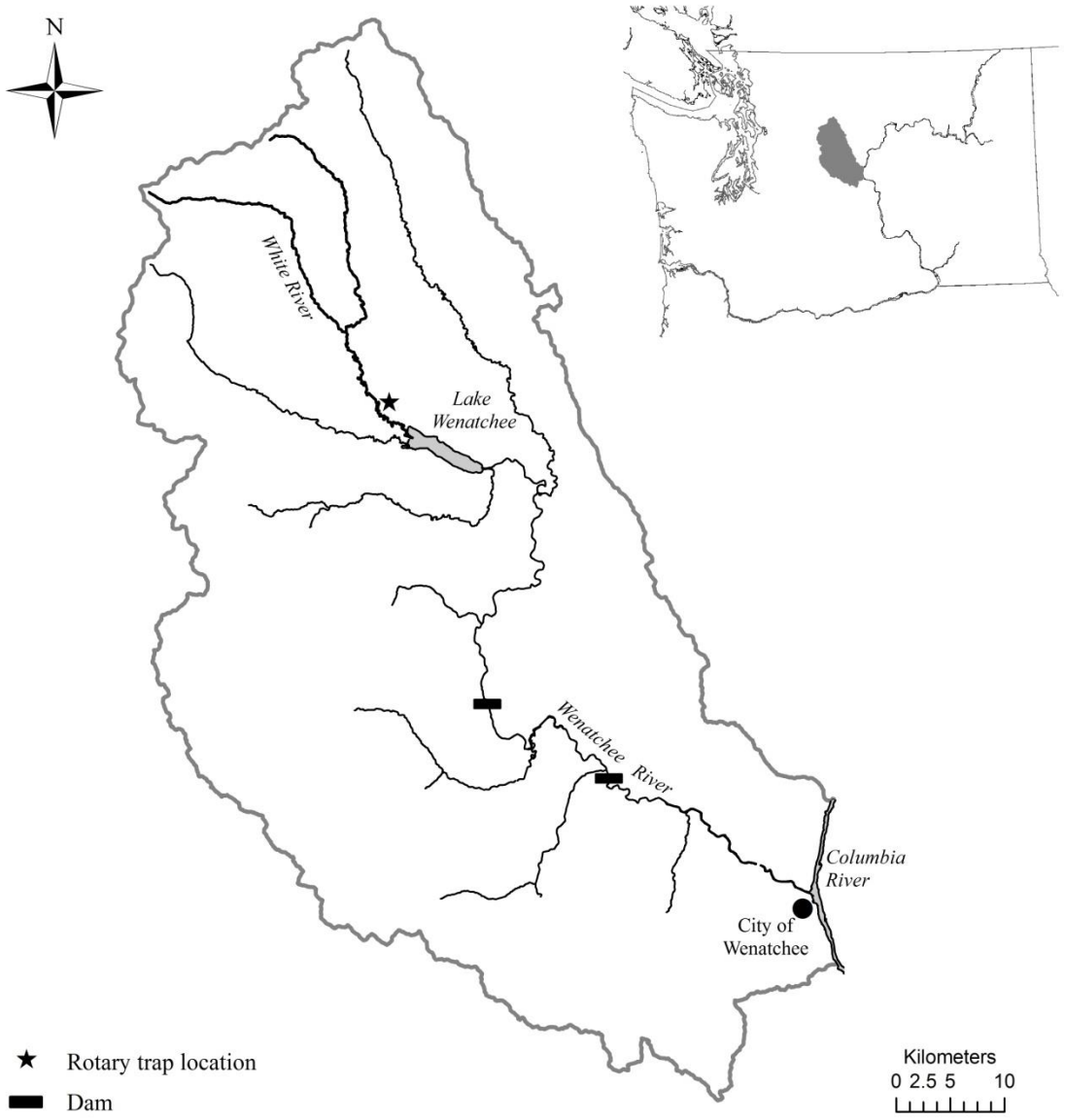


Figure 1. Map of the Wenatchee River subbasin with White River rotary trap location.

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 RK 9.9 of White River. Operation of this station by WDOE is currently maintained with funding provided by GCPUD. In 2015, daily mean stream discharge ranged from 87cfs to 4,280cfs (Figure 2) while mean daily stream temperatures ranged from 0.2°C to

15.9°C (Figure 3). Discharge and temperature data provided by WDOE should be considered provisional and are presented in **Appendix A**.

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

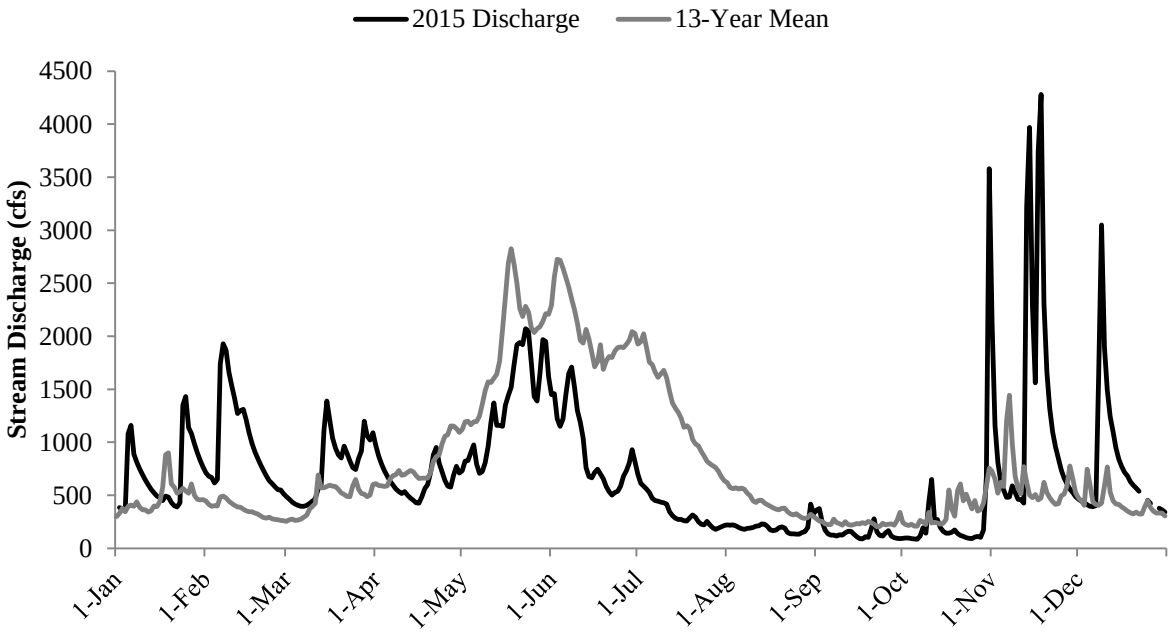


Figure 2. Mean daily stream discharge at the White River DOE stream monitoring station at Sears Creek Bridge in 2015.

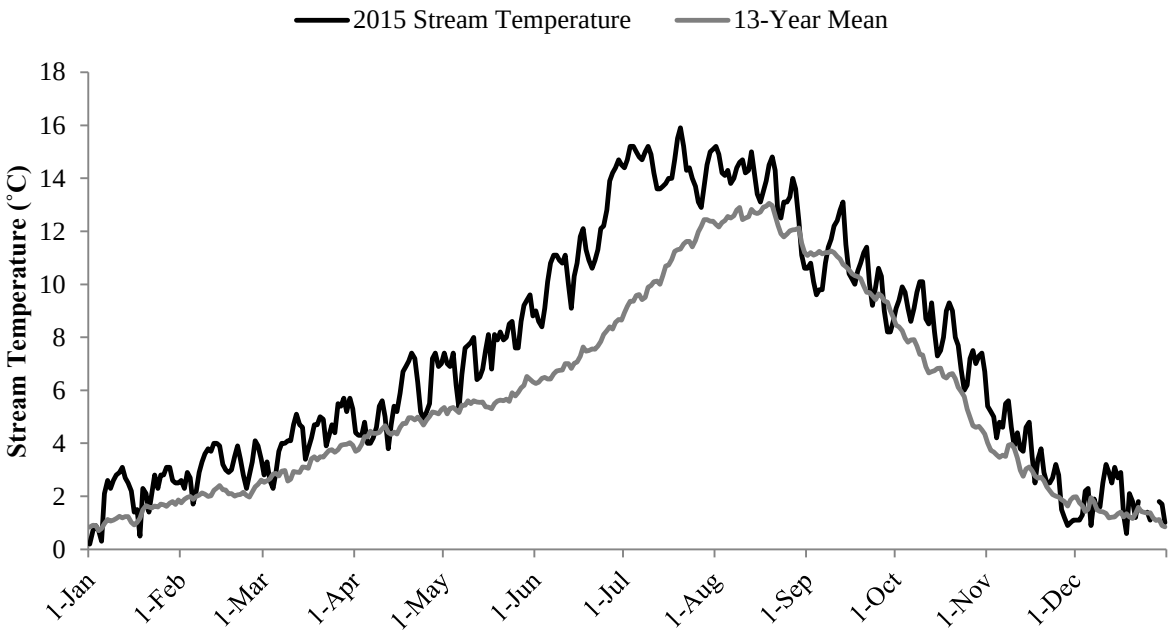


Figure 3. Mean daily water temperatures at the White River DOE stream monitoring station at Sears Creek Bridge in 2015.

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 *Salvilinus 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 2015, 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 five-gallon plastic buckets with lids to a stream-side, portable sampling station. Fish were anesthetized in a solution of MS-222 to facilitate sampling and reduce handling stress. Fork length (FL) and weight were recorded for all fish, except large numbers of sockeye (*Oncorhynchus nerka*) fry. For these fish, a representative sample of 25 individuals was measured and weighed 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 were taken from spring Chinook salmon and steelhead (small, upper lobe caudal fin clip) and applied to blotter sheets. Samples from both species 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 samples were not collected in 2015.

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 RK upstream to the release location at Sears Creek Bridge (RK 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 of ≥ 60mm were PIT tagged unless the health of an individual was in question (e.g.- fungus). 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:

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

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):

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

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% 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 2015, we 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), mechanical malfunctions, or periods of suspended trapping due to issues relating to lapsed liability insurance. Trapping was interrupted or intentionally suspended for a total of 49 days (Table 1).

Table 1. Summary of White River smolt trap operation, 2015.

Trap Status	Description	Days
Operating	Continuous data collection	226
Interrupted	Interrupted by debris, ice, tampering, or improper positioning	7
Pulled	Intentionally pulled due to flooding risk or administrative reasons	42

3.2 Daily Captures and Biological Sampling

3.2.1 Wild Spring Chinook Yearlings (BY2013)

A total of 32 wild yearling Chinook smolts were collected between March 1 and June 30, with peak catch occurring on April 9 (n = 4; Figure 4). Mean fork-length (FL) was 103mm (n = 32; SD = 6.9) and mean weight was 13.0g (n = 31; SD = 2.8); see Table 2. PIT tags were implanted into 32 smolts. Genetic samples were also taken from the same 32 fish. An additional two suspected BY2013 Chinook were captured after July 1. Mean FL for these fish was 145mm (n = 2; SD = 13.4) and mean weight was 35.15g (n = 2; SD = 11.4); see Table 2. These fish were identified as precocial parr by their large size, timing of capture, and release of milt during handling. All precocial parr were excluded from emigration estimates. There were no BY2013 spring Chinook mortalities incurred (See **3.4 ESA Compliance**).

3.2.2 Wild Spring Chinook Subyearlings (BY2014)

Spring Chinook fry were captured at the trap between March 15 and June 13 (n = 11). During this period there were no fry trapping mortalities incurred. A total of 151 wild subyearling Chinook parr were collected between July 13 and November 30, with peak catch occurring on September 5 (n = 15; Figure 5). The mean FL for subyearling parr was 96mm (n = 151; SD = 7.4) and the mean weight was 9.9g (n = 148; SD = 2.3); see Table 2. PIT tags were implanted into a total of 149 subyearling Chinook parr. Genetic samples were taken from 150 parr. One Chinook parr was not tagged due to a visible external injury. Additionally, one tag was shed during the 24hr holding period (Table 4). There were no BY2014 spring Chinook mortalities during the 2015 trapping season (See **3.4 ESA Compliance**).

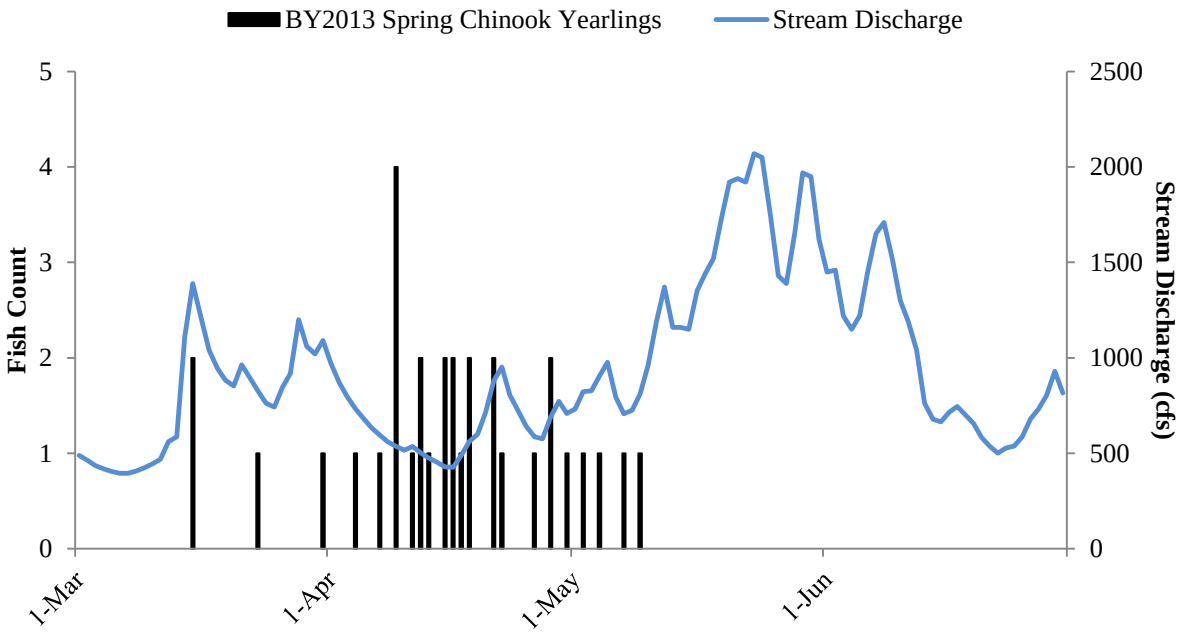


Figure 4. Daily catch of yearling spring Chinook smolt with mean daily stream discharge at the White River rotary trap, March 1 to June 30, 2015.

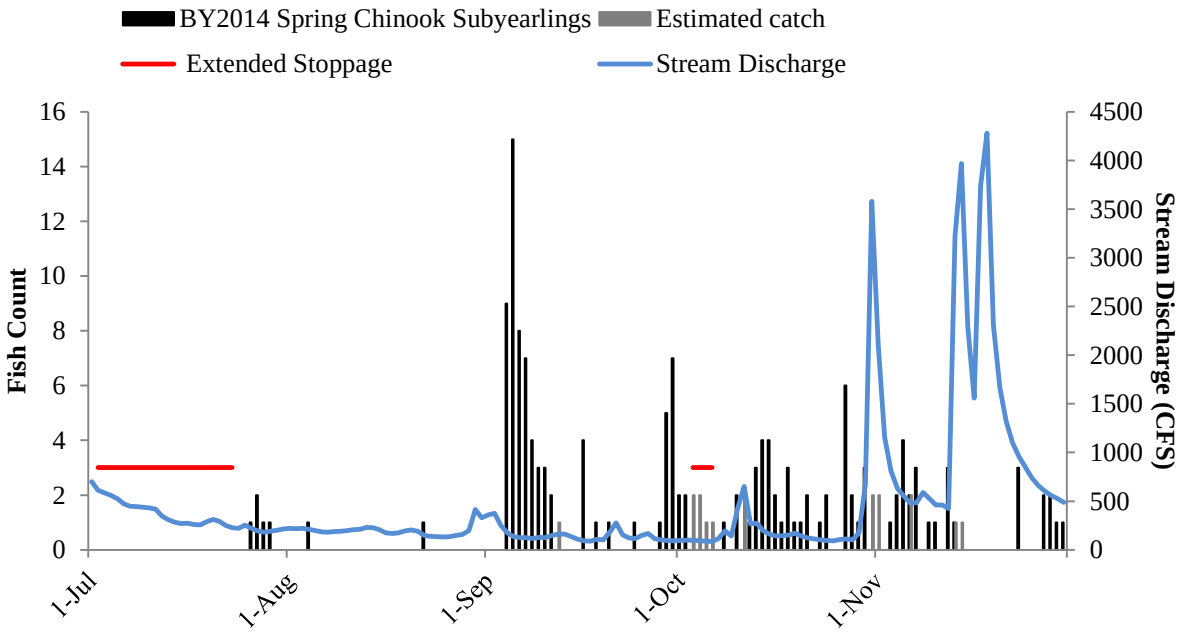


Figure 5. Daily catch of wild subyearling spring Chinook with mean daily stream discharge at the White River rotary trap, July 1 to November 30, 2015.

3.2.3 Hatchery Spring Chinook Yearlings (BY2013)

Hatchery-origin yearling Chinook released downstream of the smolt trap are sometimes caught in the summer months as precocial parr. Direct releases of BY2013 spring Chinook were not performed in the White River or in close proximity to is confluence with Lake Wenatchee (netpen-rearing). There were no hatchery-origin spring Chinook captured at the smolt trap in 2015. Hatchery fish captured at the trap are identified by the presence of CWT tags.

Table 2. Summary of length and weight sampling of juvenile spring Chinook captured at the White River rotary trap in 2015.

Brood Year	Origin/Species/Stage	Fork Length (mm)			Weight (g)			K-factor
		Mean	n	SD	Mean	n	SD	
2013	Wild Yearling Smolt	103	32	6.9	13.0	31	2.8	1.14
2013	Wild Yearling Precocial Parr	145	2	13.4	35.2	2	11.4	1.14
2014	Wild Subyearling Fry	38	11	3.3	0.5	10	0.2	0.86
2014	Wild Subyearling Parr	96	151	7.4	9.9	148	2.3	1.11

3.3 Trap Efficiency Calibration and Population Estimates

3.3.1 Wild Spring Chinook Yearlings (BY 2013)

Due to low abundance, no BY2013 natural yearling Chinook efficiency trials were performed in 2015. A composite regression model using previous year's (2008-2012) efficiency trials showed statistical significance ($r^2 = 0.57$; $p = 0.001$) for a 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 2015. 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 2014, we estimated that 2,461 (± 779; 95% CI) BY2013 subyearlings emigrated past the trap. In the spring of 2015, we estimated that 3,023 (± 2,728; 95% CI) emigrated past the trap. Combining the two estimates, total BY2013 wild spring Chinook emigrants was 5,484 (± 2,836; 95% CI; Table 3).

3.3.2 Wild Spring Chinook Subyearling (BY 2014)

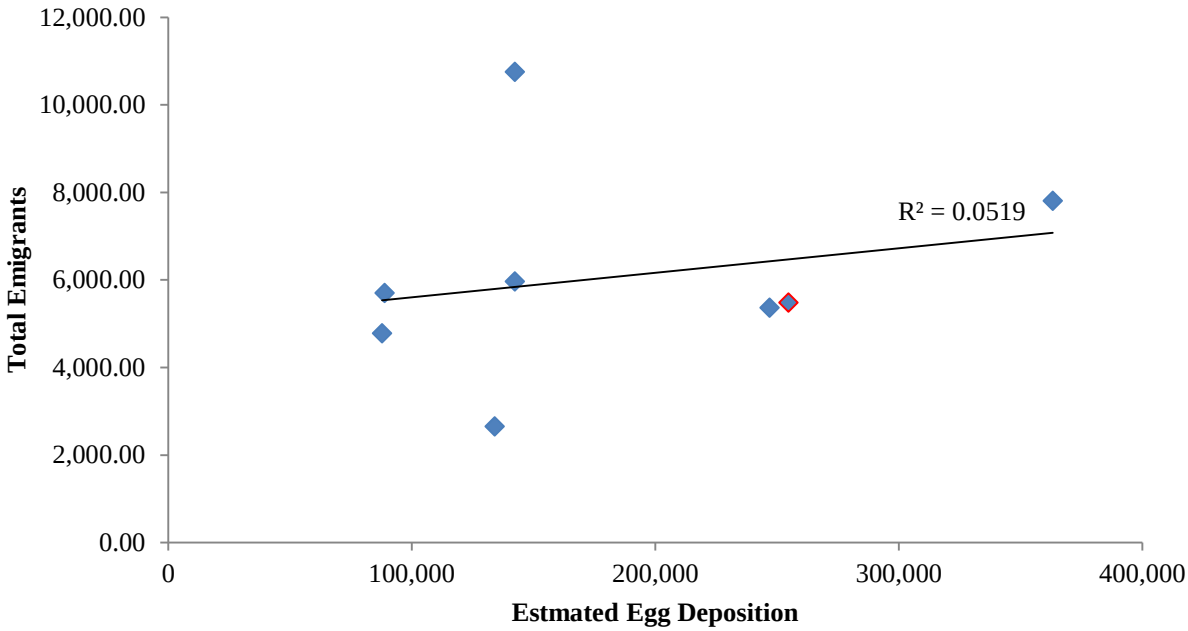
Low parr abundance presented only one opportunity to perform a mark-group release in 2015. Despite being smaller ($n = 39$) than the previously-set minimum mark group size of 50 parr, the efficiency trial was performed due to the low cfs being tested (89.5cfs). Our current strategy to improve the flow-efficiency model includes targeting mark-group releases at discharge levels where data is currently lacking. The updated multi-year composite regression was applied to BY2014 subyearling emigrants. The 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 2015, 1,449 (± 421; 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	642	2,004	2,646 ± 1,597	2.0%	85
2007	20	4,441	88,820	2,293	3,399	5,692 ± 2,214	6.4%	285
2008	31	4,592	142,352	5,552	5,193	10,745 ± 3,837	7.5%	347
2009	54	4,573	246,942	2,485	2,939	5,424 ± 2,522	2.2%	100
2010	33	4,314	142,362	1,859	4,121	5,980 ± 3,455	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	3,905	3,995	7,900 ± 3,898	2.2%	92
2013	54	4,716	254,664	2,461	3,023	5,484 ± 2,836	2.2%	102
2014	26	4,045	105,170	1,449	—	—	—	—
Avg	41	4,446	182,508	2,791	3,292	6,082	4.0%	179

[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.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Total Emigrants
R² = 0.0519
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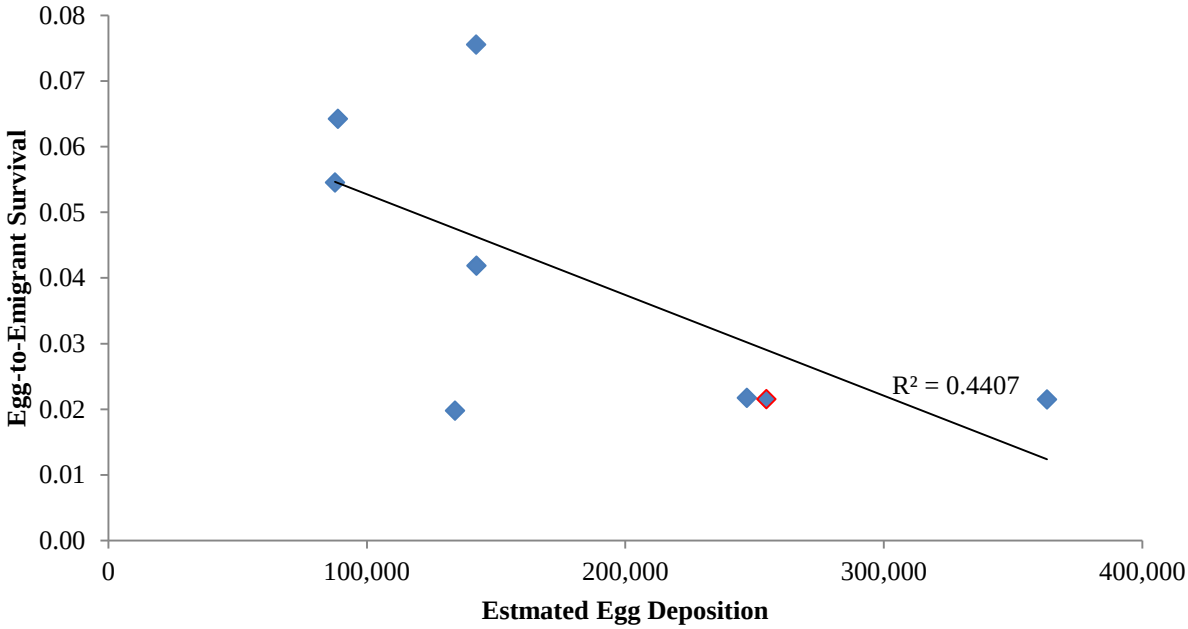
200,000
300,000
400,000
Estmated Egg Deposition


0.08
0.07
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Egg-to-Emigrant Survival
R² = 0.4407
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100,000
200,000
300,000
400,000
Estmated Egg Deposition

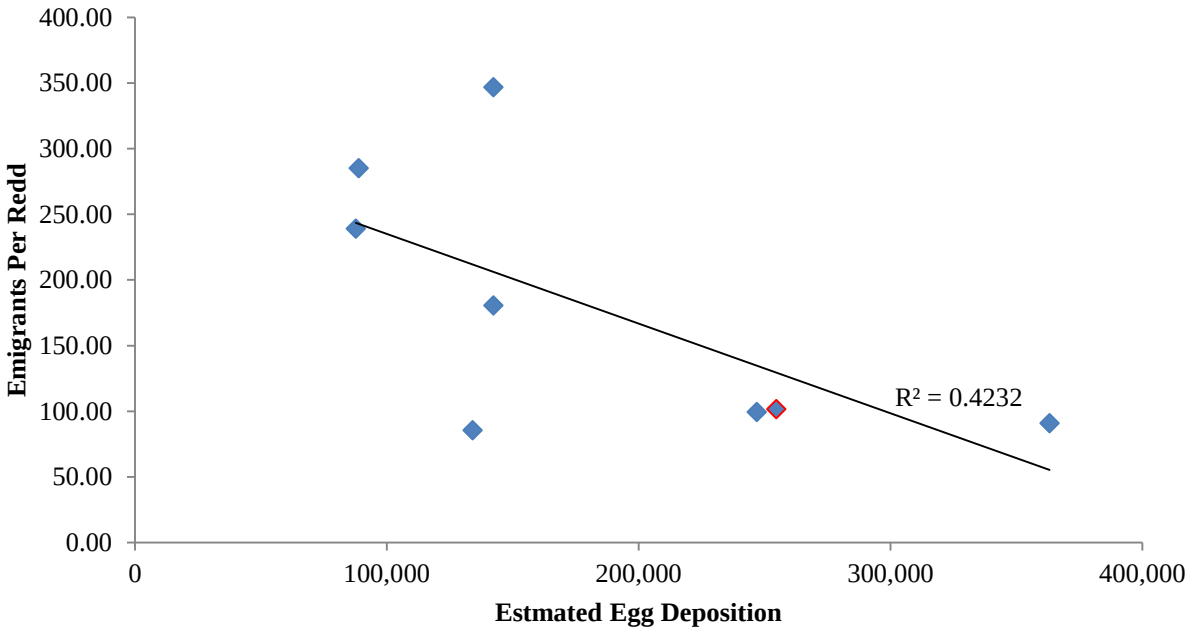

Figure 6. Relationships between estimated egg deposition and total emigrants produced, egg-to-emigrant survival, and emigrants per redd for White River spring Chinook, BY 2007 to 2013. *BY2013 values denoted by red border.

3.4 PIT Tagging

In 2015, a total of 185 spring Chinook and 6 steelhead were PIT tagged at the trap. PIT tag retention after 24 hours of observation was 100% for all species/stages, with the exception of 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 in 2015.

Brood Year	Species/Stage	Total Catch	Total PIT Tagged	Percent Tagged	Percent Tags Shed
2013	Yearling Chinook Smolt	32	32	100.00%	0.0%
2013	Yearling Chinook Precocial Parr	2	2	100.00%	0.0%
2014	Subyearling Chinook Parr	151	149	98.68%	0.7%
*	Steelhead Parr	6	6	100.00%	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 *Salvelinus confluentus*, eastern brook trout *Salvelinus fontilalis*, 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 *Oncorhynchus nerka*, 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 in 2015.

Species	Total Count	Fork Length (mm)			Weight (g)		
		Mean	*n*	SD	Mean	*n*	SD
Bull Trout Fry	1	28	1	—	—	—	—
Bull Trout Parr	8	147	8	56.3	43.0	8	34.7
Eastern Brook Trout	1	245	1	—	145	1	—
Longnose Dace	12	59	12	22.8	4.0	9	1.9
Mountain Whitefish	93	87	93	36.8	9.5	88	20.4
Northern Pikeminnow	37	128	37	47.7	35.8	35	53.0
Rainbow Trout/Steelhead Parr	6	158	5	54.5	52.3	5	38.6
Redside Shiner	147	73	147	16.2	5.6	142	3.0
Sculpin	172	45	170	22.3	3.1	97	4.4
Sockeye - Kokanee	5	203	5	9.1	90.1	5	10.5
Sockeye Fry	7,212	28	1,200	1.2	—	—	—
Sockeye Parr	5	73	5	10.5	3.7	5	1.5
Sucker	37	140	37	104.6	90.0	28	107.5
Westslope Cutthroat	30	221	30	34.9	103.3	28	50.7

3.6 ESA Compliance

There were no ESA species mortalities incurred in 2015 (Table 6). All fish handled were inspected prior to tagging or further sampling, with only one wild spring Chinook parr warranting immediate release (injury).

Table 6. Summary of White River ESA listed species catch and mortality in 2015.

Species/Stage	Total Catch	Total Mortality	Total % Mortality
Yearling Chinook Smolt	32	0	0.00%
Yearling Chinook Precocial Parr	2	0	0.00%
Subyearling Chinook Parr	151	0	0.00%
Subyearling Chinook Fry	11	0	0.00%
Total Wild Spring Chinook	**196**	**0**	**0.00%**
Bull Trout	9	0	0.00%
Steelhead/Rainbow Trout	6	0	0.00%

4.0 DISCUSSION

Estimations of White River yearling (BY2013) and subyearling (BY2014) wild spring Chinook emigrants in 2015 were calculated using multi-year compounded regressions. Given the overall low abundance of White River spring Chinook, the ability to use mark-group releases over multiple years provides the most effective means of expansion, when pooled and year-specific regression models are impracticable.

Using the multi-year yearling Chinook model, we estimated that 3,023 BY2013 spring Chinook emigrated past the trap in the spring of 2015. This estimation of smolt migrants falls below the 8-year yearling average (n = 3,292), despite above average redd counts. Combined with the previous estimate of 2,482 subyearling emigrants, the total emigrant expansion of 5,505 BY2013 was also below the 8-year average (n = 6,085). Although above-average egg deposition produced a below average estimate of emigrant abundance, the observed BY2013 egg-to-emigrant survival rate (2.2%) was consistent with the inverse relationship between total egg deposition and egg-to-emigrant survival previously observed in the White River. This suggest that the total estimated emigrants for BY2013 although potentially low, is not necessarily atypical of the system.

Base flows extending into mid-September and a brief increase in subyearling catch provided an important opportunity to expand the breadth of our subyearling regression. Efficiency trials at the high and low ends of the hydrograph are generally unfeasible due to inadequate mark-group sizes; active emigration is low at base flows and trap efficiency is extremely low at very high flows. A single, yet significant mark-group release effectively set the lower bound of the subyearling regression at 89.5cfs, a flow representative of near-base discharge on the White River. Using the improved regression model, we estimated that 1,449 BY2014 parr emigrated past the trap in 2015.

Compared to other upper-Wenatchee River tributaries (Chiwawa River and Nason Creek), the White River was the only tributary that did not have an increasing trend in egg-to-emigrant survival for the 2013 brood (Figure 7). Egg-to-emigrant survival in Nason Creek is generally lower than that of the White River, yet the inverse is seen in BY2013. This deviation from previous trends is attributed in part to a suspected overestimate of Nason Creek emigrant abundance; the product of new regression models skewed by limited trials (Ishida et al. 2016). We speculate that the eventual recalculation of the estimate may decrease Nason Creek's BY2013 estimated survival markedly.

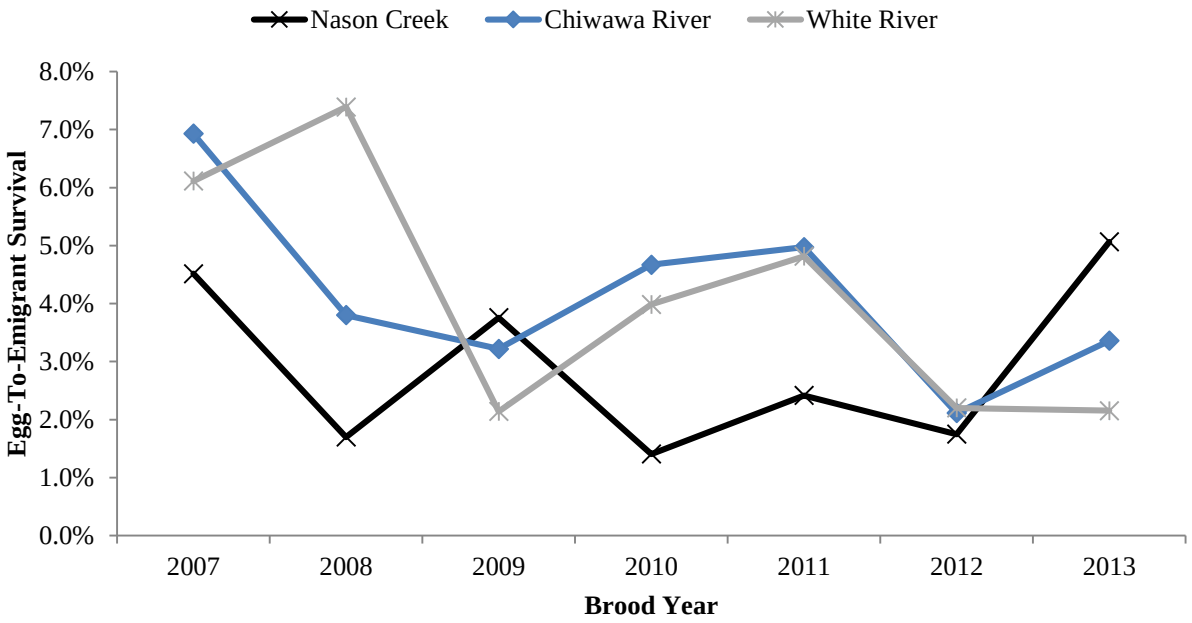

Figure 7. Comparison of wild spring Chinook abundance estimates (BY2007-2013) made at the White R., Nason Cr., and Chiwawa R. smolt traps. Chiwawa R. data provided by Hillman et al. (2015).

In 2016 we will continue to use the methodologies described in this report. Our priority will again be the strengthening of both our subyearling and yearling models through efficiency trials first vetted for adequate size and potential redundancies prior to release. While limited by the low abundance of White River spring Chinook, we remain confident that improvement to our models, albeit potentially slow, will persist through our refined methodologies.

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APPENDIX A: White River Temperature and Discharge Data

Date	Stream Discharge (CFS)	Water Temperature (˚C)
3/1/2015	488	2.8
3/2/2015	464	3.3
3/3/2015	435	2.6
3/4/2015	418	2.3
3/5/2015	405	2.9
3/6/2015	395	3.7
3/7/2015	395	4.0
3/8/2015	406	4.0
3/9/2015	423	4.1
3/10/2015	443	4.1
3/11/2015	468	4.7
3/12/2015	561	5.1
3/13/2015	586	4.7
3/14/2015	1110	4.6
3/15/2015	1390	3.4
3/16/2015	1210	3.8
3/17/2015	1040	4.2
3/18/2015	945	4.7
3/19/2015	883	4.7
3/20/2015	852	5.0
3/21/2015	964	4.9
3/22/2015	896	3.9
3/23/2015	825	4.3
3/24/2015	762	4.7
3/25/2015	742	4.4
3/26/2015	845	5.5
3/27/2015	918	5.4
3/28/2015	1200	5.7
3/29/2015	1060	5.2
3/30/2015	1020	5.7
3/31/2015	1090	5.3
4/1/2015	970	4.4
4/2/2015	870	4.3
4/3/2015	796	4.3
4/4/2015	732	4.8
4/5/2015	681	4.0
4/6/2015	633	4.0
4/7/2015	594	4.2
4/8/2015	560	4.6
4/9/2015	536	5.4
4/10/2015	516	5.6
4/11/2015	536	5.0
4/12/2015	503	3.8
4/13/2015	475	4.7
4/14/2015	453	5.4
4/15/2015	428	5.2
4/16/2015	426	5.9
4/17/2015	488	6.7
4/18/2015	561	6.9
4/19/2015	600	7.1
4/20/2015	714	7.4
4/21/2015	881	7.2
4/22/2015	952	6.3
4/23/2015	804	5.2
4/24/2015	721	4.9
4/25/2015	642	5.2
4/26/2015	586	5.5
4/27/2015	576	7.2
4/28/2015	689	7.4
4/29/2015	772	6.9
4/30/2015	709	7.0
5/1/2015	732	7.4
5/2/2015	823	7.0
5/3/2015	828	6.9
5/4/2015	904	7.4
5/5/2015	977	6.2
5/6/2015	794	5.3
5/7/2015	706	6.6
5/8/2015	726	7.6
5/9/2015	810	7.7
5/10/2015	962	7.8
5/11/2015	1190	8.0
5/12/2015	1370	6.4
5/13/2015	1160	6.5
5/14/2015	1160	6.8
5/15/2015	1150	7.5
5/16/2015	1350	8.1

5/17/2015	1440	6.8	7/1/2015	699	14.4
5/18/2015	1520	8.1	7/2/2015	612	14.7
5/19/2015	1730	7.9	7/3/2015	587	15.2
5/20/2015	1920	8.2	7/4/2015	559	15.2
5/21/2015	1940	7.9	7/5/2015	525	15.0
5/22/2015	1920	8.0	7/6/2015	473	14.8
5/23/2015	2070	8.5	7/7/2015	449	14.7
5/24/2015	2050	8.6	7/8/2015	444	15.0
5/25/2015	1750	7.6	7/9/2015	436	15.2
5/26/2015	1430	7.6	7/10/2015	430	14.9
5/27/2015	1390	8.6	7/11/2015	418	14.2
5/28/2015	1650	9.2	7/12/2015	346	13.6
5/29/2015	1970	9.4	7/13/2015	310	13.6
5/30/2015	1950	9.6	7/14/2015	283	13.7
5/31/2015	1620	8.8	7/15/2015	271	13.8
6/1/2015	1450	9.0	7/16/2015	273	14.0
6/2/2015	1460	8.6	7/17/2015	259	14.0
6/3/2015	1220	8.4	7/18/2015	257	14.7
6/4/2015	1150	9.1	7/19/2015	288	15.5
6/5/2015	1220	10.1	7/20/2015	314	15.9
6/6/2015	1450	10.8	7/21/2015	293	15.2
6/7/2015	1650	11.1	7/22/2015	250	14.3
6/8/2015	1710	11.1	7/23/2015	226	14.4
6/9/2015	1520	10.9	7/24/2015	222	14.0
6/10/2015	1300	10.8	7/25/2015	254	13.7
6/11/2015	1190	11.1	7/26/2015	221	13.1
6/12/2015	1040	10.0	7/27/2015	194	12.9
6/13/2015	762	9.1	7/28/2015	180	13.7
6/14/2015	678	10.3	7/29/2015	190	14.5
6/15/2015	664	10.8	7/30/2015	202	15.0
6/16/2015	715	11.8	7/31/2015	215	15.1
6/17/2015	746	12.1	8/1/2015	220	15.2
6/18/2015	701	11.3	8/2/2015	218	14.9
6/19/2015	657	10.9	8/3/2015	220	14.2
6/20/2015	582	10.6	8/4/2015	211	14.1
6/21/2015	536	10.9	8/5/2015	198	14.3
6/22/2015	501	11.3	8/6/2015	186	13.8
6/23/2015	527	12.1	8/7/2015	180	14.0
6/24/2015	537	12.2	8/8/2015	189	14.4
6/25/2015	586	12.8	8/9/2015	191	14.6
6/26/2015	680	13.9	8/10/2015	197	14.7
6/27/2015	732	14.2	8/11/2015	208	14.2
6/28/2015	803	14.4	8/12/2015	212	14.3
6/29/2015	930	14.7	8/13/2015	230	15.0
6/30/2015	815	14.5	8/14/2015	228	14.2

8/15/2015	206	13.4	9/29/2015	94.7	8.2
8/16/2015	173	13.1	9/30/2015	92.3	8.6
8/17/2015	167	13.5	10/1/2015	95.7	9.1
8/18/2015	173	13.9	10/2/2015	98.4	9.4
8/19/2015	195	14.5	10/3/2015	97.4	9.9
8/20/2015	204	14.8	10/4/2015	91.5	9.7
8/21/2015	190	14.3	10/5/2015	89	9.1
8/22/2015	149	12.6	10/6/2015	86.6	8.6
8/23/2015	138	12.5	10/7/2015	116	9.1
8/24/2015	136	13.1	10/8/2015	193	9.7
8/25/2015	133	13.1	10/9/2015	142	10.1
8/26/2015	134	13.3	10/10/2015	429	10.1
8/27/2015	149	14.0	10/11/2015	650	8.7
8/28/2015	157	13.6	10/12/2015	268	8.5
8/29/2015	196	12.4	10/13/2015	273	9.3
8/30/2015	416	11.1	10/14/2015	197	8.2
8/31/2015	328	10.6	10/15/2015	161	7.3
9/1/2015	359	10.6	10/16/2015	142	7.5
9/2/2015	376	10.8	10/17/2015	144	8.0
9/3/2015	249	10.1	10/18/2015	153	9.0
9/4/2015	173	9.6	10/19/2015	172	9.3
9/5/2015	136	9.8	10/20/2015	140	9.0
9/6/2015	126	9.8	10/21/2015	123	8.0
9/7/2015	126	10.8	10/22/2015	111	7.7
9/8/2015	115	11.4	10/23/2015	101	6.8
9/9/2015	129	11.7	10/24/2015	95.4	6.0
9/10/2015	126	12.2	10/25/2015	92.5	6.2
9/11/2015	147	12.4	10/26/2015	107	7.2
9/12/2015	160	12.8	10/27/2015	111	7.5
9/13/2015	162	13.1	10/28/2015	102	7.0
9/14/2015	134	11.5	10/29/2015	174	7.3
9/15/2015	108	10.4	10/30/2015	675	7.4
9/16/2015	92.8	10.2	10/31/2015	3580	6.7
9/17/2015	89.5	10.0	11/1/2015	2110	5.4
9/18/2015	110	10.5	11/2/2015	1160	5.2
9/19/2015	103	10.8	11/3/2015	809	5.0
9/20/2015	181	11.2	11/4/2015	633	4.2
9/21/2015	278	11.4	11/5/2015	551	4.8
9/22/2015	154	10.0	11/6/2015	480	4.6
9/23/2015	123	9.2	11/7/2015	483	5.5
9/24/2015	115	9.9	11/8/2015	589	5.6
9/25/2015	147	10.6	11/9/2015	525	4.6
9/26/2015	167	10.3	11/10/2015	462	3.9
9/27/2015	114	9.0	11/11/2015	459	4.4
9/28/2015	101	8.2	11/12/2015	426	3.8

11/13/2015	3220	3.7
11/14/2015	3970	4.6
11/15/2015	2290	4.8
11/16/2015	1560	3.5
11/17/2015	3740	2.5
11/18/2015	4280	3.4
11/19/2015	2310	3.8
11/20/2015	1670	2.9
11/21/2015	1320	2.5
11/22/2015	1100	2.5
11/23/2015	958	2.7
11/24/2015	850	3.2
11/25/2015	740	2.8
11/26/2015	662	1.5
11/27/2015	606	1.2
11/28/2015	560	0.9
11/29/2015	525	1.0
11/30/2015	486	1.1

APPENDIX B: Daily Trap Operation Status

Date	Trap Status	Comments
3/1/2015	No Op.	Pulled-administrative
3/2/2015	No Op.	Pulled-administrative
3/3/2015	No Op.	Pulled-administrative
3/4/2015	No Op.	Pulled-administrative
3/5/2015	No Op.	Pulled-administrative
3/6/2015	No Op.	Pulled-administrative
3/7/2015	No Op.	Pulled-administrative
3/8/2015	No Op.	Pulled-administrative
3/9/2015	No Op.	Pulled-administrative
3/10/2015	Op.	
3/11/2015	Op.	
3/12/2015	Op.	
3/13/2015	Op.	
3/14/2015	Op.	
3/15/2015	No Op.	Stopped-debris
3/16/2015	Op.	
3/17/2015	Op.	
3/18/2015	Op.	
3/19/2015	Op.	
3/20/2015	Op.	
3/21/2015	Op.	
3/22/2015	Op.	
3/23/2015	Op.	
3/24/2015	Op.	
3/25/2015	Op.	
3/26/2015	Op.	
3/27/2015	Op.	
3/28/2015	Op.	
3/29/2015	Op.	
3/30/2015	Op.	
3/31/2015	Op.	
4/1/2015	Op.	
4/2/2015	Op.	
4/3/2015	Op.	
4/4/2015	Op.	
4/5/2015	Op.	
4/6/2015	Op.	
4/7/2015	Op.	
4/8/2015	Op.	
4/9/2015	Op.	
4/10/2015	Op.	
4/11/2015	Op.	
4/12/2015	Op.	
4/13/2015	Op.	
4/14/2015	Op.	
4/15/2015	Op.	
4/16/2015	Op.	
4/17/2015	Op.	
4/18/2015	Op.	
4/19/2015	Op.	
4/20/2015	Op.	
4/21/2015	Op.	
4/22/2015	Op.	
4/23/2015	Op.	
4/24/2015	Op.	
4/25/2015	Op.	
4/26/2015	Op.	
4/27/2015	Op.	
4/28/2015	Op.	
4/29/2015	Op.	
4/30/2015	Op.	
5/1/2015	Op.	
5/2/2015	Op.	
5/3/2015	Op.	
5/4/2015	Op.	
5/5/2015	Op.	
5/6/2015	Op.	
5/7/2015	Op.	
5/8/2015	Op.	
5/9/2015	Op.	
5/10/2015	Op.	
5/11/2015	Op.	
5/12/2015	No Op.	Stopped-debris
5/13/2015	Op.	
5/14/2015	Op.	
5/15/2015	Op.	
5/16/2015	Op.	
5/17/2015	Op.	
5/18/2015	Op.	
5/19/2015	Op.	
5/20/2015	Op.	

5/21/2015	Op.		7/5/2015	No Op.	Pulled - administrative
5/22/2015	Op.		7/6/2015	No Op.	Pulled - administrative
5/23/2015	Op.		7/7/2015	No Op.	Pulled - administrative
5/24/2015	Op.		7/8/2015	No Op.	Pulled - administrative
5/25/2015	Op.		7/9/2015	No Op.	Pulled - administrative
5/26/2015	No Op.	Stopped-tampering	7/10/2015	No Op.	Pulled - administrative
5/27/2015	Op.		7/11/2015	No Op.	Pulled - administrative
5/28/2015	Op.		7/12/2015	No Op.	Pulled - administrative
5/29/2015	Op.		7/13/2015	No Op.	Pulled - administrative
5/30/2015	No Op.	Stopped-debris	7/14/2015	No Op.	Pulled - administrative
5/31/2015	Op.		7/15/2015	No Op.	Pulled - administrative
6/1/2015	Op.		7/16/2015	No Op.	Pulled - administrative
6/2/2015	Op.		7/17/2015	No Op.	Pulled - administrative
6/3/2015	Op.		7/18/2015	No Op.	Pulled - administrative
6/4/2015	Op.		7/19/2015	No Op.	Pulled - administrative
6/5/2015	Op.		7/20/2015	No Op.	Pulled - administrative
6/6/2015	Op.		7/21/2015	No Op.	Pulled - administrative
6/7/2015	Op.		7/22/2015	No Op.	Pulled - administrative
6/8/2015	Op.		7/23/2015	No Op.	Pulled - administrative
6/9/2015	Op.		7/24/2015	Op.	
6/10/2015	Op.		7/25/2015	Op.	
6/11/2015	Op.		7/26/2015	Op.	
6/12/2015	Op.		7/27/2015	Op.	
6/13/2015	Op.		7/28/2015	Op.	
6/14/2015	Op.		7/29/2015	Op.	
6/15/2015	Op.		7/30/2015	Op.	
6/16/2015	Op.		7/31/2015	Op.	
6/17/2015	Op.		8/1/2015	Op.	
6/18/2015	Op.		8/2/2015	Op.	
6/19/2015	Op.		8/3/2015	Op.	
6/20/2015	Op.		8/4/2015	Op.	
6/21/2015	Op.		8/5/2015	Op.	
6/22/2015	Op.		8/6/2015	Op.	
6/23/2015	Op.		8/7/2015	Op.	
6/24/2015	Op.		8/8/2015	Op.	
6/25/2015	Op.		8/9/2015	Op.	
6/26/2015	Op.		8/10/2015	Op.	
6/27/2015	Op.		8/11/2015	Op.	
6/28/2015	Op.		8/12/2015	Op.	
6/29/2015	Op.		8/13/2015	Op.	
6/30/2015	Op.		8/14/2015	Op.	
7/1/2015	Op.		8/15/2015	Op.	
7/2/2015	No Op.	Pulled - administrative	8/16/2015	Op.	
7/3/2015	No Op.	Pulled - administrative	8/17/2015	Op.	
7/4/2015	No Op.	Pulled - administrative	8/18/2015	Op.	

8/19/2015	Op.		10/3/2015	No Op.	Pulled-high flows
8/20/2015	Op.		10/4/2015	No Op.	Pulled-high flows
8/21/2015	Op.		10/5/2015	No Op.	Pulled-high flows
8/22/2015	Op.		10/6/2015	No Op.	Pulled-high flows
8/23/2015	Op.		10/7/2015	Op.	
8/24/2015	Op.		10/8/2015	Op.	
8/25/2015	Op.		10/9/2015	Op.	
8/26/2015	Op.		10/10/2015	Op.	
8/27/2015	Op.		10/11/2015	No Op.	Stopped-debris
8/28/2015	Op.		10/12/2015	Op.	
8/29/2015	Op.		10/13/2015	Op.	
8/30/2015	Op.		10/14/2015	Op.	
8/31/2015	Op.		10/15/2015	Op.	
9/1/2015	Op.		10/16/2015	Op.	
9/2/2015	Op.		10/17/2015	Op.	
9/3/2015	Op.		10/18/2015	Op.	
9/4/2015	Op.		10/19/2015	Op.	
9/5/2015	Op.		10/20/2015	Op.	
9/6/2015	Op.		10/21/2015	Op.	
9/7/2015	Op.		10/22/2015	Op.	
9/8/2015	Op.		10/23/2015	Op.	
9/9/2015	Op.		10/24/2015	Op.	
9/10/2015	Op.		10/25/2015	Op.	
9/11/2015	Op.		10/26/2015	Op.	
9/12/2015	No Op.	Stopped-out of position	10/27/2015	Op.	
9/13/2015	Op.		10/28/2015	Op.	
9/14/2015	Op.		10/29/2015	Op.	
9/15/2015	Op.		10/30/2015	Op.	
9/16/2015	Op.		10/31/2015	No Op.	Pulled-high flows
9/17/2015	Op.		11/1/2015	No Op.	Pulled-high flows
9/18/2015	Op.		11/2/2015	Op.	
9/19/2015	Op.		11/3/2015	Op.	
9/20/2015	Op.		11/4/2015	Op.	
9/21/2015	Op.		11/5/2015	Op.	
9/22/2015	Op.		11/6/2015	No Op.	Stopped-debris
9/23/2015	Op.		11/7/2015	Op.	
9/24/2015	Op.		11/8/2015	Op.	
9/25/2015	Op.		11/9/2015	Op.	
9/26/2015	Op.		11/10/2015	Op.	
9/27/2015	Op.		11/11/2015	Op.	
9/28/2015	Op.		11/12/2015	Op.	
9/29/2015	Op.		11/13/2015	No Op.	Pulled-high flows
9/30/2015	Op.		11/14/2015	No Op.	Pulled-high flows
10/1/2015	Op.		11/15/2015	Op.	
10/2/2015	No Op.	Pulled-high flows	11/16/2015	Op.	

11/17/2015	Op.	
11/18/2015	No Op.	Pulled-high flows
11/19/2015	No Op.	Pulled-high flows
11/20/2015	Op.	
11/21/2015	Op.	
11/22/2015	Op.	
11/23/2015	Op.	
11/24/2015	Op.	
11/25/2015	Op.	
11/26/2015	Op.	
11/27/2015	Op.	
11/28/2015	Op.	
11/29/2015	Op.	
11/30/2015	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 (cfs)
Wild Chinook Yearlings	4/10/2008	25	2	0.120	0.354	229
Wild Chinook Yearlings	3/26/2009	24	5	0.250	0.524	191
Wild Chinook Yearlings	3/30/2009	34	4	0.147	0.394	193
Wild Chinook Yearlings	4/2/2009	37	10	0.297	0.577	206
Wild Chinook Yearlings	4/5/2009	59	15	0.271	0.548	205
Wild Chinook Yearlings	4/10/2009	36	3	0.111	0.340	385
Wild Chinook Yearlings	3/12/2010	25	1	0.080	0.287	300
Wild Chinook Yearlings	3/16/2010	30	5	0.200	0.464	278
Wild Chinook Yearlings	3/20/2010	21	1	0.095	0.314	283
Wild Chinook Yearlings	4/5/2010	37	1	0.054	0.235	340
Wild Chinook Yearlings	4/9/2010	31	4	0.161	0.413	310
Wild Chinook Yearlings	4/12/2010	58	4	0.086	0.298	288
Wild Chinook Yearlings	4/16/2010	73	2	0.041	0.204	381
Wild Chinook Yearlings	4/14/2012	48	1	0.042	0.206	527

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 (cfs)
Wild Chinook Subyearlings	8/20/2009	20	2	15.00%	0.398	311
Wild Chinook Subyearlings	8/29/2009	34	4	14.71%	0.394	227
Wild Chinook Subyearlings	10/7/2009	22	2	13.64%	0.378	95
Wild Chinook Subyearlings	10/16/2009	34	6	20.59%	0.471	134
Wild Chinook Subyearlings	11/17/2009	35	3	11.43%	0.345	375
Wild Chinook Subyearlings	11/23/2009	21	0	4.76%	0.22	313
Wild Chinook Subyearlings	11/21/2011	39	2	7.69%	0.281	172
Wild Chinook Subyearlings	10/4/2012	33	5	18.18%	0.441	140
Wild Chinook Subyearlings	10/24/2012	87	6	8.05%	0.288	268
Wild Chinook Subyearlings	10/28/2012	36	1	5.56%	0.238	711
Wild Chinook Subyearlings	10/31/2013	46	7	17.39%	0.43	258
Wild Chinook Subyearlings	11/6/2013	38	9	26.32%	0.539	248
Wild Chinook Subyearlings	11/9/2013	40	6	17.50%	0.432	251
Wild Chinook Subyearlings	11/13/2013	29	2	10.34%	0.327	422
Wild Chinook Subyearlings	11/23/2013	25	3	16.00%	0.412	406
Wild Chinook Subyearlings	11/27/2013	24	0	4.17%	0.206	335
Wild Chinook Subyearlings	9/17/2015	39	4	12.82%	0.366	89.5

Appendix D. Historical Morphometric Data

Spring Chinook (2007-2015)

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	152	7.5	10.4	149	6.3	1.2

[a] Includes residualized non-precocial smolts caught after June 30
[b] "Fry" classification based on age despite FL ≥ 50mm

Appendix M

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					

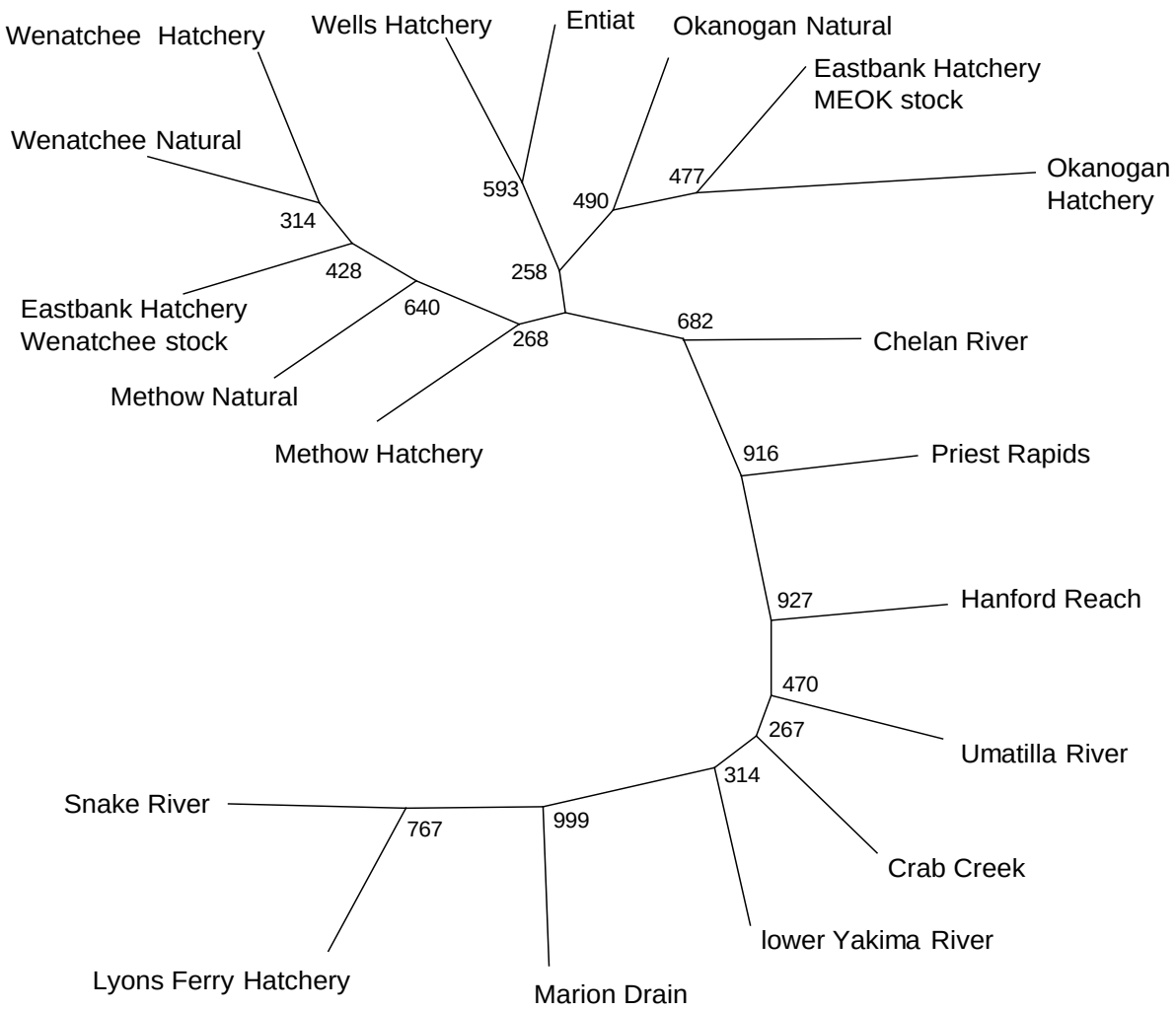

Figure 1. Relationship of natural- and hatchery-origin Chinook collections from the upper Columbia River basin using Cavalli-Sforza and Edwards (1967) chord distance. Bootstrap values are shown at each node.

Appendix N

Summer Chinook Spawning Ground Surveys in the Methow River Basin and Chelan River, 2015

BioAnalysts, Inc.℠
4725 North Cloverdale Road, Ste. 102
Boise ID 83713

March 10, 2016

To: Chelan and Grant Public Utility Districts

From: Denny Snyder, Keith Watson, and Mark Miller

Re: 2015 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 program. The tasks and objectives associated with implementing Grant and Chelan PUDs' Hatchery M&E Plan for 2015 are outlined in Hillman et al. (2013). Figures and tables are presented at the end of this memo. In 2015, The Okanogan Basin was surveyed by the Colville Confederated Tribes (CCT).

METHODS

Spawning ground surveys were conducted by foot and raft beginning the last 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 through sampling reaches and recorded the location and numbers of redds each week. Observers recorded the date, water temperature, river mile, and constructed 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.

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 PIT tag detection. 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. We only report the escapement and number of redds for the Okanogan Basin.

RESULTS

Methow

There were 1,231 summer Chinook redds counted within seven reaches of the Methow River (Table 1). No redds were counted in the Chewuch and Twisp Rivers this year. This was the fifth highest redd count observed in the last 25 years for the Methow River (Table 3). Spawning began the last week of September, peaked in early October, and ended the third week of November (Figure 1). Spawning may have started the third week of September given the unusually large number of Chinook on spawning grounds. Stream temperatures in the Methow River when spawning began varied from 9.0-10.0°C in late September. Spawning peaked the last week of September in reaches M6 and M7, while peak spawning occurred in reaches M3-M5 the first week of October. Spawning peaked the second week of October in reaches M1and M2.

Most redds (78%) were located in reaches from the mouth to the town of Twisp (M1-M3) (Table 1). In 2015, reach M1 experienced a dramatic increase in spawning with 350 redds compared to 9 redds observed in 2014. This increase is most likely because the fine sediments that covered spawning areas in 2014, as a result of the Carlton Complex Fires and landslides, were flushed from the system during high spring flows in 2015. Estimated escapement based on expansion of redd counts from the sex-ratio observed at Wells Dam during broodstock collection indicates that 3,952 summer Chinook (1,231 redds x 3.21 fish/redd) escaped to the Methow River.

There were 839 summer Chinook salmon carcasses sampled within the seven reaches of the Methow River (Table 2). The presence or absence of an adipose fin could not be determined on one fish. Twenty-one percent of the fish returning to the Methow River were sampled based on the estimated escapement of 3,952 summer Chinook. Ad-clipped hatchery fish made up 19% and naturally produced fish (adipose fin present) made up 81% of the fish sampled (Table 2). Most (94%) of the ad-clipped hatchery fish were located in reaches M1-M3, while naturally produced fish were more evenly distributed among survey reaches (Figure 2). Naturally produced fish made up 100% of the fish sampled in upper reaches (M6 and M7). Females made up 49% of the carcasses examined. Based on sampling 413 female carcasses, average egg voidance was 99%. Seven females (2 %) died before spawning (i.e., they retained all their eggs).

Chelan River

There were 448 redds counted in the Chelan River. Spawning activity began the first week of October and peaked two weeks later (Figure 3). Spawning continued into the last week of November. As more information is collected on time of spawning, the average spawn time will likely not appear bimodal. The majority of spawning occurred in the Powerhouse tailrace (48%), Columbia River tailrace (24%), and in the Habitat channel (20%) (Table 1). Estimated escapement based on expansion of redd counts from the sex-ratio observed at Wells Dam during broodstock collection indicates that 1,438 summer Chinook (448 redds x 3.21 fish/redd) escaped to the Chelan River.

There were 363 summer Chinook carcasses sampled in the Chelan River (Table 2). Twenty-five percent of the summer Chinook returning to the Chelan River were sampled based on the estimated escapement of 1,438 fish. Ad-clipped hatchery fish made up 47% and naturally produced fish were 53% of the fish examined. The distribution of ad-clipped hatchery fish and naturally produced fish was similar, except in the pool upstream of the habitat channel where only hatchery fish were recovered (Figure 4). A disproportionate number of fish (compared to redds counts) were sampled in the Columbia River tailrace, because carcasses drifted from upstream spawning areas and settled in the Columbia River tailrace. Females made up 77% of the carcasses examined (Table 2). Mean egg voidance from 281 female carcasses was 84%. Twenty females (7 %) died before spawning. Five Coho were sampled within the Chelan River and these data were submitted to the Yakima Nation (Peshastin Office).

Okanogan Basin

In 2015, CCT conducted summer Chinook surveys in the Okanogan Basin. A total of 4,128 redds were counted in the Okanogan Basin. Based on expanded redd counts, the estimated escapement for the Okanogan basin was 13,272 summer Chinook (Personal Communication, Andrea Pearl, CCT).

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Hillman, T., M. Miller, M. Johnson, C. Moran, M. Tonseth, A. Murdoch, C. Willard, L. Keller, B. Ishida, C. Kamphaus, T. Pearsons, and P. Graf. 2015. Monitoring and evaluation of the Chelan and Grant County PUDs hatchery programs: 2014 annual report. Report to the HCP and PRCC Hatchery Committees, Wenatchee and Ephrata, WA.

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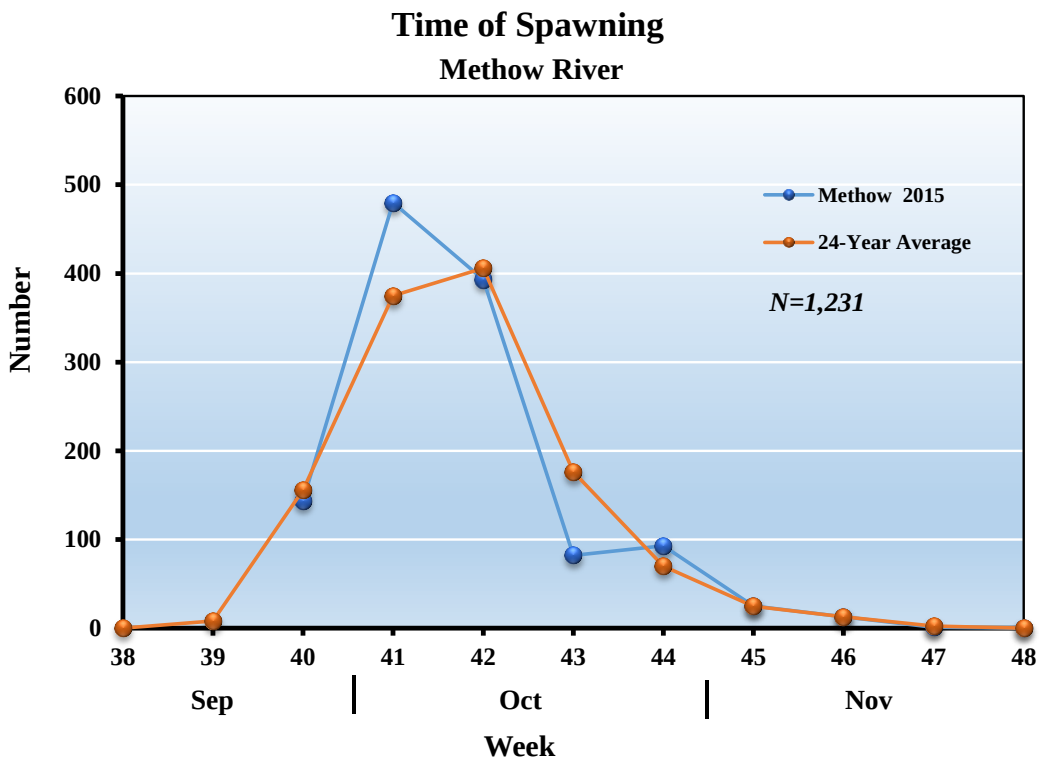

Figure 1. Number of new redds counted each week from late September to mid-November. The figure displays the beginning, peak and end of spawning for summer Chinook in the Methow River in 2015 compared to a 24-year average (1991-2014).

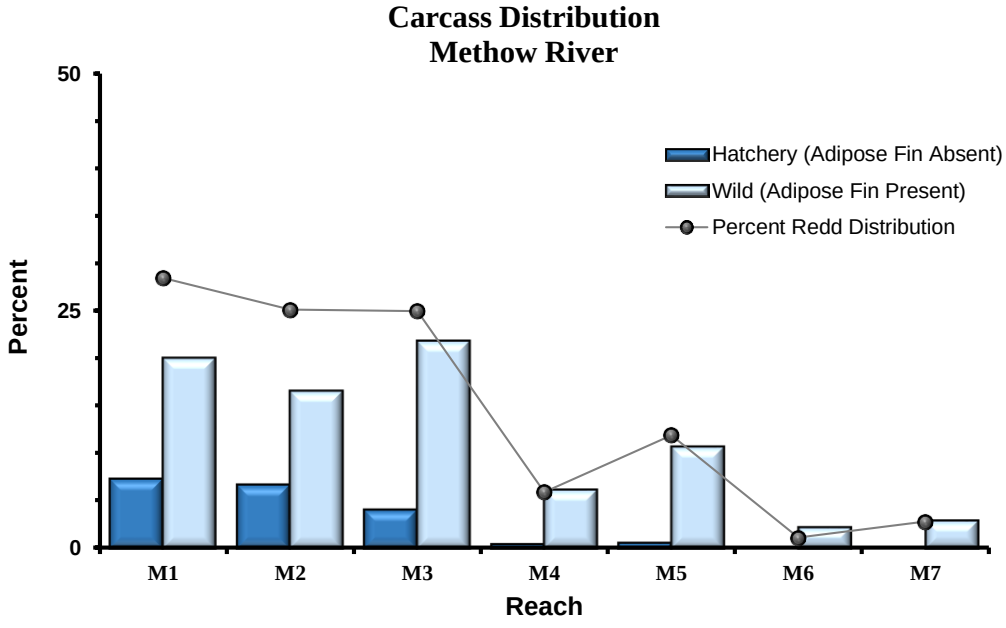

Figure 2. Percent distribution of ad-clipped hatchery and naturally produced fish plotted against the percent distribution of redds observed in reaches on the Methow River, 2015.

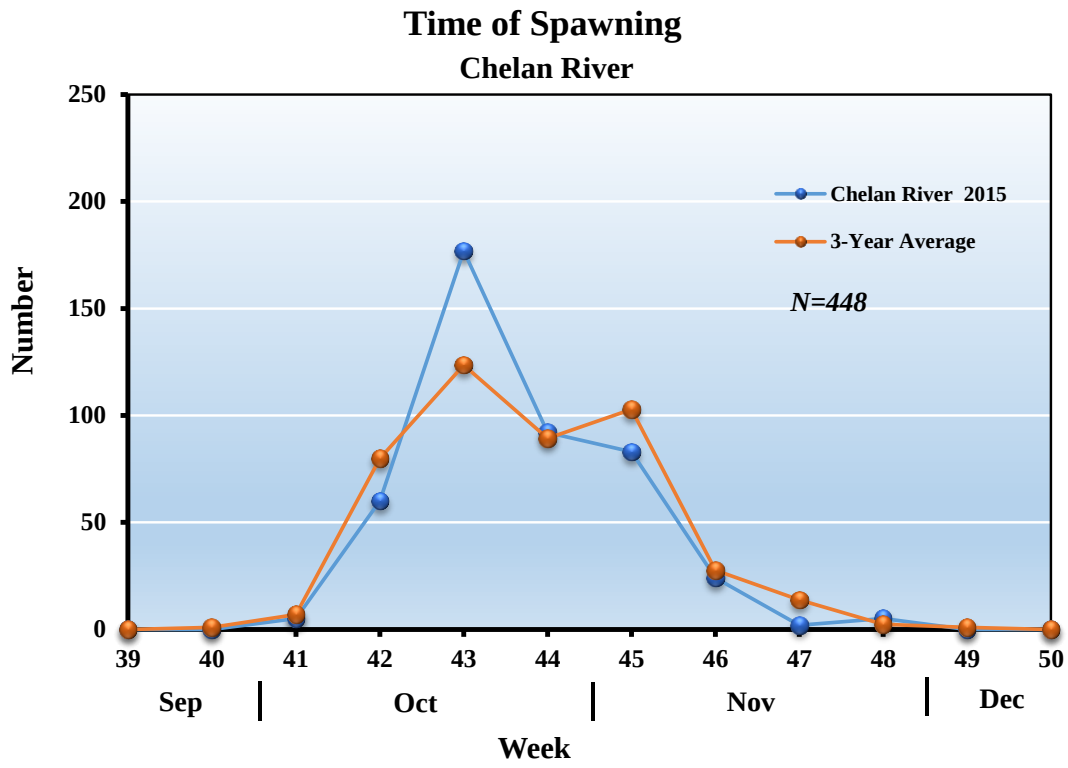

Figure 3. Number of new redds counted each week from late September to mid-November. The figure displays the beginning, peak and end of spawning for summer Chinook in the Chelan River in 2015 compared to a 3-year average (2012-2014).

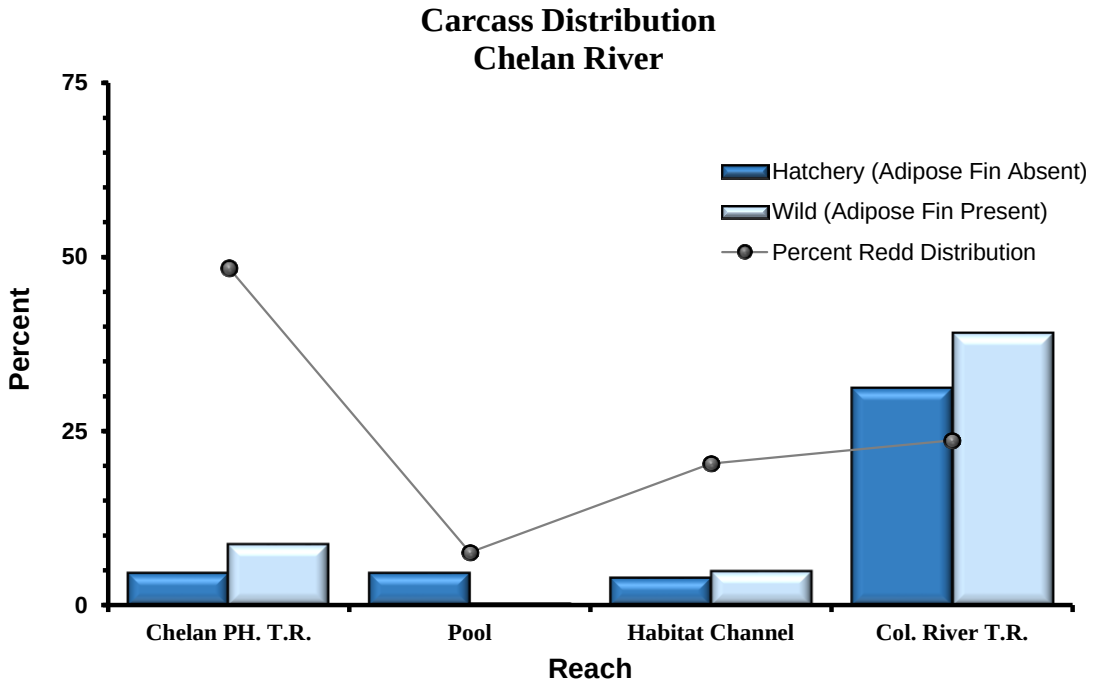

Figure 4. Percent distribution of ad-clipped hatchery and naturally produced fish plotted against the percent distribution of redds observed in reaches on the Chelan River, 2015.

Table 1. Number of summer Chinook redds observed each week within the Methow and Chelan rivers, 2015. Dashes indicate that no survey occurred.

Reach	Location (Rkm)	Sep	Oct					Nov				Dec	Total	Percent
		20-26	27-3	4-10	11-17	18-24	25-31	1-7	8-14	15-21	22-28	29-5		
		39	40	41	42	43	44	45	46	47	48	49		
Methow River														
M1	0.0-23.8	--	0	85	108	37	85	21	13	1	--	--	350	28
M2	23.8-43.8	--	30	120	124	23	8	4	--	--	--	--	309	25
M3	43.8-63.7	--	42	150	104	11	0	0	--	--	--	--	307	25
M4	63.7-72.3	--	9	41	20	2	0	0	--	--	--	--	72	6
M5	72.3-80.1	--	31	74	32	9	0	--	--	--	--	--	146	12
M6	80.1-83.0	--	11	0	2	0	0	--	--	--	--	--	13	1
M7	83.0-96.1	--	21	10	3	0	--	--	--	--	--	--	34	3
Total:		**--**	**144**	**480**	**393**	**82**	**93**	**25**	**13**	**1**	**--**	**--**	**1,231**	**100**
Chelan River														
Powerhouse Tailrace		--	0	2	21	98	48	25	18	1	4	0	217	48
Columbia R. Tailrace		--	0	1	7	25	32	37	3	1	0	0	106	24
Pool		--	0	0	13	15	3	3	0	0	0	0	34	8
Habitat Channel		--	0	2	19	39	9	18	3	0	1	0	91	20
Total:		**--**	**0**	**5**	**60**	**177**	**92**	**83**	**24**	**2**	**5**	**0**	**448**	**100**

Table 2. Number and percent of hatchery (ad-clipped) and naturally produced (not ad-clipped) summer Chinook collected in Methow and Chelan rivers, 2015. The origin of three fish sampled could not be determined in the Methow River.

Reach	Location (Rkm)	Ad-Clipped Hatchery				Naturally Produced				Reach Total
		Male	Female	Total	Percent	Male	Female	Total	Percent	
Methow River										
M1	0.0-23.8	29	31	60	26	71	97	168	74	**229**[1]
M2	23.8-43.8	43	12	55	28	78	61	139	72	**194**
M3	43.8-63.7	22	16	38	17	98	85	183	83	**221**
M4	63.7-72.3	4	0	4	7	20	32	52	93	**56**
M5	72.3-80.1	3	2	5	5	38	52	90	95	**95**
M6	80.1-83.0	0	0	0	0	9	10	19	100	**19**
M7	83.0-96.1	0	0	0	0	11	14	25	100	**25**
Total		**101**	**61**	**162**	**19**	**325**	**351**	**676**	**81**	**839**
Chelan River										
Powerhouse Tailrace		2	15	17	35	3	29	32	65	**49**
Columbia R. Tailrace		24	89	113	44	40	102	142	56	**255**
Pool		5	12	17	94	1	0	1	6	**18**
Habitat Channel		3	20	23	56	4	14	18	44	**41**
Total		**34**	**136**	**170**	**47**	**48**	**145**	**193**	**53**	**363**

[1] **Origin of one female carcass in Reach 1 could not assigned.**

Table 3. Historical aerial and ground redd counts of summer Chinook in the Methow, Chelan, Okanogan, and Similkameen rivers, 1956-2015.

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

[1.] The redd count here is for the entire Okanogan Basin (Similkameen + Okanogan rivers).