YKFP WDFW M&E Report

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1. Executive Summary

a. Fish Population RM&E

The Yakima/Klickitat Fisheries Project's (YKFP) monitoring and evaluation (M&E) project was established to evaluate critical uncertainties associated with spring Chinook salmon supplementation in the Yakima Basin. The YKFP is co-managed by the Yakama Nation (lead entity) and the Washington Department of Fish and Wildlife with guidance from the Northwest Power Planning Council and is funded predominantly by the Bonneville Power Administration. The M&E project historically, and is currently collecting information under several disciplines associated with the supplementation of spring Chinook, including ecological interactions and ecological risk containment monitoring, domestication monitoring, genetic monitoring, competition/capacity/habitat saturation monitoring, natural production monitoring, and monitoring the relative reproductive success of fish in the program, consistent with the Columbia Basin's Fish and Wildlife Program. Results from the project have been presented in public and professional forums, and are intended to inform others throughout the region on the information learned under the project. The current investigations included in this report provide summarized results of ongoing studies and should be considered preliminary until published in the peer reviewed literature.

Status and trends in abundance, productivity, distribution, and diversity of spring Chinook, and non-target taxa were collected during this contract period. Preliminary results of ongoing studies suggests operating the YKFP's production program has provided a demographic benefit to the population, has not impacted valued fish taxa beyond acceptable levels and the risk containment monitoring program is working as planned, some small levels of domestication in the context of predation vulnerability and competitive dominance have occurred although the effects are likely small, genetic stock partitioning of mixed stock smolt migrants remains a viable method to estimate population specific juvenile chinook smolt abundance and productivity, rearing habitat saturation has likely been met in several years under current conditions, and very low levels of naturally produced precociously maturing chinook have been observed on the spawning grounds.

b. Hatchery RM&E

The YKFP M&E project was established to monitor the progress of the Cle Elum Supplementation and Research Facility (CESRF) at meeting spring Chinook production and biological objectives established for the YKFP's production program. The objectives were explicitly stated in the YKFP's monitoring and evaluation plan (Busack et al. 1997) and more recently, as Quantitative Objectives for the project. The project Quantitative Objectives provide benchmark values against which the performance of the project can be monitored and evaluated relative to fixed standards. Quantitative objectives have been established for the Spring Chinook supplementation program and include objectives for natural production, harvest, genetics, ecology, habitat, and science. While all of these objectives evaluate the performance of the Yakima/Klickitat fisheries project at some level, we focused on the hatchery RM&E strategy for natural production and uncertainties research in this report. Monitoring the demographic benefit of the supplementation program has been thoroughly described in the Yakama Nation annual reports. This report extends the findings to cover uncertainty research of factors that may limit supplementation success and the projects performance relative to the natural production, ecological, and genetic quantitative objectives. It should be noted that the program strategies of hatchery RM&E and fish population RM&E are highly intertwined under this project and are not necessarily independent from one another. The hatchery RM&E evaluations included in this report provide summarized results of ongoing studies and should be considered preliminary until published in the peer reviewed literature.

2. Introduction

For each Fish and Wildlife Program Strategy briefly discuss how your project informs/supports the program sub strategies and associated management questions. The content may be reorganized into any format of your choosing.

The Yakima-Klickitat Fisheries Project is a cooperatively managed project with the Yakama Nation (YN; lead entity) and the Washington Department and Fish and Wildlife (WDFW) with support in large part by the Bonneville Power Administration (BPA) with the oversight and guidance from the Northwest Power and Conservation Council (NPCC; Sampson et al. 2013). The Yakima Klickitat Fisheries Project's (YKFP) Monitoring and Evaluation (M&E) program has been described as the "Omnibus" scientific component of the broader YKFP (ISRP review 20060831). The M&E project provides a rigorous assessment of the assumptions of supplementation and the application in the Yakima Basin to increase the natural production of salmon throughout the basin. The YKFP is an adaptively managed supplementation program designed "to test the assumption that new artificial production can be used to increase harvest and natural production while maintaining the long-term genetic fitness of the fish population being supplemented and keeping adverse genetic and ecological interactions with non-target species or stocks within acceptable limits" (BPA 1996). The M&E project was designed to evaluate the YKFP progress towards addressing these four questions:

1) Can integrated hatchery programs be used to increase long-term natural production?
2) Can integrated hatchery programs limit genetic impacts to non-target Chinook populations?
3) Can integrated hatchery programs limit ecological impacts to non-target populations?
4) Does supplementation increase harvest opportunities?

This contract supports ongoing M&E activities and research conducted by the Washington Department of Fish and Wildlife under the YKFP. The WDFW previously produced a minimum of 4 technical reports annually as deliverables under this contract (Competition/Capacity monitoring; Ecological Risk Containment monitoring; Genetics; Domestication Research and Monitoring) but with the new streamlined BPA reporting guidance and requirements, the reporting structure (and timelines) have been reduced to the summarized information herein, although in some cases detailed reporting for various topics are included in Appendix C for completeness. The work and reporting under each of the topics of ecological interactions, domestication selection, competition/capacity, and genetic investigations are in varying stages of development and should be considered in preliminary until published in the peer reviewed literature. Finally, this project has produced numerous publications that provide detailed evaluations under each topical research area (Appendix B).

a. Fish Population RM&E

F&W Program Strategy: Assess the status and trend of adult natural and hatchery origin abundance of fish populations for various life stages.

F&W Program Management Question: What are the status and trend of adult abundance of natural and hatchery origin fish populations?

Adult status and trend data for spring Chinook salmon are collected and presented annually under the Yakama Nation contracts and reports associated with the YKFP (Project 1995-063-25; Sampson et al. 2013).

Adult status and trend population data are also collected for non-target taxa (NTT) under the ecological risk containment monitoring program under this WDFW contract. The status and trends of 15 non-target taxa of concern (NTTOC) are collected annually to ensure the operation of the YKFP's production scale salmon supplementation program does not adversely affect the status of these taxa. Benchmark values were established during the pre-supplementation period and changes in the population status for these NTTOC are judged relative fixed standards termed containment objectives. Acceptable levels of change were established as containment objectives for each NTTOC under the project (Pearsons et al. 1998) and change in the population status are monitored within the risk containment and adaptive management framework (Temple and Pearsons 2012).

F&W Program Strategy: Assess the status and trend of juvenile abundance and productivity of natural origin fish populations.

F&W Program Management Question: What are the status and trend of juvenile abundance and productivity of fish populations?

Non-target taxa of concern monitoring

Juvenile status and trend data for the target taxon (spring Chinook) are collected and presented annually under the Yakama Nation contracts and reports associated with the YKFP (Project 1995-063-25; Sampson et al. 2013). Juvenile status and trend population data are also collected for non-target taxa under the ecological risk containment monitoring program under this WDFW contract. The status and trends of 15 non-target taxa of concern (NTTOC) are collected annually to ensure the operation of the YKFP's production scale salmon supplementation program does not adversely affect the status of these taxa. Benchmark values were established during the pre-supplementation period and changes in the population status for these NTTOC are judged relative to the benchmark levels. Acceptable levels of change were established as containment objectives for each NTTOC under the project (Pearsons et al. 1998) following a formal risk assessment process (Pearsons and Hopley 1999) and change in the population status are monitored within the risk containment and adaptive management framework (Appendix C; Temple and Pearsons 2012).

Spring Chinook habitat saturation and limiting factors

The status and trend of juvenile spring Chinook salmon abundance and productivity are collected annually for rearing spring Chinook (target-taxa) under the spring Chinook competition/carrying capacity program under the YKFP's, M&E contract. The carrying capacity of a watershed is an important factor in determining whether supplementation is a viable technique of increasing natural production. In the Yakima River Basin, carrying capacity can limit the number of naturally produced spring Chinook salmon even when supplementation mechanics are operating perfectly (Busack et al. 1997). Preliminary analysis suggests that density dependent mechanisms affecting spring Chinook survival exist in the upper Yakima River after fall spawning and prior to or during the parr stage the following fall (Johnson et al. 2009). If the Yakima River is at capacity for rearing sub-yearlings in some years, then supplementation efforts can only serve to increase the number of naturally produced smolts when natural production is below that capacity. Therefore, identifying the factors that limit natural production is critical if restoration efforts aimed at maintaining or increasing natural production are to achieve their intended biological goals. The spring Chinook habitat saturation and limiting factors work aims to identify juvenile life-stage survival bottlenecks that may limit supplementation success in some years.

Spring Chinook residual/precocious male monitoring

Artificial propagation of Chinook salmon has the potential to alter the age that fish mature and result in undesirable interactions with natural origin fish (Knudsen et al. 2006). This is a particular concern for conservation hatcheries where the goal is to increase natural production while maintaining the characteristics of the natural population (Mobrand et al. 2005). Although most Chinook salmon are anadromous (Healey 1983), some salmon complete their entire life cycle in freshwater, even when they have access to the ocean. These salmon are generally small, male, precociously mature, short-lived and are referred to as residents, precocious males, or minijacks (Gebhards 1960; Mullan et al. 1992; Zimmerman et al. 2003). The occurrence of precocity in salmon has been credited to genetic factors and environmental and physiological cues (Thorpe 1987; Bohlin et al. 1990; Foote et al. 1991). Age-at-maturation has been shown to be heritable in salmon (Heath et al. 1994; Unwin et al. 1999); and although it has been known for some time that hatcheries can produce large numbers of precocious Chinook salmon (Robertson 1957; Mullan et al. 1992; Larsen et al. 2004; Beckman and Larsen 2005), there have been relatively few studies that have investigated the abundance and distribution of these fish in rivers during the spawning season. Previous research indicated that the Yakima Supplementation and Research Facility had produced and released an average of 129,249 precocious males/year into the upper Yakima basin between 1999 and 2008 (Larsen et al. 2004; Larsen et al. 2008; Yakima/Klickitat Fisheries Project, Unpublished data). Our primary objectives are to 1) estimate the annual abundance of hatchery origin precocious males on the spawning grounds, and 2) quantify the annual distribution of hatchery precocious males on and away from the spawning grounds. We also present information about the abundance and distribution of natural origin precocious males so that we can determine how hatchery precocious males might differ.

Spring Chinook reproductive success/spawning channel

Although hatcheries have been extensively utilized in Chinook salmon management for over 100 years, only recently have rigorous experiments been developed to measure the relative reproductive success of hatchery- and natural-origin spawners in a shared natural setting. Some of the difficulty in designing informative studies has stemmed from the challenges of controlling entry to natural spawning areas and collecting representative samples of recently hatched fry. Furthermore, if control could be established over the potential spawners in the spawning area, the measurement of individual reproductive output still would require a means of associating individual fish captured in one year with individuals that spawned in a previous year. The spawning behavior of Chinook salmon adds to the complexity of quantifying individual reproductive output through behavioral observations: at a redd site, a female might be courted by several males that compete for access to the female, providing opportunities for multiple paternity in a single redd. In areas with moderate to high spawning densities, males might attend females on several adjacent redds. Microsatellites, a class of highly polymorphic, codominant DNA markers, provide a means to quantify individual spawners' reproductive output. A suite of 10 to 15 highly variable microsatellites can resolve individual identity in a moderate to large population, and through a simple inheritance model, can illuminate parent-offspring relationships.

Washington Department of Fish and Wildlife (WDFW) and the Yakama Nation (YN) are cooperating on a study of Chinook salmon reproductive success in a presumably closed access spawning observation channel at the Cle Elum Hatchery. Viewing blinds line the channel, allowing researchers to observe spawning activities.

Chinook salmon carrying visible external marks were released into the spawning channel in September 2011. Hatchery-control line (three generations of hatchery influence) males and females were released into three of six shared spawning areas and supplementation hatchery line (one generation of hatchery influence) males and females were released into the other three shared spawning areas to select and compete for mates. Prior to the release of the potential spawners, researchers collected and preserved samples of fin tissue to enable genetic characterization of the potential spawners and to allow subsequent inference of parent/offspring relationships after juveniles were collected and genotyped. One group of researchers examined morphological characteristics of these potential parents and observed and recorded spawning area behaviors and interactions. The results of the morphological and behavioral work are described in a separate report.

The potential parents' fin tissue samples and the collected progeny (fry) were delivered to the WDFW Molecular Genetics Laboratory in Olympia, Washington for genetic screening and parentage analysis following the same protocols that have been used from 2002 – 2007, 2009 – 2012 (Young and Kassler 2005, Kassler 2005, Kassler 2006, Kassler and Von Bargen 2007, 2008, and 2010, Kassler et al. 2011; Kassler and Peterson 2012). The genetic analyses provide direct, quantitative estimates of fry production by individual spawning Chinook salmon. In this report, we present the parentage results for the 2011 – 2012 Cle Elum spawning channel experiments.

Spring Chinook Genetic stock separation-juveniles

Production and survival of the Yakima River basin spring Chinook stocks (American River, Naches River, and upper Yakima River) are monitored, as part of the Yakima/Klickitat Fishery Project supplementation evaluation program. However, in the lower Yakima River, where the best facilities to collect samples exist, the three spring Chinook stocks are mixed with one another and with the Marion Drain and Yakima River fall Chinook stocks, during downstream juvenile migration. Thus, methodologies for discriminating stocks in an admixture are vital for development of stock-specific estimates. Domestication monitoring plans require discrimination of the three spring Chinook salmon stocks in the basin, and a complete analysis of migration timing and stock abundance for all Chinook requires discrimination of the two fall stocks as well. Accurate assignments of Chinook smolts captured at the Chandler fish passage facility to population-of-origin will allow researchers and managers to estimate production by the three spring Chinook stocks, assess smolt-to-smolt survival of the three spring Chinook stocks, and could be utilized to evaluate stock-specific environmental condition factors.

F&W Program Strategy: Assess the status and trend of spatial distribution of fish populations.

F&W Program Management Question: What are the status and trend of spatial distribution of fish populations?

The spatial distribution of adult Spring Chinook salmon (target taxa) are best described in the Yakama Nation's annual reports (Sampson et al. 2013) where the spawning distribution for spring Chinook is intensively monitored and reported annually.

The spatial distribution of rearing naturally produced spring Chinook in the upper Yakima basin is monitored under the ecological risk containment monitoring program and is of interest in the context of distributional overlap with non-target taxa of concern. Lack of spatial overlap between the target taxa and non-target taxa are thought to preclude negative effects of species interactions. The distribution of spring Chinook is monitored annually in tributary and mainstem Yakima River index monitoring sites. The ecological effects of distributional overlap with NTTOC are currently monitored in the risk containment monitoring framework (Temple and Pearsons 2012).

Spatial distribution of early rearing spring Chinook in the upper Yakima basin is monitored under the spring Chinook Competition/Capacity program. Previous work in the upper Yakima River (Johnson et al. 2009) has suggested density dependent constraints to spring Chinook production prior to fall estimates of abundance. One primary objective of this program is to identify life-stage specific factors limiting to survival and development in the natural environment. Such data can then be used to educate management decisions in selecting actions to most effectively increase natural production.

F&W Program Strategy: Assess the status and trend of diversity of natural and hatchery origin fish populations.

F&W Program Management Question: What are the status and trend of diversity of natural and hatchery origin fish populations?

Operating a production scale supplementation program have unintended effects that alter the diversity of both natural and hatchery origin fish populations through selective forces imposed by the hatchery environment. The domesticating effects of hatchery culture are being intensively monitored for spring Chinook in the Yakima under the Domestication monitoring program. The YKFP's domestication monitoring plan was developed to determine if the spring Chinook supplementation program affects a large number of phenotypic and morphometric traits of the Yakima population (Busack et al. 2006).

Domestication Predation and competitive dominance description

Raising fish in hatcheries can cause unintended behavioral, physiological, or morphological changes in Chinook salmon due to domestication selection. Domestication selection is defined by Busack and Currens (1995) as: "changes in quantity, variety, or combination of alleles within a captive population or between a captive population and its source population in the wild as a result of selection in an artificial environment." Selection in artificial environments could be due to intentional or artificial selection, biased sampling during some stage of culture, or unintentional selection (Busack and Currens 1995). Genetic changes can result in lowered survival in the natural environment (Reisenbichler and Rubin 1999). The goal of supplementation or conservation hatcheries is to produce fish that will integrate into natural populations and increase the number of grandchildren relative to fish that live entirely in natural environments. Conservation hatcheries attempt to minimize intentional or biased sampling so that the hatchery fish are similar to naturally produced fish. However, the selective pressures in hatcheries are dramatically different than in the wild, which can result in genetic differences between hatchery and wild fish. The selective pressures may be particularly prominent during the freshwater rearing stage where most mortality of wild fish occurs. We are attempting to evaluate the effects of domestication on the vulnerability of spring Chinook to Predators, and on competitive dominance of spring Chinook salmon.

b. Hatchery RM&E

F&W Program Strategy: Evaluate the effectiveness of hatchery safety-net/conservation programs and the effectiveness of hatchery reform actions on the achievement of biological performance objectives.

F&W Program Management Question: Are hatchery improvement programs and actions achieving the expected biological performance objectives?

The YKFP has a long history built upon a strong foundation of hatchery RM&E. The larger YKFP was built upon developing responsible hatchery operations and production protocols consistent with many of the general (and specific) hatchery reform actions and recommendations that have recently been advised by the Hatchery Scientific Review Group for many hatchery programs throughout the Columbia basin and much of the Pacific Northwest. Much of the hatchery effectiveness monitoring information is presented in the Yakama Nation's annual technical report of the YKFP (Sampson et al. 2013). The YKFP established a long list of performance measures, termed quantitative objectives, and the project's performance relative to these standards are monitored and reported annually (Fritts 2012; Appendix D).

F&W Program Strategy: Assess and investigate as appropriate critical uncertainties regarding the effects of artificial propagation on the viability of wild fish populations.

F&W Program Management Question: What deleterious effects does artificial production have on natural populations of anadromous fish?

This M&E project was founded upon monitoring and evaluating the effects of artificial production on natural populations and anadromous fish. The monitoring tasks described throughout this report covering the disciplines of domestication, genetics, ecological investigations, and competition/capacity work all strive towards answering critical uncertainties associated with artificial production in the Yakima Basin. Results from this work are intended to inform others throughout the Columbia River Region.

F&W Program Strategy: Monitor and evaluate annual PNI, NOS, pHOS values, marking rates, production information and describe the relevance to HSRG recommendations or HGMPs.

F&W Program Management Question: To what extent are hatchery programs meeting mitigation production requirements and operational objectives in Master Plan, HGMP, and Annual Operating Plan?

The Yakama Nation annual technical reports for the YKFP (e.g., Sampson et al. 2013) provide robust assessments of the projects monitoring and evaluation of PNI, NOS, pHOS, marking rates, and production information relative to operational guidelines (e.g., HGMP's, Master Plans, and M&E plan).

Location details: For each F&W Program Strategy above, insert maps, aerial photos, or pictures of where your work was conducted. Below are links to existing project or contract map options created in cbfish.org or insert your own.

Project Map:

http://www.cbfish.org/Project.mvc/Map/1995-063-25

Contract Map(s):

http://www.cbfish.org/Contract.mvc/Map/53279

http://www.cbfish.org/Contract.mvc/Map/61480

3. Methods: Protocols, Study Designs, and Study Area

Protocol Title: Ecological Interactions (1995-063-25) v1.0

Protocol Link: http://www.monitoringmethods.org/Protocol/Details/113

Protocol Title: Genetics (1995-063-25) v1.0

Protocol Link: http://www.monitoringmethods.org/Protocol/Details/115

Protocol Title: Natural Production (1995-063-25) v1.0

Protocol Link: http://www.monitoringmethods.org/Protocol/Details/116

4. Results

a. Fish Population RM&E

Non-target taxa of concern monitoring

General approach

Comparisons of environmental variables measured in main stem and tributary areas before and during supplementation indicated that there were generally no significant differences in the variables evaluated (Table 1). We did observe a significant increase ($P = 0.01$) in the minimum daily stream discharge in the main stem Yakima River during the supplementation period. We hypothesize that increased minimum daily stream discharge would benefit NTT and would not confound our evaluation because the increase was not significantly correlated with our monitoring variables (e.g., age 1 rainbow trout size, $P = 0.24$; cutthroat trout size, $P = 0.63$).

Table 1. Environmental variables and results from two-sample student's t-tests of before versus during supplementation periods in the Yakima Basin. Main stem variables were based on water year (October through September of the following year as recorded at three gauging stations) and tributary mean, minimum (Min.), and maximum (Max.) discharge and mean air temperature (based on August through July of the following year) recorded at a single tributary gauging station or weather station. Main stem standard deviation of stream width was recorded at the time of sampling. Tributary mean summer discharge, mean wetted width, mean thalweg depth, and standard deviation of thalweg depth were recorded at the time of sampling. Degrees of freedom (df) were 22 for all comparisons unless otherwise noted.

Environmental Variable	Before Mean	During Mean	*t*	P
Main stem				
Mean Daily Discharge (m^3/s)	61.20	56.41	0.78	0.45
Min. Daily Discharge (m^3/s)	13.42	17.21	-2.72	0.01
Max. Daily Discharge (m^3/s)	239.39	207.16	0.65	0.52
Mean Daily Water Temperature (°C)	8.69	8.86	-0.71	0.48
Min. Daily Water Temperature (°C)	0.30	0.67	-1.06	0.30
Max. Daily Water Temperature (°C)	17.81	18.36	-1.23	0.23
Standard Deviation of Stream Width (m)[a]	12.28	12.81	-0.66	0.52
Tributary				
Mean Daily Discharge (m^3/s)	10.13	9.50	0.41	0.68
Min. Daily Discharge (m^3/s)	0.29	0.38	-1.80	0.08
Max. Daily Discharge (m^3/s)	100.62	80.77	0.91	0.37
Mean Summer Discharge (m^3/s)[a]	0.22	0.30	-1.03	0.32
Mean Daily Air Temperature (°C)	3.93	4.20	-0.94	0.36
Mean Wetted Width (m)[b]	6.18	6.99	-1.72	0.10
Mean Thalweg Depth (m)[b]	0.30	0.32	-1.25	0.23
Standard Deviation of Thalweg Depth (m)[b]	0.15	0.13	1.38	0.19

[a] Degrees of freedom (df) was 19 for these tests.

[b] Degrees of freedom (df) was 18 for these tests.

Field Methods

Results from statistical tests indicated that using the mixture analysis to determine the parameter estimates from the length frequencies of main stem age 1 rainbow trout were valid. Chi squared tests of known versus predicted proportions of main stem rainbow trout in the population were not significantly different in the LCYN ($X^2 = 0.14$; 4

df; $P > 0.99$), UCYN ($X^2 = 0.03$; 2 df; $P > 0.99$), EBURG ($X^2 = 0.10$; 4 df; $P > 0.98$), THORP ($X^2 = 0.12$; 4 df; $P > 0.99$), or CELUM index sites ($X^2 = 0.10$; 4 df; $P > 0.99$) during 2003. In addition, student's t-tests of the known versus model predicted mean length of age 1 main stem rainbow trout were not significantly different in the LCYN ($t = -2.96$; 404 df; $P>0.99$), UCYN ($t = -0.85$; 270 df; $P > 0.60$), EBURG ($t = -0.44$; 262 df; $P > 0.34$), THORP ($t = -0.89$; 174 df; $P > 0.63$), or CELUM ($t = -0.16$; 200 df; $P > 0.13$) index sites.

Residualization of Hatchery Smolts

Many spring Chinook salmon did not migrate to the ocean after release (residuals) and may have interacted with trout. In contrast, very few coho salmon residuals were observed (Table 2). Residuals were most concentrated below acclimation sites during the spring and summer, but were found in all reaches of the main stem that we sampled. Residuals were observed approximately 1-2 km upstream of the acclimation site in the North Fork Teanaway during most years and also migrated upstream into the Cle Elum River. Many residuals were observed in the main stem as late as September and October.

Table 2. Estimated annual abundance (fish/km) of spring Chinook and coho salmon residuals in the main stem Yakima River sampling sections. The LCYN section is the Lower Canyon, UCYN is the Upper Canyon, EBURG is Ellensburg, THORP is Thorp, and CELUM is Cle Elum section.

Year	LCYN	UCYN	EBURG	THORP	CELUM	TOTAL
			Spring Chinook Residuals			
1999	12	5	3	5	0	25
2000	12	5	1	25	3	46
2001	196	71	85	71	0	423
2002	6	0	7	2	0	15
2003	23	8	3	5	0	39
2004	80	21	29	9	0	139
2005	34	29	3	0	0	66
2006	1	1	0	0	0	2
2007	5	9	6	8	0	28
2008	8	10	4	9	0	31
2009	102	43	22	23	0	190
2010	25	19	5	2	0	51
2011	8	10	4	9	0	30
2012	25	34	17	35	0	110
2013	29	36	22	64	0	150
			Coho Salmon Residuals			
1999	7	0	0	0	4	11
2000	0	0	0	0	0	0
2001	3	0	0	0	0	3
2002	0	0	0	0	0	0
2003	0	0	0	0	0	0
2004	0	0	11	0	7	18
2005	0	0	25	0	0	25
2006	0	0	5	0	0	5
2007	0	0	20	0	0	20
2008	0	0	0	0	0	0
2009	0	0	0	0	0	0
2010	0	0	0	0	0	0
2011	0	0	0	0	0	0
2012	0	0	0	0	0	0
2013	0	0	0	0	0	0

Natural Production

We did not observe coho salmon in our tributary index monitoring sites before the supplementation period (0 coho/33 sites). During the supplementation period (1999-2012) we observed coho in 8 of our index sites (between 25 and 36 sites sampled annually) averaging 29 ± 23 per km (mean ± 95%). This increase was statistically significant (t = -2.14; 22 df; *P* = 0.04). Our coho abundance index calculated for the main stem Yakima River averaged 0.10 + 0.25 (mean + 95%) coho per river km before supplementation releases. During supplementation, we estimated 2.5 + 1.4 coho per km (mean + 95%). The increase in main stem coho salmon abundance was nearly statistically significant (t = -2.07, 19 df, *P* = 0.05). Since 2009, snorkelers have observed increasing numbers of coho in mixed pods with Chinook salmon in the Yakima River upstream from our monitoring sites relative to previous years (WDFW unpublished data).

Before supplementation, our abundance index was 23 + 17 (mean + 95%) spring Chinook salmon per km in our tributary monitoring sites and during the supplementation period it was 62 + 31 per km (mean + 95%). The observed increase during this period was not statistically significant (t=-1.95; 22 df; *P* = 0.06) with the addition of 2013 data due to the increased variation associated with the large abundance in 2013 relative to previous years. In the main stem Yakima River, our abundance index for spring Chinook salmon indicated there were approximately 1,987 + 843 fish per km during the baseline period while there were 2,403 + 769 fish per km during the supplementation period. The observed difference was not significant (t=-0.68; 19 df; *P* = 0.50).

Overlap

The degree of trout overlap with salmon was highest in main stem areas, intermediate for cutthroat and rainbow trout in tributaries, and absent for bull trout (Figure 1). There was no overlap of salmon and bull trout in our index sites. In fact, the shortest distance between the uppermost distribution of Chinook salmon and the lowermost distribution of bull trout was approximately 8 km. Cutthroat trout and supplemented spring Chinook overlapped in distribution in both tributary and main stem Yakima River areas. The distributional overlap in tributary streams was approximately 11%, confined to relatively moderate elevations, and was less than the 40% containment objective (Figure 1). Salmon overlapped 100% of the main stem distribution of cutthroat trout (Figure 1). In tributaries, salmon overlapped 50% of the distribution of rainbow trout. Overlap was predominately confined to lower portions of tributaries (e.g., Swauk Creek 1 and Umtanum Creek 1) and farther upstream in the North Fork Teanaway River. However, salmon did not overlap rainbow trout in high elevation portions of tributaries.

There was also extensive overlap between rainbow trout, sucker species, and mountain whitefish and salmon in the main stem (100%; Figure 1). Salmon overlapped in distribution with longnose dace (58%) and speckled dace (72%) in tributaries, although mean overlap was less than the containment objectives for both species. Salmon overlapped sculpin species 19% in tributaries but this was less than the containment objective. Finally, there was 23% overlap in distribution between sucker species and salmon in tributary streams, although this was also less than the containment objective.

Data that were collected at similar times and sites by snorkeling and electrofishing methods were consistent with each other. For example, in areas that we found salmon, rainbow trout, cutthroat trout or bull trout, they were detected with both electrofishing and snorkeling methods. In addition, we did not capture any salmon when we electrofished areas where bull trout were present.

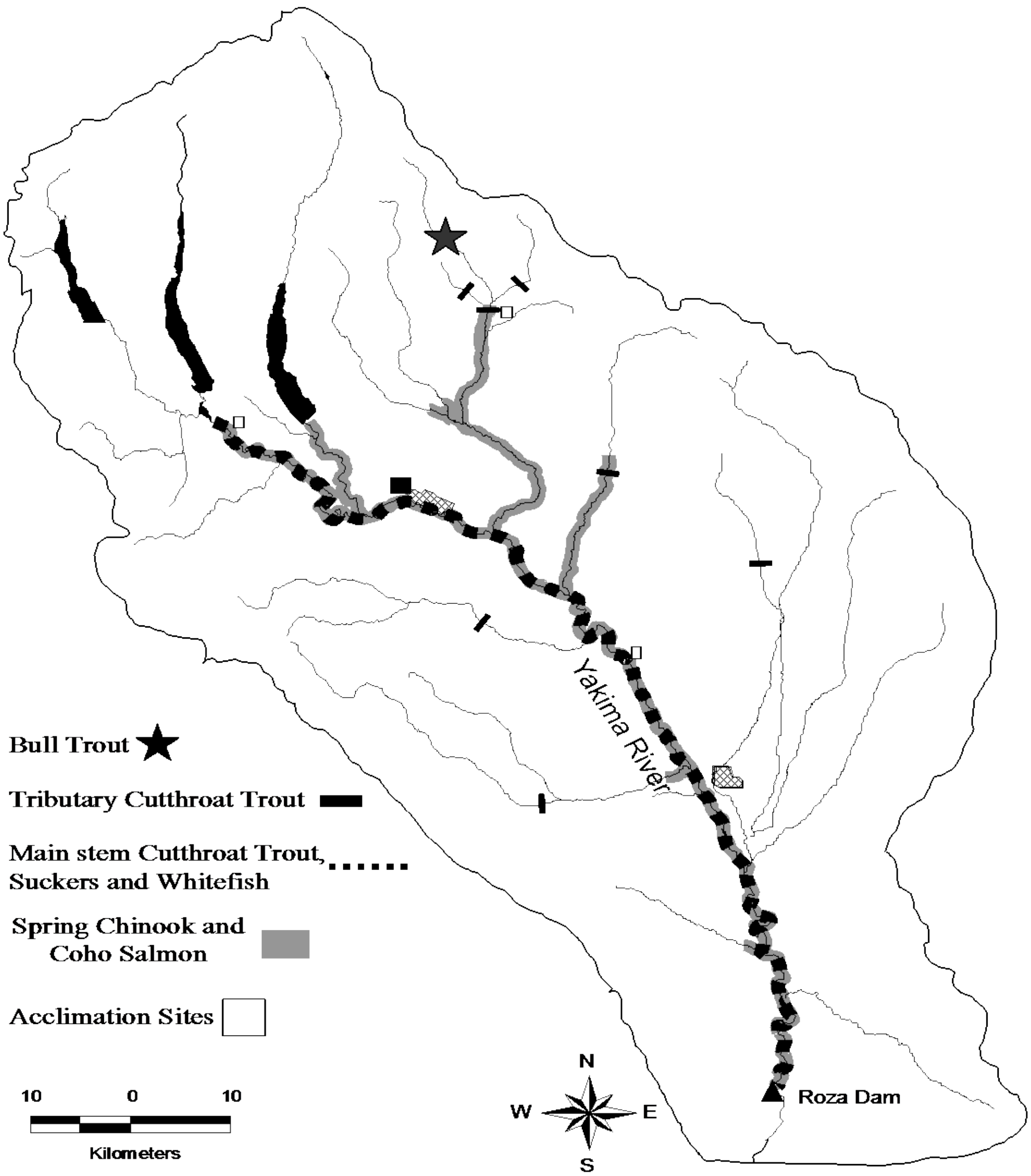

Figure 1. Map of species distributions in the upper Yakima Basin. Spring Chinook and coho salmon distributions are shaded grey. The lowest elevation observations of bull trout and cutthroat trout in tributary streams are marked with stars and bars, respectively. Cutthroat trout, suckers and mountain whitefish distribution in the main stem is marked as a dashed line. The Cle Elum hatchery facility is marked with a black square and hatchery acclimation sites are marked with open squares. Rainbow trout are widely distributed throughout the basin and are not marked on the map.

Before-After Analysis

Rainbow (age 1) and cutthroat trout (<250mm), mountain whitefish, and sucker species in the main stem, and rainbow trout in tributaries (all ages; analog for steelhead) exhibited distributional overlap with salmon that were outside the containment objectives and therefore we compared their abundance, size, and biomass (salmonids) before and after stocking began. The mean abundance and 90% CL of sympatric rainbow trout (all ages) was 34 ± 17% higher in the tributaries and 33 ± 16% higher in the main stem (age 1) in the years when supplementation occurred than during the baseline phase. The mean abundance of cutthroat trout (<250 mm) was 446 ± 361% CL higher in the main stem during supplementation than during the baseline phase (Table 3; Figure 2). The mean abundance of sub-adult mountain whitefish increased 109 ± 43% CL during supplementation period, while the mean abundance of sucker species adults decreased 44 ± 8% CL and the decrease was significant ($P = 0.001$), although it was within our containment objectives (Figure 3). Finally, we observed a 27 ± 24% CL increase in sub-adult sized sucker abundance (analog for mountain sucker) during supplementation and the lower 90% CL did not exceed our containment objective (Figure 3).

During the supplementation period, the mean and 90% CL of rainbow trout size (age 1) in the main stem indicated that size decreased by 5 ± 2% (Table 3; Figure 2). Slopes between log length-log weight of age 1 rainbow trout in the main stem were not significantly different before and during supplementation ($P = 0.79$). An ANCOVA revealed the average weight of fish for a given length was significantly greater during the supplementation period ($P = 0.004$, Figure 4). In addition, biomass increased by 13 ± 17% CL. Similarly, the mean and 90% CL of cutthroat trout size (<250 mm) in the main stem indicated a 0 ± 3% CL decrease, and an increase in biomass of 713 ± 858% CL (Table 3; Figure 2). The size of rainbow trout in the tributaries (all ages) was similar during both periods (1 ± 2% CL; Table 4; Figure 2). Slopes between log length-log weight for rainbow trout in tributaries (all ages) were not significantly different before and during supplementation ($P = 0.30$). An ANCOVA indicated the mean weights at each length were significantly greater during the supplementation period than the before period ($P < 0.001$; Figure 4). Additionally, tributary rainbow trout biomass (all ages) increased by 28 ± 12% CL (Table 4; Figure 2). Our index of mountain whitefish size indicated that the proportions of subadults observed increased 10 ± 2% CL during the supplementation period (Figure 3). Our index of sucker species size indicated that the proportion of adults decreased 41 ± 10% during supplementation, and although the decrease was significant ($P < 0.001$), it was still well within our containment objectives (Figure 3). Our index of mountain sucker size indicated a 28 ± 10% CL increase in the proportion of subadults during the supplementation period (Figure 3).

The mean abundance, size, and biomass of catchable sized main stem rainbow trout (>249 mm) did not decrease during supplementation. The mean abundance of rainbow trout greater than 249 mm increased by 15 ± 14% (mean ± 90% CL), mean size increased by 2 ± 1%, and biomass increased by 42 ± 17% during supplementation when compared to baseline conditions.

The only NTT with parameter estimates outside of the containment objectives was steelhead, which uses rainbow trout as an analog. The lower 90% CL for age 1 rainbow trout size in the main stem and rainbow trout size (all ages) in the tributaries were exceeded, so we tested whether the decrease was caused by supplementation.

Table 3. Annual abundance (fish/km), size (mm, FL), and biomass (kg/km) estimates and associated 95% confidence intervals of age 1 rainbow trout and cutthroat trout less than 250 mm fork length in the main stem Yakima River.

	Abundance		Size		Biomass	
Year	RBT	CUT	RBT	CUT	RBT	CUT
1990			210 ± 33	237 ± 5		
1991	189 ± 67	11 ± 14	205 ± 27	237 ± 11	19 ± 14	1.6 ± 3.2
1992	151 ± 28	1	217 ± 31	242	18 ± 7	0.1
1993	193 ± 48	6 ± 17	232 ± 36	238 ± 3	27 ± 11	0.8 ± 3.5
1994	180 ± 33	2 ± 1	217 ± 32	225 ± 17	21 ± 8	0.3 ± 1.4
1995	190 ± 54	6 ± 17	235 ± 34	239 ± 6	28 ± 12	0.9 ± 3.5
1996	182 ± 27	5 ± 11	217 ± 32	239 ± 10	22 ± 7	0.7 ± 2.4
1997	272 ± 49	10 ± 44	203 ± 35	239 ± 5	27 ± 10	1.4 ± 8.9
1998	130 ± 20	16 ± 84	212 ± 34	230 ± 5	15 ± 6	2.0 ± 16.8
1999	182 ± 25	12 ± 25	217 ± 33	236 ± 5	22 ± 7	1.8 ± 5.1
2000	214 ± 40	13 ± 1	210 ± 36	227 ± 13	24 ± 10	1.8 ± 1.4
2001	384 ± 81	18 ± 85	206 ± 32	238 ± 7	41 ± 16	2.5 ± 17.1
2002	207 ± 39	7 ± 42	203 ± 31	232 ± 6	20 ± 9	0.9 ± 8.4
2003	230 ± 41	10 ± 34	207 ± 30	234 ± 7	24 ± 9	1.3 ± 7.0
2004	275 ± 19	16 ± 34	223 ± 32	234 ± 5	35 ± 15	2.3 ± 6.9
2005	272 ± 20	28 ± 142	213 ± 32	229 ± 5	30 ± 9	3.4 ± 28.6
2006	150 ± 12	16 ± 11	216 ± 34	235 ± 5	17 ± 7	2.1 ± 2.5
2007	233 ± 17	22 ± 35	210 ± 33	233 ± 5	26 ± 8	3.1 ± 7.1
2008	264 ± 26	24 ± 61	204 ± 33	229 ± 7	26 ± 9	3.0 ± 12.3
2009	156 ± 29	44 ± 138	188 ± 29	231 ± 3	12 ± 3	5.8 ± 27.8
2010	233 ± 48	32 ± 111	197 ± 36	230 ± 5	21 ± 7	4.1 + 22.3
2011	273 ± 23	39 ± 63	199 ± 34	227 ± 4	26 ± 9	5.0 ± 12.8
2012	270 ± 30	70 ± 250	192 ± 33	226 ± 5	23 ± 8	8.7 ± 50.3
2013	359 + 38	237 ± 335	196 ± 27	290 ± 9	32 ± 10	75.6 ± 68.0

Table 4. Annual abundance (fish/km), size (mm, FL), and biomass (kg/km) estimates and associated 95% confidence intervals for rainbow trout in Yakima River Basin tributary streams.

Year	Abundance	Size	Biomass
1990	241 ± 129	136 ± 8	8 ± 13
1991	204 ± 102	131 ± 8	6 ± 8
1992	375 ± 240	130 ± 5	11 ± 24
1993	317 ± 158	131 ± 7	9 ± 17
1994	328 ± 129	132 ± 8	11 ± 15
1995	213 ± 118	139 ± 8	7 ± 14
1996	165 ± 109	133 ± 8	5 ± 11
1997	294 ± 119	132 ± 5	8 ± 11
1998	442 ± 174	138 ± 7	15 ± 25
1999	288 ± 175	135 ± 8	12 ± 27
2000	318 ± 135	144 ± 8	11 ± 21
2001	464 ± 178	129 ± 3	12 ± 17
2002	321 ± 131	132 ± 6	10 ± 15
2003	291 ± 142	132 ± 5	8 ± 14
2004	243 ± 135	142 ± 5	9 ± 15
2005	349 ± 163	127 ± 5	9 ± 16
2006	434 ± 171	134 ± 5	13 ± 20
2007	368 ± 153	138 ± 4	12 ± 18
2008	331 ± 166	138 ± 7	11 ± 19
2009	256 ± 123	138 ± 12	9 ± 19
2010	548 ± 243	127 ± 5	15 ± 25
2011	486 ± 215	124 ± 7	12 ± 20
2012	490 ± 163	124 ± 4	13 ± 15
2013	571 ± 232	129 ± 5	16 ± 24

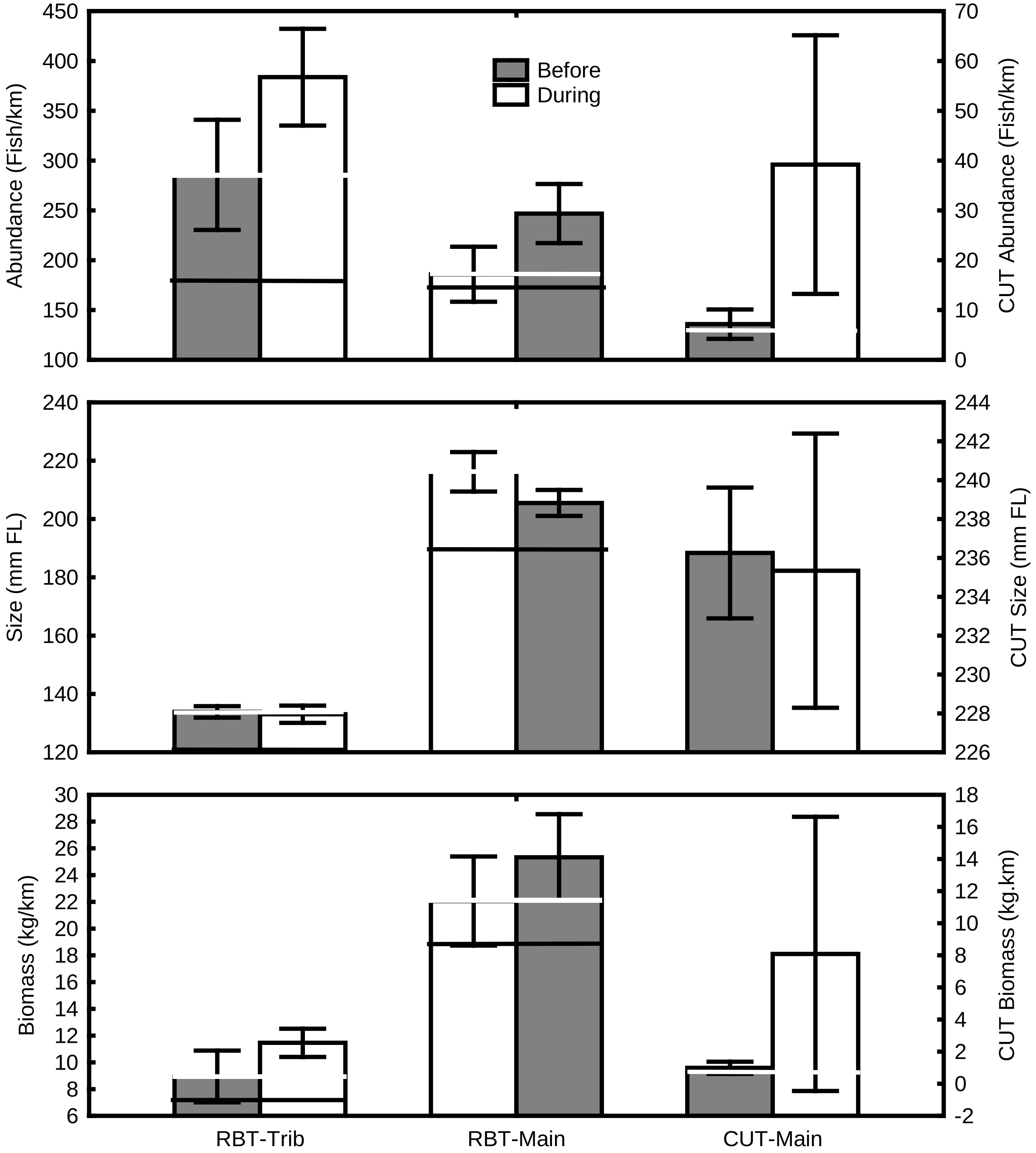

Figure 2. Abundance (n/km), size (FL mm), and biomass (kg/km) of tributary rainbow trout, main stem Yakima River rainbow trout (age 1) and cutthroat trout (<250 mm) before and during supplementation. Main stem cutthroat trout abundance, size, and biomass are associated with the right *y*-axis. The horizontal dashed line represents the 0% containment objective (CO) for steelhead in the main stem and tributaries, and the 10% CO for mainstem cutthroat trout. The solid horizontal line represents the 10% CO for main stem rainbow trout and 40% CO for tributary rainbow trout. Error bars represent 90% confidence intervals.

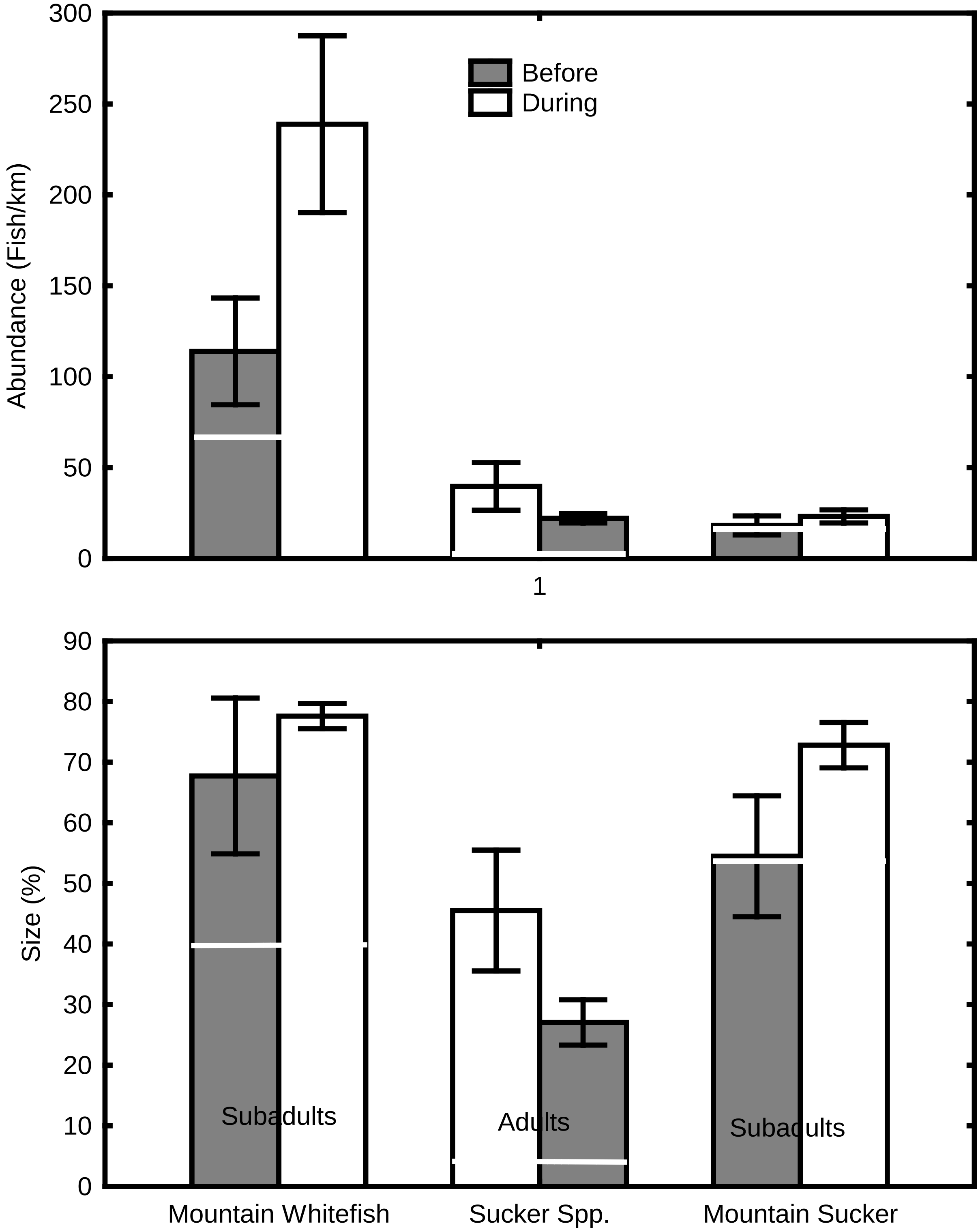

Figure 3. Abundance (fish/km) and size (percent by size class) of mountain whitefish, suckers, and mountain suckers before and during supplementation. Error bars represent the 90% confidence interval. Dashed lines represent the 40% containment objectives for mountain whitefish, 90% for sucker species (Spp), and 5% for mountain suckers.

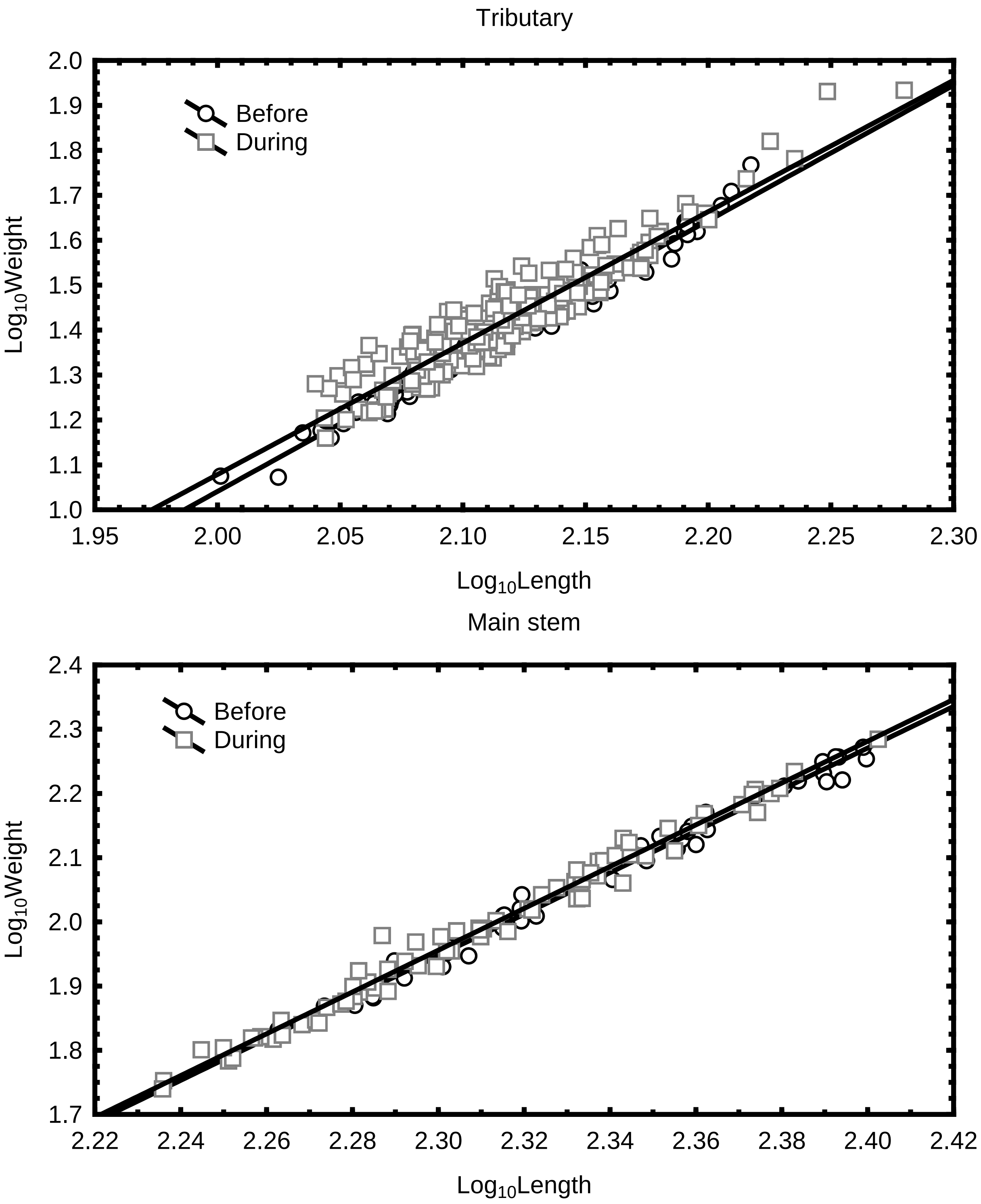

Figure 4. Mean length-weight relationships of tributary and age 1 main stem Yakima River rainbow trout before (1990-1998) and during (1999-2013) the supplementation period. Each data point represents the mean from a sample site.

Causation

Since the lower 90% confidence limit for our steelhead size index was exceeded in both the Yakima River main stem (age 1 rainbow trout) and Yakima Basin tributaries (all ages of rainbow trout), we tested if the changes could be reasonably attributed to supplementation. We did not detect a statistically significant decrease in our steelhead size index (age 1 rainbow trout; BACIP; $P = 0.79$) or our steelhead biomass index (age 1 rainbow trout; BACIP; $P = 0.50$) in the main stem downstream from the Clark Flats acclimation facility. Interestingly, we did not detect a significant relationship between our steelhead abundance and size index relationship ($R^2 = 0.11$; $P = 0.12$) suggesting density dependence was probably not influencing our steelhead size index. For tributary comparisons, we did not detect significant differences in our steelhead size index in comparisons between the North Fork Teanaway River down stream from the Jack Creek acclimation facility (treatment sites) and the West (BACIP; $P = 0.14$) and Middle Fork (BACIP; $P = 0.41$) Teanaway River reference sites. Additional comparisons of our steelhead size index in the main stem Teanaway River relative to the West and Middle Fork Teanaway River reference sites were not consistent with an impact (i.e. all changes were positive). Thus, at this time, the weight-of-evidence suggests declines in our steelhead size index are not likely the result of salmon supplementation activities in the basin.

Although the before vs. after comparisons of rainbow trout abundance did not indicate declines warranting a refined analysis of abundance, we erred on the side of caution and conducted the analysis given our concerns related to the depressed steelhead size index. A comparison of rainbow trout abundance in index monitoring sites located downstream from the Jack Creek Acclimation Facility (e.g., North Fork and Main stem Teanaway Rivers) relative to reference sites in the Middle and West Fork Teanaway Rivers revealed significant reductions in the abundance of rainbow trout relative to the control streams (BACIP). We attempted to account for factors that may influence abundance such as movement and angler induced mortality. Motion activated cameras mounted in both treatment and reference sites during the open angling season in 2011 indicated that the reduction in abundance was probably not angler induced. In addition, we have not detected large scale movements of tagged rainbow trout between treatment and reference streams that would be consistent with a largescale displacement of trout. However, we do have evidence that the North Fork of the Teanaway River produces a higher proportion of anadromous steelhead smolt migrants than the reference streams and significant migrant production may contribute to reduced resident trout abundance. We will continue this investigation in the coming year in cooperation with the Yakima Steelhead VSP project.

Spring Chinook habitat saturation and limiting factors

Post-emergent growth

In a multiple year analysis (2009-2013), observed growth rate differed significantly among years (Homogeny of slopes model: $F_{4,\,151} = 4.36$, $P < 0.01$; Figure 5). Mean length, accounting for sampling date, was also detectably different among years (separate slopes model: $F_{4,\,151} = 6.02$, $P < 0.01$). Post-hoc analysis revealed significantly greater mean length in 2010 in comparison to all other years except 2011.

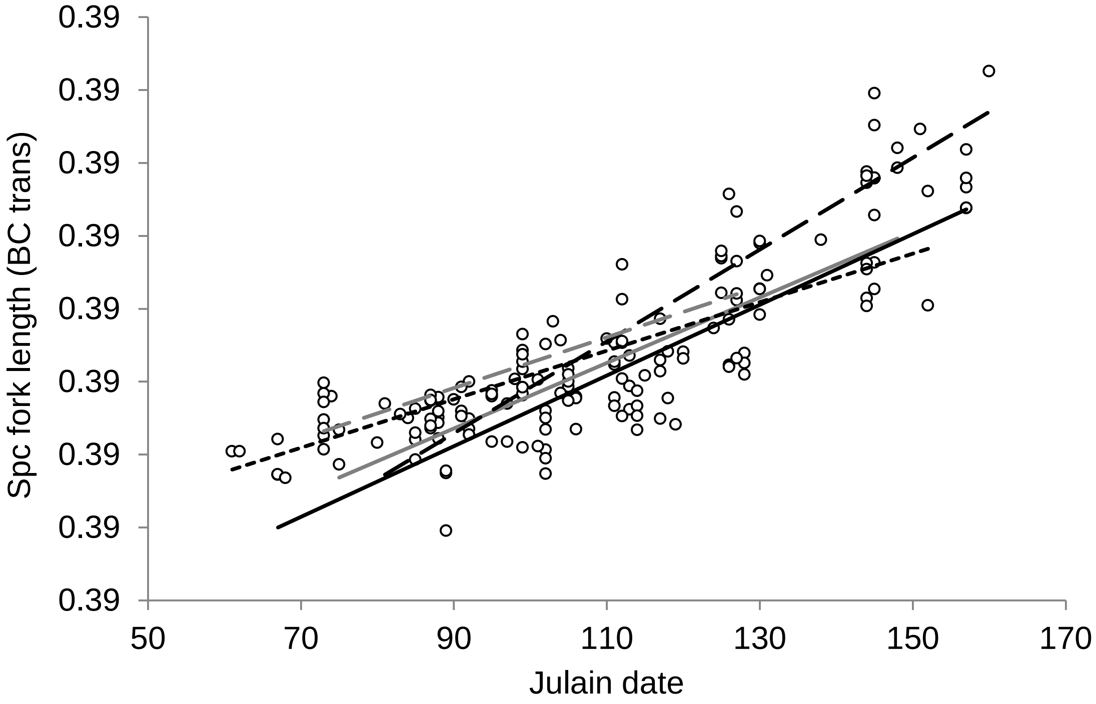

Figure 5. Comparison of mean growth rate among years in the upper Yakima River basin 2009-2012. The observed rate of growth was greater in 2010 (heavy dashed line) when compared to other survey years; 2009 (solid grey line), 2011 (smaller dashed line), 2012 (solid black line), and 2013 (dashed grey line).

Rearing abundance and habitat use

A total of 133 sites were surveyed in the three study reaches between July 10 and September 4, 2012 (Table 5.) for a total of 266 total replicates. We did not detect a significant difference in sub-yearling Chinook density among study reaches in 2012 (ANOVA: $F_{2,\,258} = 5.04$, $P < 0.60$), or in a multi-year analysis (ANOVA: $F_{1,\,828} = 0.23$, $P < 0.63$). Mean density was significantly greater in 2010 when compared all other years (ANOVA: $F_{4,\,828} = 10.67$, $P < 0.01$; Tukey post-hoc: $P < 0.01$; Figure 6.). Summer and fall mean spring Chinook densities trended, but were not significantly correlated ($R^2 = 0.63$, $P = 0.11$). A significant difference in spring Chinook density among habitat types was detectable in 2012 (ANOVA: $F_{5,\,258} = 1.88$, $P = 0.01$; Table 5), and also among years (ANOVA: $F_{5,\,863} = 8.44$, $P < 0.01$; Figure 7). A total of 67 sites were surveyed in the three study reaches between July 2nd and August 29th, 2013 (Table 5.) for a total of 134 total replicates. We did not detect a significant difference in sub-yearling Chinook density between study reaches in 2013 ($t = -0.9$, df = 134, $P = 0.36$), or in a multi-year analysis (ANOVA: $F_{1,\,963} = 0.02$, $P = 0.90$). Mean density was significantly greater in 2010 when compared all other years (ANOVA: $F_{5,\,963} = 8.40$, $P < 0.01$; Tukey post-hoc: $P < 0.01$; Figure 6.). Summer and fall mean spring Chinook densities trended, but were not significantly correlated ($R^2 = 0.54$, $P = 0.09$). We did not detect a significant difference in spring Chinook density among habitat types in 2013 (ANOVA: $F_{5,\,130} = 1.32$, $P = 0.26$; Table 5). However, differences were detectable among habitat types in a multi-year analysis (ANOVA: $F_{5, 996} = 9.27$, $P < 0.01$). Post-hoc analysis suggests significantly greater densities of summer rearing spring Chinook in pool and deep riffle habitats (Tukey HSD, P < 0.01; Figure 7).

Table 5. Physical parameters of 2013 snorkeling survey sites by sampling reach.

Habitat Classification	n	Mean site length (m)	SD	n	Site width (m)	SD
			Easton 2012			
Deep Riffle	10	70.0	19.8	10	17.8	4.2
Glide	13	72.4	22.2	13	21.9	5.7
Pool	5	52.6	8.5	5	23.2	3.8
Rapid	1	60.0	na	1	21.3	na
Riffle	5	43.6	16.2	5	18.0	5.5
Run	17	79.1	23.8	17	19.2	5.3
			Nelson 2012			
Deep Riffle	9	57.0	15.5	9	25.7	7.2
Glide	6	94.7	7.8	6	28.9	2.8
Pool	3	73.3	42.2	3	30.8	7.2
Rapid	5	58.0	24.0	5	30.1	8.4
Riffle	7	75.9	20.1	7	28.9	3.9
Run	10	90.1	16.1	10	30.8	3.7
			Bullfrog 2012			
Deep Riffle	10	70.6	18.2	10	23.8	5.9
Glide	10	89.3	17.0	10	27.2	5.2
Pool	5	33.0	7.3	5	21.0	4.2
Rapid	2	57.0	1.4	2	22.8	13.0
Riffle	6	86.0	21.7	6	27.5	1.5
Run	9	89.1	17.5	9	24.2	6.1
			Easton 2013			
Deep Riffle	7	42.3	14.5	7	19.1	5.5
Glide	7	83.7	19.6	7	21.0	6.4
Pool	4	32.3	7.1	4	19.1	4.8
Rapid	1	42.0	n/a	1	24.0	n/a
Riffle	6	52.3	22.9	6	21.3	4.8
Run	9	57.8	20.9	9	18.9	3.2
			Nelson 2013			
Deep Riffle	7	60.4	14.9	7	27.3	7.7
Glide	8	91.1	10.7	8	31.1	4.1
Pool	2	50.0	14.1	2	25.2	0.2
Rapid	2	49.5	4.9	2	35.8	1.6

Riffle	6	73.0	16.3	6	31.1	2.9
Run	8	84.8	16.9	8	29.4	3.5

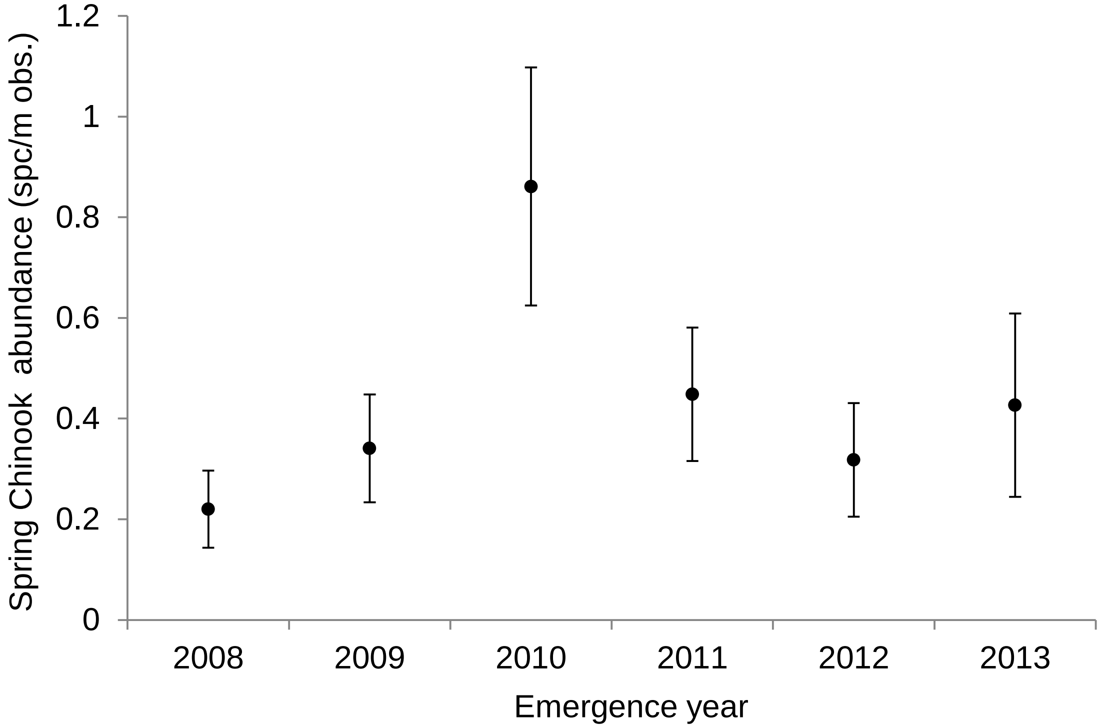

Figure 6. Multi-year analysis of observed abundance among years in two upper Yakima River study reaches with similar temperature and flow characteristics (Easton and Nelson). Significantly greater densities of spring Chinook sub-yearlings were detected in 2010.

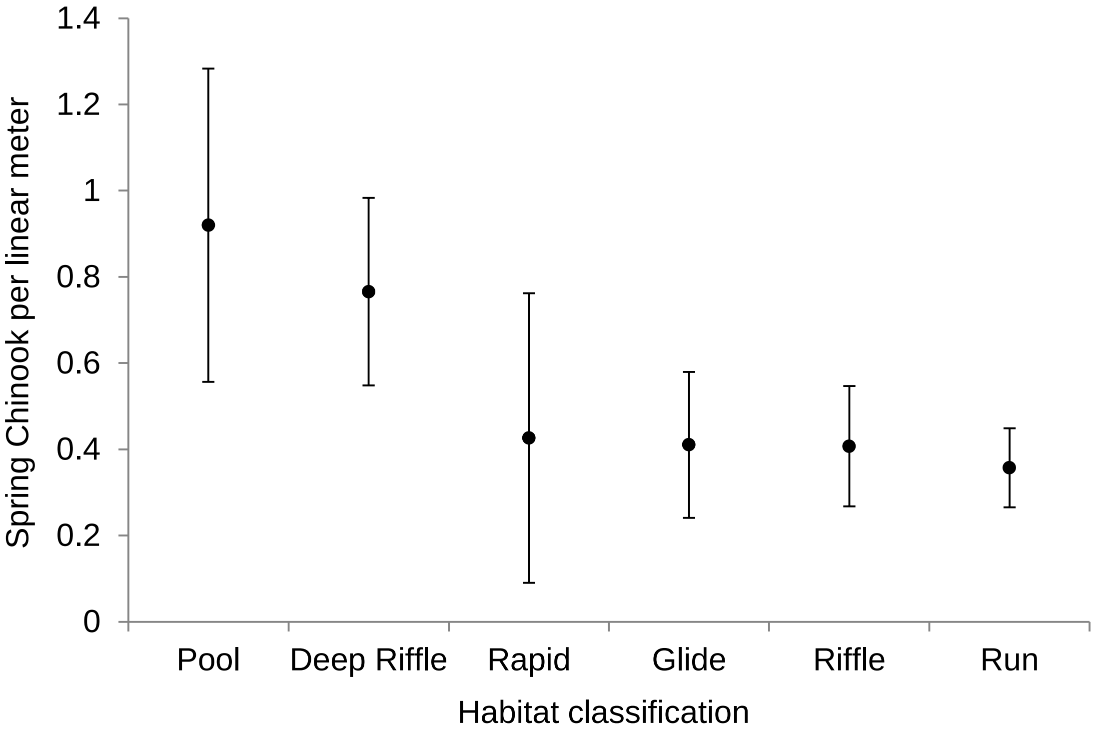

Figure 7. Mean spring Chinook observed abundance by habitat type, 2008-2013. Error bars represent 95 percent confidence intervals.

Water temperatures during sampling ranged between 11 and 18 degrees Celsius (mean, 15.4; SD, 1.6). Temperatures at sampling were not detectably different between survey reaches (t = -1.6, df = 131, P = 0.12). Overall, temperature was not significantly correlated with observed abundance of spring Chinook (n = 130, $R^2 < 0.01$ $P < 0.65$). Visibility while sampling ranged between 0.9 and 3.9 meters (mean, 2.2; SD, 0.5) and was not significantly correlated with estimates of abundance ($R^2 < 0.01$, $P = 0.85$).

Territory size (log transformed) was significantly correlated with fish fork length (mm) ($R^2 = 0.28$, $P < 0.01$; Figure 8). The proportions of feeding strikes were significantly different between categorical distances (1-4 body lengths) from the focal position (Friedman ANOVA: $\chi^2_{3,\ 634} = 1074.9$; $P < 0.01$; Figure 9). Agonistic strikes were also significantly different between categorical distances (1-4 body lengths) from the focal position (Friedman ANOVA: $\chi^2_{3,\ 281} = 150.6$, $P < 0.01$; Figure 9). The observed ratios of agonistic to feeding were not significantly different among grouped distances from the focal position (Friedman ANOVA: $\chi^2_{8,\ 3} = 5.9$, $P = 0.12$; Figure 10).

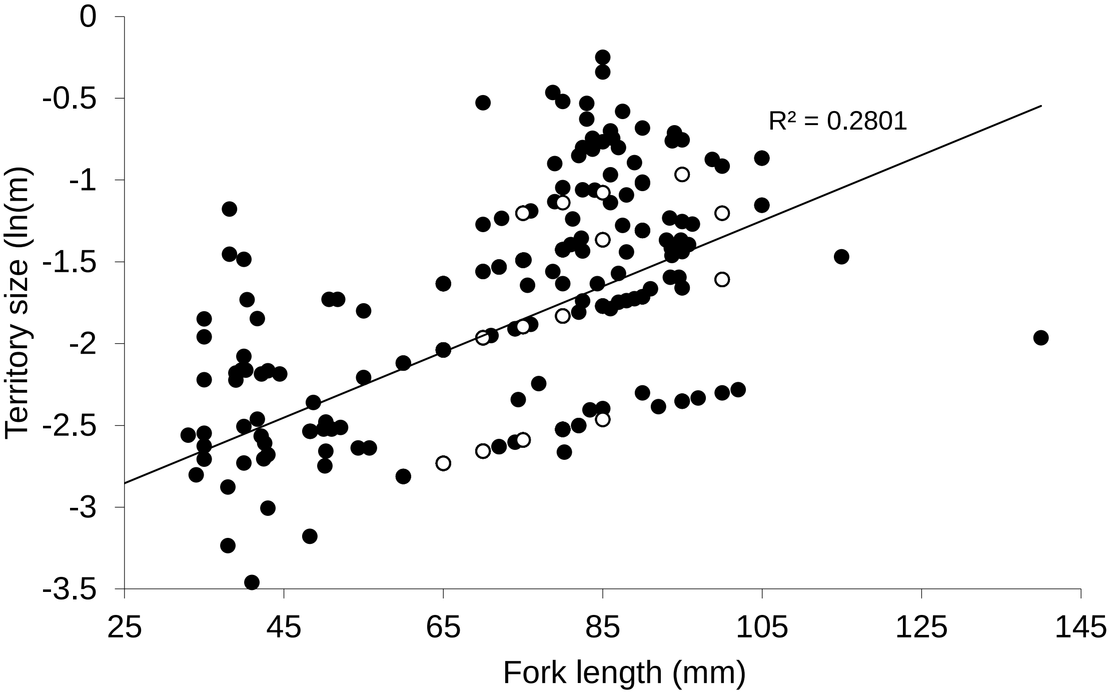

Figure 8. Relationship between spring Chinook fork length (mm) and observed territory size in the spring and summer of 2006-2012 (black points) and 2013 (white points).

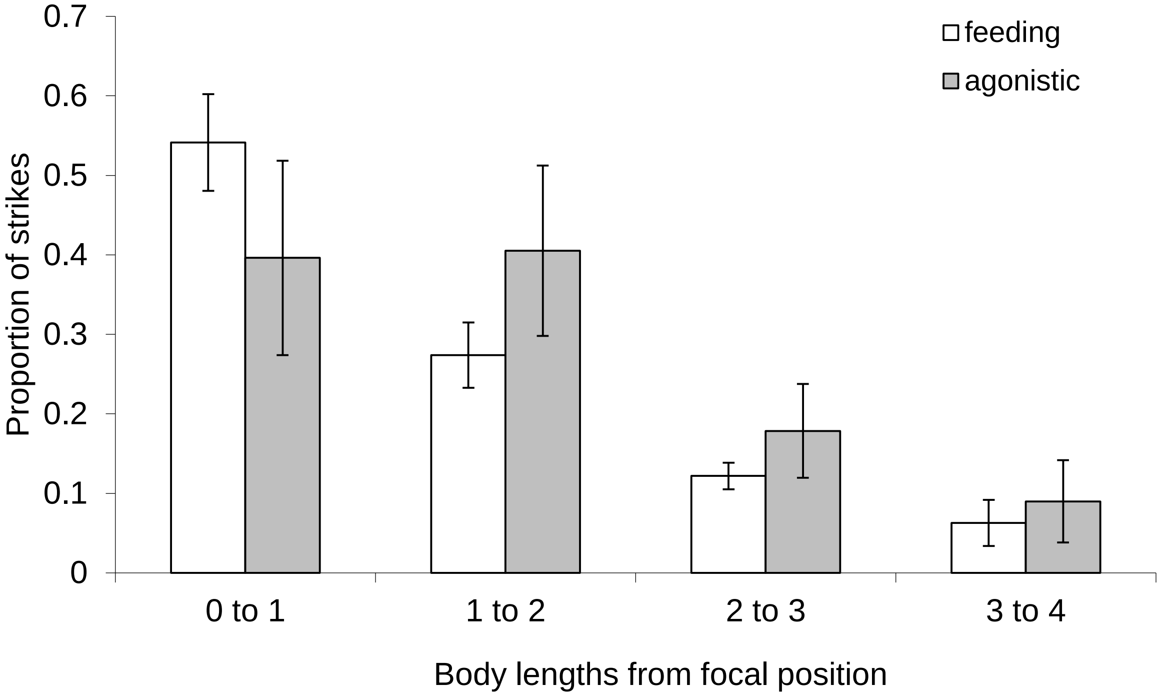

Figure 9. Proportion of rearing spring Chinook feeding and agonistic strikes with increasing distance from the observed focal position in body lengths 2006-2013.

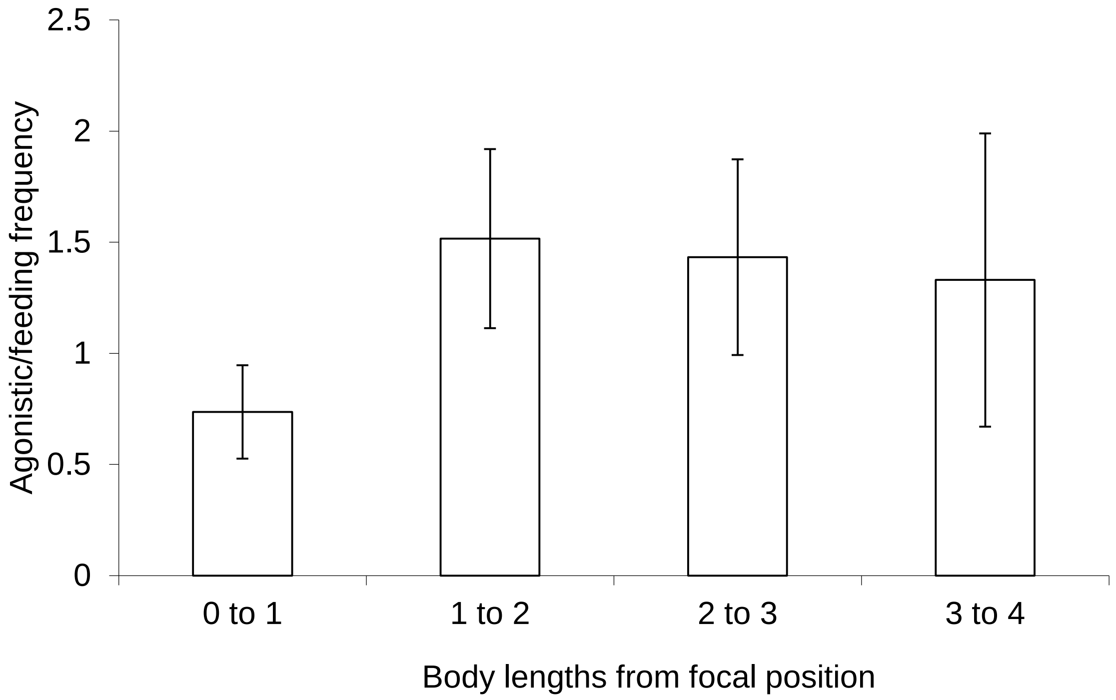

Figure 10. Mean ratio of agonistic strikes per feeding strike with increasing distance from the observed focal position 2006-2013.

Velocities were higher adjacent to spring Chinook focal positions in 72.1 percent of the observations in 2012, and 86.0 percent of the observation in 2013. A summary of microhabitat variable measured around Chinook focal positions is presented in table 6.

Table 6. Summary physical parameters measured at observed spring Chinook focal positions in 2012 and 2013.

	Temp °C	Spc length (mm)	Focal depth (m)	Total depth (m)	Focal velocity (m/s)
			2012 (n=111)		
Mean	15.9	69.7	0.3	1.3	0.2
SD	1.0	7.4	1.1	5.7	0.1
			2013 (n=43)		
Mean	16.3	79.5	2.3	0.9	0.2
SD	0.7	9.5	9.3	4.2	0.1

Spring Chinook residual/precocious male monitoring

The estimated number of natural origin age 0, natural origin age 1, and hatchery precocious males on the spawning grounds during the peak of spawning ranged from 5 to

718, 0 to 92, and 0 to 78 between 1999 and 2013 respectively (Table 7). Differences in the number of observed precocious males on or associated with active redds were detectable among age classes, and origin. Differences in the mean abundance of precociously mature males of different age and origin were detectable among years (ANOVA: $F_{2, 42} = 14.1$, $P < 0.01$). Post-hoc analysis determined that natural production age 0 precocious males were greater in abundance than both natural and hatchery production age-1 males (Tukey test: $P < 0.01$). There were no detectable differences in abundance between age 1 natural and hatchery production precocious males (Tukey test: $P = 0.96$). Among years, age 0 precocious males were found on a greater proportion of redds sampled than either age 1 or hatchery origin (ANOVA: $F_{2, 42} = 13.0$, $P < 0.01$; Tukey test: $P < 0.01$), and were greater in number per active redd (ANOVA: $F_{2, 42} = 19.4$, $P < 0.01$; Tukey test: $P < 0.01$; Table 8.).

Table 7. Number of observed and estimated totals of natural (age 0 and age 1) and hatchery origin precocious males by age class at the peak of spawning activity in the upper Yakima River. Estimated totals are extrapolations over redds and/or portions of reaches not sampled.

Survey year	Active redds	(%) Redds surveyed	(%) Spawning area sampled	Observed			Estimated total		
				Age 0	Age 1	Hatchery	Age 0	Age 1	Hatchery
1999	36	100	87	4	11	17	5	16	19
2000	316	66	87	103	42	8	128	42	11
2001	276	62	87	336	11	26	555	21	53
2002	304	81	87	138	15	8	228	25	14
2003	230	78	100	204	25	19	267	35	24
2004	1662	27	100	195	16	21	718	65	78
2005	655	99	100	357	17	0	360	17	0
2006	198	90	100	148	2	0	177	3	0
2007	92	100	100	55	0	0	55	0	0
2008	173	82	100	69	55	42	85	67	52
2009	105	99	100	87	15	34	88	15	34
2010	499	48	100	133	42	12	280	92	21
2011	418	73	100	124	40	0	171	55	0
2012	243	63	100	44	17	3	70	27	5
2013	166	66	100	76	10	3	115	15	5

Table 8. Means of the presence and abundance of natural (age 0 and age 1) and hatchery origin precocious males per active redd at the peak of spawning activity in the upper Yakima River.

Survey year	Active redds	Presence/Active redd			Abundance/Active redd		
		Age 0	Age 1	Hatchery	Age 0	Age 1	Hatchery
1999	36	0.11	0.14	0.19	0.14	0.44	0.53
2000	316	0.18	0.10	0.02	0.41	0.13	0.03
2001	276	0.31	0.03	0.04	2.01	0.08	0.19
2002	304	0.23	0.03	0.03	0.75	0.08	0.05
2003	230	0.31	0.06	0.06	1.16	0.15	0.10
2004	1662	0.05	0.01	0.01	0.43	0.04	0.05
2005	655	0.24	0.02	0	0.55	0.03	0
2006	198	0.75	0.04	0	0.89	0.02	0
2007	92	0.18	0	0	0.60	0	0
2008	173	0.08	0.21	0.16	0.49	0.39	0.30
2009	105	0.24	0.09	0.13	0.84	0.14	0.33
2010	499	0.15	0.05	0.03	0.56	0.18	0.05
2011	418	0.24	0.07	0	0.41	0.13	0
2012	243	0.13	0.08	0.02	0.29	0.11	0.02
2013	166	0.20	0.07	0.04	0.69	0.09	0.03

Hatchery precocious males were distributed differently than natural origin age 0, and natural origin age 0 and age 1 combined on the spawning grounds (G-test; $P < 0.03$). A significant difference was not detected between natural origin age 0 and natural origin age 1 fish (G-test; $P = 0.57$), or between natural origin age 1 and hatchery precocious males (G-test; $P = 0.21$; Figure 11). An average of 24 percent of all hatchery precocious males observed on the spawning grounds were in the lowest spawning reach examined, whereas only 3 percent of natural origin age 0, and 8 percent of natural origin precocious males were observed in this reach (Figure 11.).

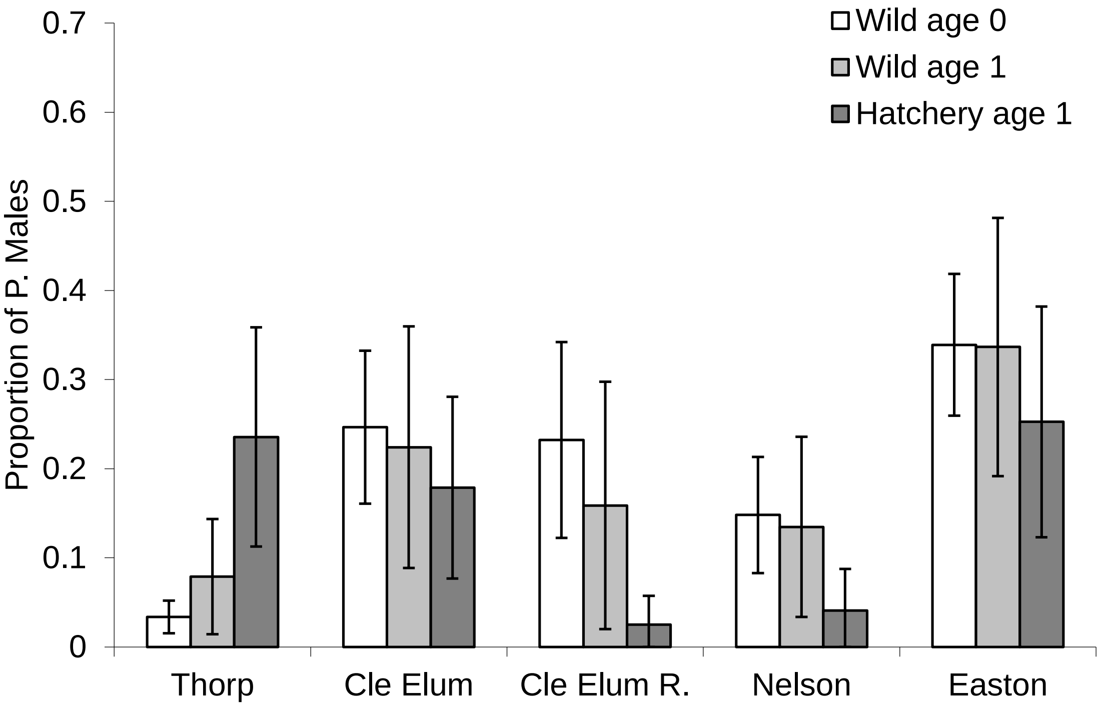

Figure 11. Mean proportion (*p*) of natural and hatchery origin precocious males by reach within the upper Yakima River at the peak of spawning activity 1999-2013. Error bars represent 95 percent confidence intervals with negative boundaries of zero.

Estimated total abundance of hatchery origin spring Chinook salmon away from redds at the time of spawning in 2013 ranged between 0 and 64 fish/km among sampling reaches (Table 9). The lower and upper Yakima Canyon averaged 59 percent of the estimated number of precocious males away from redds between 1999 and 2012, and 34 percent in 2013 (Figure 12). The annual abundance of hatchery precocious males away from redds was not significantly correlated with the number observed on redds (P = 0.06).

Table 9. Estimated abundance of hatchery origin spring Chinook salmon (HSPC) away from redds in the main stem Yakima River in the fall of 2013. The maximum number of fish netted (n) in one of two electrofishing surveys completed in consecutive weeks is presented (LCYN is the Lower Canyon, UCYN is the Upper Canyon, EBURG is Ellensburg, THORP is Thorp, and CELUM is Cle Elum). Capture probability was generated using rainbow trout of approximately the same size range as hatchery spring Chinook salmon.

Section	n	Capture prob.	Section est.	Section km	HSPC/km	Reach km	Total est.
LCYN	11	0.08	137	4.8	29	19.2	547
UCYN	22	0.12	188	5.2	36	13.4	485
EBURG	12	0.13	93	4.2	22	21.2	471
THORP	24	0.07	363	5.7	64	24.1	1533
CELUM	2	0.07	29	7.4	0	16.2	0
Total	71	n/a	n/a	n/a	150	94.1	3036

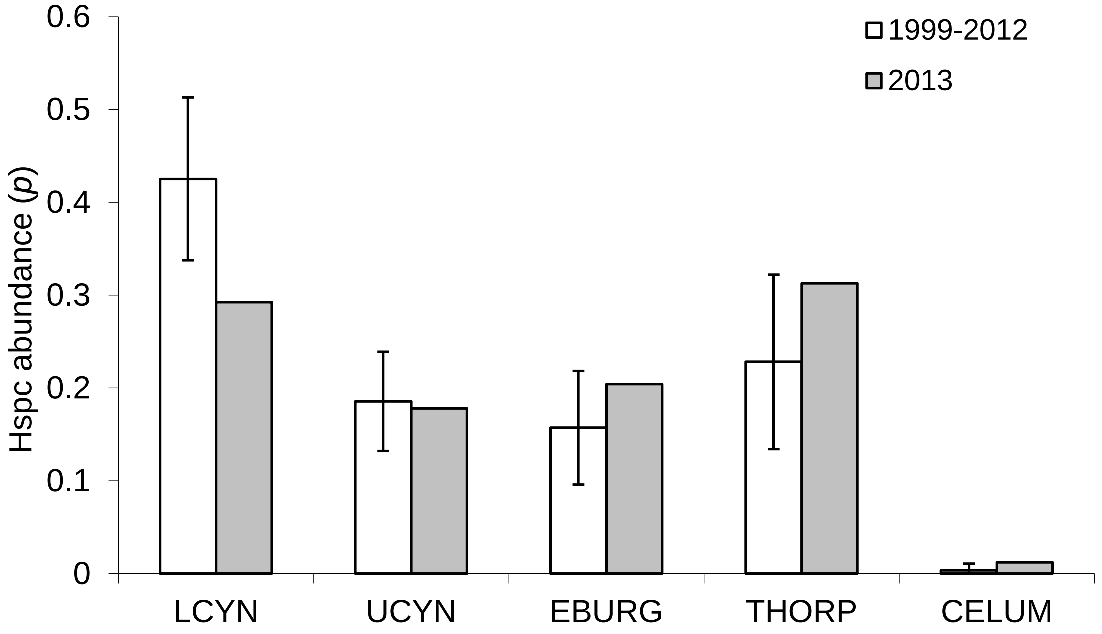

Figure 12. Proportional abundance (*p*) of hatchery spring Chinook sampled away from redds in the fall of 2013, and the mean proportional abundance between 1999 and 2012.

Domestication monitoring program-predation mortality and competitive dominance

Predation Mortality

The mean lengths of the predators were not different between net pens in either year (ANOVA, $P > 0.05$). The rainbow trout ranged from 160 mm FL to 255mm FL and the torrent sculpin ranged from 97 mm TL to 157 mm TL (Table 10). No significant differences were found between the mean lengths of the three origins of fry within each net pen at introduction (ANOVA, $P > 0.05$). Mean lengths never varied more than 0.02 mm (Table 11). The weights of the fry at introduction did not statistically differ in either year. The condition factors were not statistically different between groups in 2012

(ANCOVA, *P* > 0.05). The mean condition factors of the fry at introduction into the net pens during 2013were 0.82 for hatchery, 0.82 for supplementation, and 0.80 for Naches and were significantly different (ANCOVA, P < 0.05).

Table 10. Dates, predator replicates, and mean lengths (ranges) of the predators for predation challenges (RBT = rainbow trout; TSC = torrent sculpin).

Date Fry Stocked	Date Fry Removed	Week #	Predator Set	RBT Length (mm FL)	TSC Length (mm TL)
4/4/12	4/4/12	1	1	198.3(165-255)	119.4(101-157)
4/6/12	4/7/12	2	2	181.2(164-190)	104.0(111-129)
4/12/12	4/14/12	3	3	190.3(166-235)	116.8(101-128)
4/18/12	4/20/12	4	4	187.4(164-217)	112.8(104-125)
4/25/12	4/28/12	5	5	183.2(168-220)	111.6(103-132)
5/2/12	5/5/12	6	6	186.3(173-201)	113.9(104-127)
5/9/12	5/13/12	7	7	198.0(168-238)	110.3(97-120)
5/18/12	5/23/12	8	8	181.0(167-204)	114.6(106-129)
3/24/13	3/26/13	1	1	184.4(159-235)	114.3(104-127)
3/26/13	3/28/13	2	2	177.5(160-211)	114.9(104-136)
4/1/13	4/4/13	3	3	176.7(160-220)	117.2(103-138)
4/7/13	4/10/13	4	4	173.0(162-206)	114.3(105-139)
4/14/13	4/17/13	5	5	189.4(162-230)	113.9(106-131)
4/21/13	4/24/13	6	6	184.7(161-242)	112.8(106-127)
4/28/13	5/2/13	7	7	177.3(161-210)	115.3(107-128)
5/5/13	5/9/13	8	8	188.5(179-201)	121.1(110-131)

Table 11. Mean fork lengths (standard deviation) of the hatchery (H), supplementation (S), and Naches (N) fry upon stocking in each net pen during the predation challenges.

	Origin	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
2012	H	36.94 (1.13)	37.05 (1.16)	37.63 (1.43)	38.51 (1.61)	39.55 (1.61)	40.46 (1.67)	42.42 (1.65)	44.43 (1.68)
	S	36.95 (1.14)	37.07 (1.16)	37.63 (1.43)	38.51 (1.61)	39.55 (1.61)	40.46 (1.68)	42.42 (1.65)	44.43 (1.68)
	N	36.95 (1.14)	37.07 (1.16)	37.62 (1.41)	38.51 (1.61)	39.56 (1.61)	40.46 (1.68)	42.42 (1.66)	44.43 (1.68)
2013	H	35.40 (1.14)	36.44 (1.22)	37.48 (1.25)	38.15 (1.34)	38.89 (1.36)	39.46 (1.43)	41.17 (1.53)	42.93 (1.90)
	S	35.40 (1.14)	36.34 (1.21)	37.47 (1.24)	38.16 (1.34)	38.88 (1.31)	39.46 (1.43)	41.17 (1.53)	42.93 (1.89)
	N	35.40 (1.14)	36.37 (1.23)	37.48 (1.27)	38.17 (1.37)	38.89 (1.33)	39.46 (1.43)	41.17 (1.53)	42.94 (1.92)

Background mortality

The mortality in the circular tanks was statistically different between groups in 2012 (ANOVA, F = 7.00, df = 2, *P* = 0.004). The mean percent mortalities were hatchery 0.16%, supplemental 0.14%, and Naches 0.06%. The mortality in the circular tanks was statistically different between groups in 2013 (ANOVA, F = 3.82, df = 2, P = 0.038). The mean percent mortality in the circular tanks was hatchery 0.24%, supplemental 0.10%, and Naches 0.13%.

Influence of size on predation

There was no statistical difference in size between surviving or consumed fry, regardless of origin in 2012 (two sample t-test, df = 178, *P* > 0.05). Surviving fry regardless of origin, were larger than those that were eaten in 2013 (two sample t-test, df = 311, P <0.01).We did not detect a difference in condition factor between the consumed and surviving fry for any of the three origins during the study (Table 12)

Table 12. Mean lengths and condition factors (kd) of the consumed and surviving fry and results of the paired t-tests of the condition factors.

	Origin	Consumed length	Survivor length	Consumed kd	Survivor kd	DF	P
2012	H	42.2	41.0	0.822	0.829	8	0.17
2012	S	41.4	41.6	0.862	0.848	7	0.14
2012	N	40.5	42.3	0.810	0.840	7	0.32
2013	H	39.4	39.7	0.801	0.817	9	0.28
2013	S	39.8	39.5	0.813	0.808	10	0.26
2013	N	38.3	40.6	0.819	0.795	7	0.11

Predation survival by origin

Overall mean survival between all origins was very similar, with supplementation fry survival being slightly higher than both hatchery and Naches, and hatchery fry survival slightly higher than Naches (Table 13). No statistical difference in survival was found between hatchery, supplemental, or Naches fry in 2012(P > 0.05, Table 14). Overall mean survival between all origins was very similar, with supplementation fry survival being slightly higher than both hatchery and Naches, and Naches fry survival slightly higher than hatchery. No statistical difference in survival was found between hatchery, supplemental, or Naches fry in 2013 (P>0.05, Table 14).

During all years of this study, survival between years has varied considerably (Figure 13). This is likely due to varying lengths of time that the fry were exposed to predation during the trials. Within year differences in survival between groups has been relatively small and in most cases the supplementation line has had a slight survival advantage over the hatchery control line. Unfortunately, the Naches group has not been available for all years but has shown greater variability in relative survival from year to year and their survival appears to be decreasing relative to the other groups the last three years (Figure 13).

Table 13. Percent of hatchery (H), supplementation (S), and Naches (N) fry surviving predator net pen trials at the end of each week and the overall mean survival and standard deviation.

Year	Week	Origin	Pen 1	Pen 2	Pen 3	Pen 4	Pen 5	Pen 6	Mean (SD)
2012	1	H	64.0	58.0	70.0	80.0	64.0	60.0	64.2(15.1)
		S	70.0	56.0	60.0	70.0	56.0	62.0	65.4(15.8)
		N	68.0	56.0	64.0	72.0	58.0	60.0	62.8(13.6)
2012	2	H	56.0	60.0	74.0	60.0	78.0		
		S	78.0	58.0	84.0	58.0	74.0		
		N	66.0	64.0	72.0	50.0	78.0		
2012	3	H	50.0	68.0	70.0	68.0			
		S	50.0	66.0	72.0	64.0			
		N	64.0	66.0	66.0	64.0			
2012	4	H		78.0	80.0	60.0	64.0	68.0	
		S		76.0	90.0	58.0	58.0	74.0	
		N		80.0	80.0	50.0	58.0	64.0	
2012	5	H	64.0	50.0	62.0	66.0	78.0	74.0	
		S	60.0	64.0	48.0	82.0	90.0	58.0	
		N	48.0	62.0	62.0	76.0	68.0	62.0	
2012	6	H		80.0	82.0	70.0	56.0	60.0	
		S		88.0	78.0	74.0	60.0	66.0	
		N		82.0	76.0	78.0	66.0	78.0	
2012	7	H	66.0	80.0	60.0	80.0	86.0	68.0	
		S	92.0	86.0	72.0	78.0	92.0	68.0	
		N	74.0	86.0	62.0	76.0	76.0	64.0	
2012	8	H	80.0	54.0	80.0	76.0	96.0	66.0	
		S	80.0	86.0	88.0	76.0	92.0	58.0	
		N	72.0	64.0	66.0	74.0	92.0	60.0	
2013	1	H	66.0	80.0	60.0	68.0	64.0	64.0	62.8(11.6)
		S	78.0	74.0	80.0	82.0	54.0	54.0	64.8(13.0)
		N	76.0	78.0	60.0	64.0	62.0	64.0	63.3(12.7)
2013	2	H	64.0	96.0	70.0	72.0	74.0		
		S	78.0	92.0	66.0	48.0	66.0		
		N	82.0	98.0	72.0	62.0	58.0		

		H	78.0	76.0	66.0	62.0	52.0	40.0
2013	3	S	82.0	78.0	86.0	60.0	56.0	40.0
		N	72.0	65.0	58.0	44.0	48.0	32.0
		H	62.0	52.0	56.0	74.0	48.0	62.0
2013	4	S	50.0	60.0	70.0	78.0	48.0	70.0
		N	58.0	66.0	68.0	70.0	46.0	54.0
		H	64.0	66.0	46.0	54.0	46.0	36.0
2013	5	S	62.0	66.0	60.0	74.0	44.0	32.0
		N	52.0	78.0	62.0	78.0	48.0	46.0
		H	64.0	68.0	60.0	74.0	68.0	58.0
2013	6	S	62.0	74.0	63.0	76.0	50.0	68.0
		N	52.0	78.0	70.0	66.0	66.0	56.0
		H	56.0	70.0	64.0	56.0	50.0	66.0
2013	7	S	66.0	74.0	58.0	48.0	68.0	54.0
		N	50.0	78.0	48.0	56.0	54.0	66.0
		H	48.0	88.0	64.0	58.0	58.0	
2013	8	S	56.0	70.0	78.0	72.0	54.0	
		N	82.0	74.0	74.0	68.0	52.0	

Table 14. Results from Wilcoxon matched pairs tests for survival between the three origins of fry, their relative survival ranking, and absolute and relative differences in the mean survivals.

Year	Pairing	Z	N	P	Relative Survival Ranking	Mean Abs. Difference	Mean Relative Difference
2012	N vs S	1.46	41	0.145	S=N	2.594%	4.13%
2012	N vs H	1.47	41	0.143	N=H	1.171%	1.82%
2012	S vs H	0.37	41	0.712	S=H	1.424%	2.27%
2013	N vs S	0.90	46	0.370	N=S	1.471%	2.32%
2013	N vs H	0.02	46	0.985	N=H	1.471%	2.34%
2013	S vs H	1.37	46	0.171	S=H	1.988%	3.17%

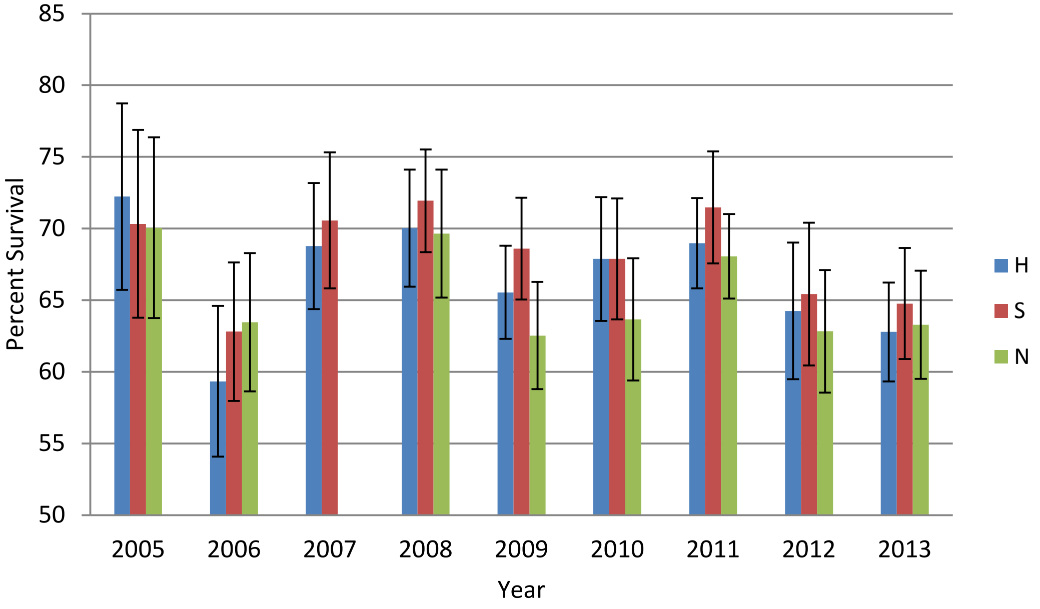

Figure 13. Percent survival by origin for all previous years of this study. Error bars are 95% confidence intervals.

Competitive Dominance

Unequal numbers of replicates occurred among pair-wise comparisons because 1) some experiments did not meet the minimum criteria or 2) fish died. The replicates that did not meet experimental criteria or cases where fish died were a small percentage of the replicates that were conducted (Table 15).

Dominance was assessed in 144 replicates of supplementation vs. Naches fish during 2012. Supplementation fry were 16% more dominant but the difference was not statistically significant (Table 16). The frequencies of different types of interactions used by supplementation and Naches fish during pair-wise comparisons were not significantly different (Table 17). Interaction rates of dominant supplementation fish were significantly lower than interaction rates of dominant Naches fish (Table 18). Supplementation fish gained more weight and grew significantly more than Naches in 2012 (Table 19).

Dominance was assessed in 140 replicates of supplementation vs. Naches fish during 2013. Supplementation fry were 9% more dominant but the difference was not statistically significant (Table 16). The frequencies of the different types of interactions used by supplementation and Naches fish during pair-wise comparisons were not significantly different (Table 17). Differences in aggression between Naches and supplementation fish were not detected (Table 18). Naches fish grew slightly more and lost slightly more weight than supplementation fish although the differences were not significant (Table 19).

Dominance was assessed in 145 replicates of supplementation vs. hatchery fish in 2012. Supplementation fish were 14% more dominant in 2012 but the difference was not statistically significant (Table 16). The frequencies of different types of interactions used by supplementation and hatchery fish during pair-wise comparisons were not significantly different (Table 17). Differences in aggression were not detected (Table 18). Supplementation fish grew significantly more than hatchery fish in 2012 (Table 19).

Dominance was assessed in 148 replicates of supplementation vs. hatchery fish in 2013. Hatchery fish were 18% more dominant than supplementation fish in 2013 (Table 16). The frequencies of different types of interactions used by supplementation and hatchery fish during pair-wise comparisons were not significantly different (Table 17). Hatchery fish were more aggressive than supplementation fish (Table 18). Hatchery fish grew slightly more and lost slightly less weight than supplementation fish (Table 19).

Dominance was assessed in 132 replicates of Naches vs. hatchery fish during 2012. Hatchery fish were 20% more dominant than Naches fish in 2012 but the difference was not statistically significant (Table 16). The frequencies of different types of interactions used by Naches and hatchery fish during pair-wise comparisons were not significantly different (Table 17). Interaction rates of dominant hatchery fish were significantly lower than dominant Naches fish in 2012 (Table 18).

Dominance was assessed in 140 replicates of Naches vs. hatchery fish during 2013. Hatchery fish were 17% more dominant than Naches fish in 2103 (Table 16). The frequencies of different types of interactions used by Naches and hatchery fish during pair-wise comparisons were not statistically significantly (Table 17). Interaction rates of Naches fish were significantly lower than hatchery fish in 2013 (Table 18). Hatchery fish grew more in length and weight than Naches fish (Table 19).

Table 15. Factors responsible for eliminating contest replicates from analysis.

Year	Origin	Died or Missing	< 10 Pellets	No Interactions	Abnormal behavior	Total
2012	Supp	1			1	
2012	Naches				1	
2012	Both		27			30
2012	Supp			1	3	
2012	Hatchery	1				
2012	Both	1	20	3		29
2012	Naches	1			1	
2012	Hatchery	3			2	
2012	Both		25			32
2013	Supp	2			1	
2013	Naches	2			1	
2013	Both	1	28	6	1	42
2013	Supp	6			2	
2013	Hatchery	2			2	
2013	Both		17	6		35
2013	Naches	3			1	
2013	Hatchery	3				
2013	Both		24	8		39

Table 16. Comparisons of mean (± 1 SD) of the % food acquisition, % habitat occupation, % agonism dominance (dom. interactions), % total dominance, sum of the scores used to assess dominance, and P values from Wilcoxon matched pairs test in contest competition experiments between supplementation (Supp.), hatchery, and Naches Chinook salmon.

Year	Origin	n	% Food	% Habitat	% Dom. Interactions	% Total Dom.	Sum Total Dom. %	P
2012	Supp	144	55 (36)	56 (42)	58 (48)	58	169 (124)	
2012	Naches	144	45 (36)	44 (42)	42 (48)	42	131 (124)	0.162
2012	Supp	145	53 (37)	55 (42)	56 (48)	57	164 (124)	
2012	Hatchery	145	47 (37)	45 (42)	44 (48)	43	136 (124)	0.486
2012	Naches	132	44 (38)	41 (42)	41 (46)	40	126 (122)	
2012	Hatchery	132	56 (56)	59 (42)	59 (46)	60	174 (122)	0.078
2013	Supp	140	55(42)	54(46)	55(48)	55	163(132)	
2013	Naches	140	45(42)	46(46)	45(48)	45	135(132)	0.330
2013	Supp	148	42(42)	42(47)	42(48)	41	125(134)	
2013	Hatchery	148	58(42)	58(47)	58(48)	59	175(134)	0.012
2013	Naches	140	39(40)	40(45)	41(48)	41	120(129)	
2013	Hatchery	140	61(40)	60(45)	59(48)	59	179(129)	0.003

Table 17. Comparisons of frequencies of interaction types initiated (mean interaction/fish in each tank (± 1 SD)) by supplementation (S), hatchery (H), and Naches (N) fish and total interactions (total ints.) by origin in contest competition experiments.

	Origin	Crowd	Threat	Chase	Butt	Nip	Total Ints.	P G-test
2012	S	3.15 (6.21)	10.55 (9.19)	7.98 (11.48)	2.62 (3.55)	1.76 (2.87)	3753	0.999
2012	N	2.57 (6.70)	10.22 (9.60)	7.69 (15.89)	2.22 (4.91)	1.37 (3.46)	3465	
2012	S	2.99 (5.09)	10.31 (9.69)	8.56 (13.00)	3.21 (7.19)	1.66 (3.60)	3871	0.998
2012	H	2.75 (5.41)	9.58 (9.81)	6.83 (13.32)	2.22 (4.32)	1.50 (3.75)	3313	
2012	N	2.46 (5.31)	9.51 (8.60)	5.96 (13.85)	2.52 (4.47)	1.21 (3.55)	2860	0.996
2012	H	2.71 (5.46)	9.46 (7.25)	7.74 (12.20)	2.36 (4.02)	1.49 (2.99)	3134	
2013	S	0.26(1.27)	5.94(12.07)	3.69(8.01)	0.56(1.56)	1.19(2.90)	1629	0.997
2013	N	0.34(4.19	7.54(16.29)	3.57(13.43)	0.54(2.47)	0.96(2.99)	1812	
2013	S	0.16(1.73)	5.92(12.67)	2.76(8.57)	0.46(1.92)	0.74(2.59)	1485	0.958
2013	H	0.15(0.72)	5.45(8.78)	5.30(13.34)	0.66(1.77)	1.18(5.18)	1886	
2013	N	0.18(0.73)	7.29(17.63)	4.71(16.91)	0.54(2.82)	0.82(2.26)	1896	
2013	H	0.16(1.73)	7.12(14.88)	5.27(12.10)	0.76(2.92)	1.33(7.52)	2051	0.997

Table 18. Interaction rates (mean interaction/fish/minute) of agonistic interactions initiated by supplementation (Supp.), hatchery and Naches fish in contest experiments.

Year	Origin	n[a]	Mean Interaction rate	1 Standard Deviation	P[b]
2012	Naches	144	1.08	1.24	0.086
	Supplementation	144	1.16	1.03	
	Naches Dominant	61	2.10	1.31	0.046
	Supp. Dominant	83	1.67	0.99	
	Naches Subordinate	83	0.33	0.34	0.396
	Supp. Subordinate	61	0.48	0.59	
2012	Supplementation	145	1.19	1.11	0.106
	Hatchery	145	1.03	1.13	
	Supp. Dominant	83	1.78	0.45	0.610
	Hatchery Dominant	62	1.88	0.47	
	Supp Subordinate	62	0.39	0.45	0.816
	Hatchery Subordinate	83	0.40	0.47	
2012	Naches	132	0.97	1.06	0.105
	Hatchery	132	1.07	0.96	
	Naches Dominant	53	1.85	1.11	0.049
	Hatchery Dominant	79	1.50	1.11	
	Naches Subordinate	79	0.39	0.43	0.996
	Hatchery Subordinate	53	0.43	0.58	
2013	Naches	140	0.56	0.94	
	Supplementation	140	0.53	0.76	0.601
	Naches Dominant	63	1.14	1.15	
	Supp. Dominant	76	0.90	0.86	0.190
	Naches Subordinate	76	0.11	0.22	
	Supp. Subordinate	63	0.10	0.18	0.976
2013	Supplementation	148	0.45	0.70	
	Hatchery	148	0.56	0.77	0.023
	Supp. Dominant	61	0.97	0.82	
	Hatchery Dominant	87	0.87	0.86	0.141
	Supp. Subordinate	87	0.08	0.20	
	Hatchery Subordinate	61	0.13	0.24	0.131
2013	Naches	140	0.61	0.99	
	Hatchery	140	0.64	0.99	0.046
	Naches Dominant	58	1.30	1.22	
	Hatchery Dominant	82	0.99	1.15	0.127
	Naches Subordinate	82	0.11	0.21	
	Hatchery Subordinate	58	0.13	0.21	0.244

[a] Number of replicates

[b] P values from Mann-Whitney U-tests are for comparisons of fish origins regardless of dominance, when they were dominant, and when they were subordinate.

Table 19. Comparisons of supplementation (Supp.), hatchery, and Naches fish growth in contest competition experiments. Replicate numbers vary a bit because when equal growth occurred, they were not analyzed.

Year	Origin	n[a]	Average Growth mm Length	Average Growth Mg Weight
	Supp.	142	1.01 (1.00)	125.57 (199.89)
2012	Naches	142	0.77 (1.07)	64.25 (206.04)
	P[b]		0.044	0.024
	Supp	142	1.06 (1.00)	95.70 (200.14)
2012	Hatchery	142	0.75 (1.07)	63.13 (187.60)
	P[b]		0.007	0.181
	Naches	131	0.94 (1.07)	60.18 (237.99)
2012	Hatchery	131	1.02 (1.14)	111.10 (199.84)
	P[b]		0.054	0.072
	Supp.	140	0.51(1.15)	-57.45(227.88)
2013	Naches	140	0.55(1.13)	-59.46(216.58)
	Pb		0.78	0.94
	Supp.	147	0.50(1.02)	-81.62(210.77)
2013	Hatchery	147	0.85(1.05)	-9.84(230.42)
	Pb		0.0060	0.0080
	Naches	139	0.48(1.27)	-91.32(359.72)
2013	Hatchery	139	0.90(0.99)	24.27(222.79)
	Pb		0.0029	0.0023

[a] number of replicates
[b] P values for statistical tests
Note: Numbers in parentheses are 1 standard deviation

Over the years, relative percent dominance between the hatchery and supplementation groups has been variable with a slight indication that the hatchery control fish were increasing in dominance during the first generation but has since leveled out (Figure 14). While not available for comparison for all years of study, the relative dominance between hatchery control and Naches fish have tracked very closely to that of hatchery versus supplementation until the last two years (Figure 14). The relative dominance between the supplementation and Naches fish does not show any clear trend relative to the other two comparisons in the earlier years but has since tracked with the hatchery versus Naches.

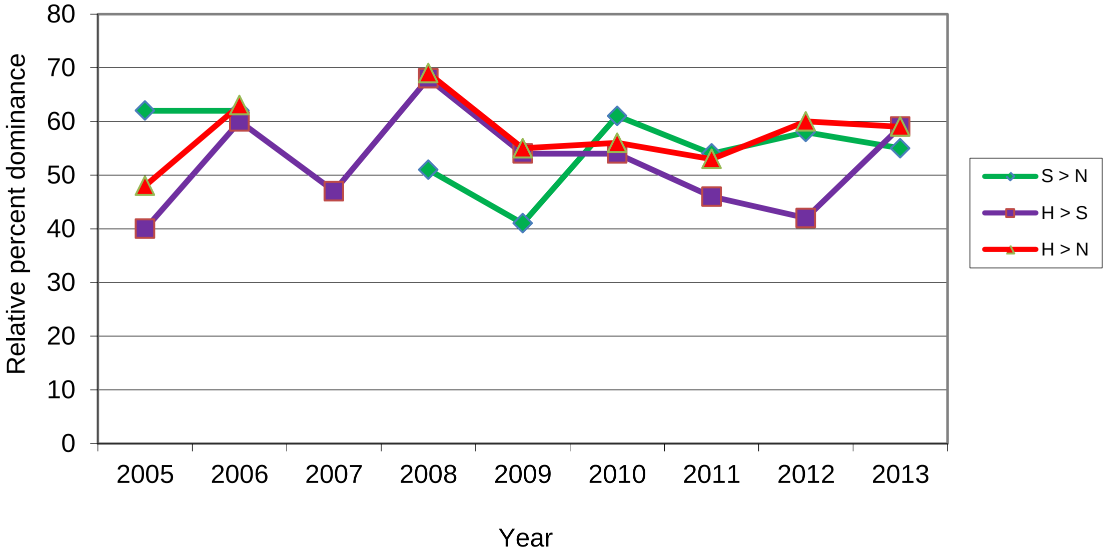

Figure 14. The relative percent dominance between the pairings of the three populations from 2005 through 2010 (H = hatchery line; S = supplementation line; N = Naches wild line). Data for 2005-2009 is from Fritts and Stockton (2010).

Spring Chinook reproductive success/spawning channel

Genetic analysis revealed that all 96 fish released or found in the spawning channel had unique genotypes. There were a total of 24 hatchery control line (HC) adult males, 24 HC adult females, 24 supplementation hatchery line (SH) adult males, and 24 SH adult females. Four HC males and four HC females were released into three of the six sections and four SH males and four SH females were released into the other three section. A total of 14 loci were screened and all 14 were used in the analysis. Number of alleles ranged from 5 - 32 (*Ots-9* and *Omm-1080* respectively) and observed heterozygosity ranged from 0.330 – 0.958 (*Ots-G474* and *Omm-1080* respectively). Individual exclusionary power was below 46.3% for five loci (*Ogo-2*, *Ogo-4*, *Ots-G474*, *Ots-3M*, and *Ots-9*) and above 60.5% for the remaining loci when neither parent was known. Exclusionary power was below 40.6% for three loci (*Ots-G474*, *Ots-3M* and *Ots-9*) and above 59.2% for the remaining loci when one parent was known. Cumulative exclusionary power was 1.000000 for analysis using all loci when one parent was known. Parentage assignments were made when genotype data was available for nine or more loci. All 96 parents were genotyped at 8 or more loci while 2,925 of the 3,000 offspring were successfully genotyped at eight or more loci. Parentage analysis was conducted independently for each of the six sections using all 96 adults as possible parents. Each fry was assigned a dam-sire-fry combinations (trios) based on the most likely candidate male parents (sires) and female parents (dams). Those assignments yielded possible.

Any fry-sire assignments with more than two mismatching loci were excluded from further consideration. Of the total 2,925 fry included in the analysis a total of 2,845 fry were assigned to a single male and female parent (2,845/2,925 = 97.3.0%).

Spring Chinook Genetic stock separation-juveniles

A total of 1,112 unknown Chinook smolts were selected and analyzed from those collected at Chandler Trap. Smolt samples that were missing data for six or more loci (N = 27) were dropped from statistical analyses. The mixture composition estimates for the entire 2012 smolt outmigration indicated that the largest overall percentage of spring smolts was from the upper Yakima River followed by the Naches River and American River in the first four strata. During the migration from January – May, the proportion of the upper Yakima River stocks was between 38.8 and 65.1% while the American River and Naches River spring stocks was between 5.3 and 31.6%. The proportion of the two fall stocks was between 0.0 – 17.0% for the first four time strata and 81.5% in the June - July time stratum. A comparison of the morphological assessment to genetic assignment was conducted for all five time strata. A total of 39 smolts in January/February, 54 smolts in March, 164 smolts in April, 214 smolts in May, and 379 in the June/July time strata were scored, and therefore included in the analysis. Results for the time strata were as follows: January/February time stratum – all 39 smolts were assigned identically using morphological and genetic methods (39 spring); March stratum – all 54 smolts were assigned identically using morphological and genetic methods (54 spring); April time stratum – 164 smolts were assigned identically using morphological and genetic methods (164 spring); May time stratum – 199 out of 214 smolts were assigned identically using morphological and genetic methods (168 spring – 31 fall), eight of the 15 discrepancies were identified as a fall by the genetic analysis and spring with morphological identification and the remaining seven were identified as a spring by the genetic analysis and fall with morphological identification ; June/July time stratum – 319 out of 379 smolts were assigned identically using morphological and genetic methods (40 spring and 279 fall), 33 discrepancies were assigned as fall by the genetic analyses while morphological identification was spring, the remaining 27 discrepancies were identified as a spring by the genetic analysis and fall with morphological identification.

b. Hatchery RM&E

The performance of the YKFP spring Chinook supplementation program has been documented relative to the project quantitative objectives and has been presented annually in the YKFP M&E project overview (Fritts 2012). Briefly, the project appears to be meeting or is making progress towards achieving the project’s objectives (Appendix D).

5. Synthesis of Findings: Discussion/Conclusions

Lessons Learned: Explain how the results of this project benefit fish and wildlife. Address each applicable sub-strategy and management question(s), provided in the Introduction for higher-level or project/program level adaptive management. If studies are incomplete, discuss preliminary findings. (Refer to the RM&E Annual Technical Reporting guidance document for more information on content to include).

a. Fish Population RM&E

Non-target taxa of concern monitoring

We failed to reject the hypothesis that early-middle stages of salmon supplementation have impacted valued trout species in the upper Yakima Basin beyond predetermined containment objectives. There were no impacts of supplementation activities on bull and cutthroat trout that inhabited tributary streams because limited or no overlap with hatchery or naturally produced salmon occurred. However, the potential existed for much overlap between salmon and bull and cutthroat trout in the tributaries of the upper Yakima Basin. For example, hatchery steelhead that were released in 1994 very close to the release site in the North Fork of the Teanaway River, migrated upstream into areas containing bull trout and cutthroat trout (McMichael and Pearsons 2001). Hatchery spring Chinook also migrated upstream of the acclimation site in the North Fork of the Teanaway River, but not nearly as far as hatchery steelhead. This finding is consistent with our earlier work and extends the findings into later stages of supplementation (Pearsons and Temple 2007).

It is possible that some overlap occurred at times and places when/where we did not sample. However, substantial overlap was unlikely because we sampled at times and places that overlap was most likely. There are certainly areas outside the upper Yakima watershed where overlap occurs at the times that we sampled. Furthermore, overlap has been detected using the methods we used (e.g. snorkeling). Salmon and bull and cutthroat trout overlap during the summer in another large tributary in the Yakima Basin that parallels the upper Yakima River. In the Naches Basin, which merges with the upper Yakima River near the city of Yakima, substantial overlap exists between bull and cutthroat trout and naturally produced Chinook salmon (T. Pearsons, unpublished data). Hatchery coho salmon are released into that basin and undoubtedly overlap with bull and cutthroat trout. Other studies have also documented overlap between salmon and cutthroat and bull/Dolly Varden trout (Glova 1984; Bisson et al. 1988; Nakano and Kaeriyama 1995; Thurow et al. 1997).

There are a variety of possible reasons why overlap was not detected in tributaries of the upper Yakima River. First, all but one of the acclimation sites for salmon were located in the main stem and the acclimation site in the tributary was located downstream of bull and cutthroat trout. Risks to bull and cutthroat trout were one of many factors that contributed to acclimation site placement. Second, the distribution of juvenile salmon has not increased substantially even though the abundance of adult salmon has increased. We had expected that the distribution of juvenile salmon would have increased with increasing abundance of spawners. Third, high abundance of rainbow trout in lower elevation portions of tributaries may competitively exclude cutthroat and bull trout to higher elevations that salmon do not occupy. Relaxation of competition could result in broader distributions of bull and cutthroat trout and the possibility of greater overlap with salmon. Fourth, salmon, bull trout, and cutthroat trout have different habitat preferences. Salmon typically occupy streams of lower gradient, lower elevation, and warmer water temperatures than cutthroat and bull trout (Glova 1987; Dunham and Rieman 1999). Glova (1987) concluded that impacts to cutthroat trout could be reduced by stocking coho in areas with gradients greater than 1% and ample fast water habitats. Faster water velocities allow for more resource partitioning and competitive dominance by trout. Most of the tributaries in the upper Yakima Basin met these criteria. We did observe overlap between salmon and cutthroat trout in the main stem, where water temperatures were more suitable for both of these species.

Contrary to our previous findings (Pearsons and Temple 2007), we did detect a significant difference in the abundance of rainbow trout in treatment areas in the North Fork Teanaway River and main stem Teanaway River relative to our control sites (Pearsons and Temple 2010). With each additional year of sampling we will have increased power to detect smaller differences (Ham and Pearsons 2000). However, it is important to note that our "Before-After" detection plan would not have triggered the "Causation" analysis that was used to detect the decline and the decline was isolated to a small area and was small relative to the total population size. Furthermore, we do not yet have evidence to support the decline was due to mortality of fish in the treatment area. Other possibilities may include displacement, and perhaps angler harvest, both of which we are currently evaluating.

Although we observed decreases in the size of rainbow trout during the post-supplementation period, the decline is unlikely to have been caused by supplementation. If supplementation had changed the size structure or growth of the steelhead size index, we would expect to detect this change in areas with high densities of salmon. We did not detect a reduction in the size of rainbow trout in the high-density areas of the target taxa below the Clark Flats acclimation site or below the release site in the North Fork Teanaway River. These areas are likely to have the greatest potential of detecting an impact. One potential explanation for the observed decrease in main stem rainbow trout size is that intraspecific density dependent mechanisms have altered the size of main stem Yakima River rainbow trout. The abundance of rainbow trout increased by approximately 30% (30% increase of age 1 fish, and 29% increase of fish greater than 249 mm) after stocking began. This information and results from small-scale enclosure experiments (McMichael et al. 1997) leads us to believe that the decline in rainbow trout lengths is most likely the result of intraspecific competition.

With the exception of the BACIP results from the Teanaway basin, the lack of detectable impacts to rainbow trout were consistent with results that were derived from smaller scale enclosure experiments between naturally produced spring Chinook salmon and rainbow trout in high elevation tributaries (McMichael and Pearsons 1998). In these experiments, growth and abundance of rainbow trout were not impacted when the density of salmonids was doubled by the addition of naturally produced spring Chinook salmon parr. However, growth of rainbow trout was suppressed when the density was doubled with rainbow trout (McMichael et al. 1997), which supports the previously mentioned idea of intraspecific impacts to rainbow trout growth in the main stem. The current results extend the findings of McMichael and Pearsons (1998) to smolts, residuals, coho salmon, and to lower elevation waters such as the main stem. Our ability to detect impacts with the BACI design and the longer experimental period in this study (higher statistical power) may explain the differences among the studies. Opportunities for cumulative impacts to manifest and larger sample sizes may be necessary to detect impacts where high natural variation occurs.

It is possible that our abundance estimates in the main stem and tributaries and the size estimates in the tributaries were influenced by the size breaks that we used in our analysis. The lower size breaks were necessary (e.g., 80 mm in tributaries and 100 mm in the main stem), because we capture very few of these fish due to our low electrofishing efficiencies on small fish and hence cannot calculate valid estimates on these fish. This could result in varying proportions of age 0 and 1 fish in our estimate if the length at age varied across years or sites. However, we do not believe that length truncations significantly affected our conclusions. For example, if fish length was negatively impacted then the distribution of fish size would have become smaller, and more age 1 fish could have been pushed below 100 mm. Regardless of how many fish may have been shifted below 100 mm, if the impact occurred to the whole age class then we should have detected a decrease in size for fish above 100 mm (e.g., the whole length frequency curve would be shifted to smaller sizes). Similarly, if many fish were impacted so that they were less than 100 mm then the abundance of age 1 fish would have been negatively biased. In other words, we would expect to detect less fish than we did prior to supplementation. If we had concluded that impacts had occurred, then our length truncations would be a more serious issue.

We did not detect impacts to non-trout NTT that could be attributed to supplementation. In the tributaries, this was because none of the non-trout NTT overlapped salmon at high enough levels to exceed the CO. All non-trout NTT in the main-stem overlapped completely, but none exceeded the containment objectives.

With the exception of minimum daily stream discharge in the main stem Yakima River, we did not detect changes in the environmental variables that were measured. We hypothesize the increased minimum daily stream discharge observed would benefit NTT. However, the increased minimum daily discharge was not significantly correlated with our NTT monitoring variables suggesting that it did not confound our results. Average and maximum stream discharge and temperature were heavily regulated by upstream irrigation reservoirs providing a relatively stable environment to conduct risk containment monitoring. Although discharge in tributaries is unregulated, summer base flows have not differed drastically during the time of sampling from year to year. The relatively stable environmental conditions observed in both tributary and main stem areas supports the use of time as a control in our evaluation.

Post-emergent growth

The development of a growth model has allowed the detection of annual differences in size and growth rate among years. This information provides insight into upper Yakima spring Chinook population dynamics in the fry-to-parr life stage, and contributes to our understanding of environmental factors and/or behavioral responses which may negatively affect growth or survival in years of high spawner density.

The Yakima Basin experienced flow conditions throughout the 2009-2010 incubation period that were lower and far less variable than average for the system (Johnson et al. 2012). These conditions may have resulted in a relaxation of environmental influences on survival, resulting in uncharacteristically high survival and, in effect, an increase in the system capacity for spring Chinook subyearlings. Our data indicate that greater growth and size were present in the early rearing period in 2010, suggesting such a relaxation in limiting factors was present within or before the spring sampling period.

High observed productivity in the fall of both 2010 and also 2011 may give indications of the time period in which density dependent constraints exist under normative conditions in the upper Yakima. Preliminary results from genetic stock separation analysis of 2011 spring Chinook smolts originating from both the Yakima and Naches River basins (2010 fry-parr; WDFW unpublished data) suggests that high productivity in 2010 was not unique to the Yakima River basin. It is possible that a relaxing of capacity constraints occurred as a result of larger scale environmental conditions affecting multiple basins. If this is true, and the larger scale trends temporary, we might expect to observe a slow decrease in productivity in years following high escapement until the system again returns to its previous capacity for spring Chinook production. If this occurs, data collected from years of unusually high system productivity may give additional insight into the specific factors, again present, affecting survival in the upper basin.

Rearing abundance and habitat use

Our data suggest a greater abundance of summer rearing spring Chinook in 2010 than in any other survey year. This is consistent with our detection of greater size and growth in the spring, and also abundance and size in the fall of 2010. These findings, along with a nearly significant correlation between estimates of summer and fall abundance, suggest our methods were successful in tracking relative productivity through three distinct subyearling spring Chinook life-stages. Over time, these data should allow identification of the life-stage in which limitations to growth and survival are occurring; a critical first step in identifying the specific factor or factors negatively affecting the population in some years.

Yakima River spring Chinook redd-to-parr productivity observed in the fall of 2010 (WDFW unpublished data) was much higher than that predicted through the use of a Beverton-Holt recruitment curve developed using data from the previous sixteen years (Johnson et al 2009). Environmental conditions in the spring of 2010 appear to have been very conducive to early survival, perhaps due to an uncharacteristically low number of high-flow events during the incubation period (Johnson et al 2012). The absence of a detectable response through subsequent life-stages when environmental conditions were not notably different (late spring, summer, and fall), suggests that capacity constraints may exist in earlier developmental periods in years where environmental conditions are more normative.

Documenting the existence of density dependent constraints post-emergence is confounded by the fact that this is often a period of high mortality, even when spawner densities are low. A system's capacity for incubating alevin is generally far greater than its capacity for juveniles, which generally results in low spawning densities, high survival to emergence, and post-emergent thinning of the population (Quinn 2005). However, during high return years, when competition exists for preferred spawning habitats, density dependent limitations to growth and survival may ultimately occur prior to first emergence. Such limitations may be attributable to a number of potentially limiting environmental factors such as increased sedimentation, scour, temperature, and/or decreased dissolved oxygen levels in less optimal spawning habitats.

Estimates of life-stage specific growth and abundance during years with a high density dependent response will be necessary to identify limiting factors with any degree of certainty. We will continue to monitor summer parr abundance and to investigate the potential relationship between our summer and fall estimates.

Perhaps as important as the documentation of abundance in the summer rearing period is the identification of the existing habitats most heavily utilized by subyearling Chinook. This information may help in the identification of limiting factors, but will also further our understanding of reach specific productivity in years of low density; a critical metric that is often missing from restoration efforts, which often concentrate only on limitations or "bottlenecks" within the population (Mobrand et al. 1997). Although we encountered high variability in abundance among sampling units, we did find higher densities of rearing Chinook in pool and deep riffle type habitats. Therefore, the summer distribution of rearing subyearlings appears to be in-part due to the presence of certain habitat types. In addition to other, larger scale, environmental factors which may affect movement and subsequent survival (e.g. temperature, flow events) the use of habitat type as an explanatory variable should be beneficial in determining relative productivity among reaches of the upper Yakima River for summer rearing spring Chinook salmon. Territory size continued to be strongly associated with spring Chinook length, which is consistent with the findings of others (Grant and Kramer 1990, Keeley and Grant 1995). These data suggest that territory may be a reasonable microhabitat metric to measure the degree of competition for space. Previous work in the Yakima Basin was unsuccessful in linking calculated territory based on local abundance to fall abundance (Pearsons et al. 2007). However, the spatial scale of those measures may have been either too large to detect changes in territorial behavior, or measured after any subsequent mortality or out-migration had occurred. Subyearling Chinook decreased the frequency of defense and foraging with increased distance from the holding position. This is consistent with our expectations that increased effort would be required to defend and utilize space away from the position of holding. The frequency of defense may be just as important as the size of the observed territory when evaluating limiting factors. For example, if food is a limiting factor, then we may observe highly defended areas of high food availability and smaller territory size, and areas of low food availability where the individual is forced to defend a larger area. These two scenarios may be energetically equivalent for the individual. Ranges of focal depth, total depth, and focal velocity during our observations were within the ranges of previous years.

Spring Chinook residual/precocious male monitoring

Despite the large numbers of precocious males that are apparently released from the CESRF annually (Larsen et al. 2004; Beckman and Larsen 2005; Larsen et al. 2006), only a small fraction of these fish appear to reach the spawning grounds. Hatchery precocious males may experience high mortality, migrate out of the study area after release, and/or fail to migrate back to the spawning grounds. Although the occurrence of some of these factors were observed in this or other studies (Larsen et al. 2004; Beckman and Larsen 2005), we do not know the relative contribution of each of these factors towards the low abundance of precocious males on the spawning grounds.

Mortality of hatchery precocious males may be due to high angler exploitation, starvation, or predation. There is considerable angling pressure focused on trout in the Yakima River and anglers have at times commented on the number of precocious Chinook males caught, particularly in 2001. However, it is illegal to keep Chinook salmon in the upper Yakima River. Furthermore, studies have shown that hatchery origin fish released into the natural environment have lower survival than natural origin fish, presumably because of their inability to find food or avoid predators (White et al. 1995; Weber and Fausch 2003).

It has been documented that some hatchery precocious males move downstream out of the spawning areas and have been detected as far downstream as Bonneville Dam on the Columbia River (Larsen et al. 2004; Beckman and Larsen 2005). In northern Oregon, precocious males were documented to have migrated at least 800 km and past three dams to reach salt water and return to the Umatilla River (Zimmerman et al. 2003). Hatchery precocious males were collected migrating both downstream in the spring and upstream during the summer (Larsen et al. 2004; Beckman and Larsen 2005). The downstream migrations occurred during the smolt out-migration period and the upstream migrations occurred at the time of adult spawning immigration. If precocious males migrate downstream and then environmental conditions turn poor before they are able to migrate back upstream, then they are likely to die. The lower Yakima River becomes lethal for salmonids during many of the hot summer months when precocious males might attempt to ascend the river. If the factors contributing to hatchery fish mortality in the river are reduced or the conditions in the river are favorable for migration back to the spawning grounds (e.g., favorable flows and low angling pressure), then presumably the number of hatchery precocious males on the spawning grounds could increase dramatically. However, the range of conditions that we evaluated in this study, which included both high and low flow years, provide a reasonable range of what can be expected in the future.

Most of the hatchery precocious males that we encountered were located downstream of spawning areas. The lower and upper Yakima Canyon typically contain less than 1% of the upper Yakima Basin redds (Yakama Nation, unpublished data) and yet averaged 59% of the estimated number of hatchery precocious males during the spawning season. Many of the hatchery precocious males on the spawning grounds were observed in a reach that had relatively little spawning activity, whereas the natural origin precocious males were mainly in the areas with high spawning activity. The spawning area where many of the hatchery precocious males were observed was at the lower end of the spawning distribution. It also happens to be located closest to the Yakima Canyon where the highest abundance of precocious males that were not on the spawning grounds was observed. In the Wenatchee River, very few hatchery precocious males were observed on the spawning grounds, but a considerable number were captured migrating upstream at a location downstream of the spawning areas (Murdoch et al. 2007). These fish may have also distributed themselves below the main spawning areas as we observed in the Yakima Watershed. This behavior is in contrast to natural origin precocious males that are rarely observed moving upstream past dams in the Yakima or Wenatchee watersheds, suggesting that natural origin precocious males have adopted a strategy of remaining on or near the spawning grounds and thus conserving energy and promoting growth and testes development. Some hypotheses as to why sexually mature hatchery precocious males, most of which are exuding milt at the time of sampling, are located in areas away from where most of the spawning activity occurs include: lack of energetic capacity to swim back upstream to the spawning grounds; inappropriate downstream migration behavior for their life-history strategy; late migration timing; and inability to locate areas with spawning females after they had migrated downstream of spawning areas. Younger salmon, such as precocious males and jacks, typically migrate back to the spawning grounds later than older salmon (Knudsen et al. 2006; Murdoch et al. 2007) and may migrate during unfavorable environmental conditions.

Cle Elum Hatchery origin fish are only released at age 1, which eliminates the possibility that age 0 hatchery precocious males will have the potential to spawn. In the absence of hatchery releases, age 0 precocious males are generally more abundant in the spawning areas than age 1 precocious males, so the hatchery is skewing the precocious male composition to an older age and larger size. This is in stark contrast to anadromous hatchery fish which typically mature earlier than wild fish and often at a smaller size-at-age (Knudsen et al. 2006). It is interesting to note that few incidences of precocious male maturation at age 0 have been observed in the Yakima hatchery (Larsen et al. 2004). In addition, attempts to experimentally produce age 0 precocious males by high feeding rates in the hatchery did not produce any precocious males in 2002 (Farrell 2003). These fish emerged at the average emergence time of the population. It is possible that only the fish that emerge very early and experience good growth have the potential to precociously mature at age 0 (Larsen et al. 2007). However, because precocious males were not used in the broodstock, we cannot eliminate the possibility that genetics also influenced the absence of precocialism (e.g., Heath et al. 1994; Unwin et al. 1999).

Hatchery age 1 fish may be competitively superior to wild precocious males because hatchery precocious males are larger. Larger salmonids typically dominate smaller ones in behavioral contests (McMichael et al. 1999). We have observed a number of instances where hatchery precocious males displaced wild precocious males from redds or from preferred locations on redds. Behavioral dominance is important because dominant fish are more likely to be close to spawning females and hence more able to fertilize eggs (Garant et al. 2003). Dominant fish are better able to choose which locations pose the best chance for spawning success. Our behavioral observations suggest that per capita fertilization rates of hatchery precocious males should be higher than that of wild precocious males. However, sneaking strategies of smaller individuals may also be successful.

We have identified some issues that could potentially contribute to the underestimation of precocious male numbers during our peak snorkel counts. We may have underestimated the number of active redds by spooking adults or by floating at times when adults are temporarily away from their redds. However, we rarely observed precocious males on redds without adults being present and this finding was also supported by work in the Salmon River drainage (Gebhards 1960). Gebhards (1960) concluded that precocious males were generally only found in areas where there was spawning activity and were usually found in the bowl of the redd, and “the yearling males remained constantly within the redd.”

Other reasons include the possibility that precocious males may have been hiding away from the redds, were scared off the redds, were moving between redds, or were present in greater numbers before or after our peak count. Additional snorkeling efforts along the banks in 1998 and 2007 did not find hatchery precocious males in hiding areas such as undercut bank in the vicinity of spawning areas, and multiple reach surveys conducted in 2007 and 2008 did not suggest greater numbers of precocious males on the spawning grounds the week before or after our peak of spawning surveys. We have also observed that repeated counts of precocious males at three different times of the day in the same reach were similar. This suggests that either our counts were accurate or that our bias was consistent. However, our estimates of fish away from redds, that were generated from electrofishing were higher in some years than those generated from snorkeling in the Thorp reach, suggesting that snorkeling may underestimate abundance. In short, if we underestimated the number of precocious males on the spawning grounds then our numbers should be treated as indices.

Our study suggests that hatchery precocious males are unlikely to contribute a high proportion of genes in the Yakima Watershed when the number of anadromous adult returns is high, but contributions could be high when anadromous adult numbers are low. The highest abundance of hatchery precocious males that we estimated on the spawning grounds during any year was 78. This is a small proportion of the spawners when anadromous spawners number in the thousands, but relatively large when the abundance of spawners is in the hundreds. This range of anadromous fish abundance has been observed in the upper Yakima Watershed. In a separate DNA pedigree study conducted in an artificial spawning channel (Schroder et al. 2006), hatchery and natural origin precocious males of the upper Yakima spring Chinook salmon stock have been documented to sire offspring. In addition, precocious maturation appears to be highly heritable in Yakima spring Chinook salmon (Pearsons et al. 2007). In short, it appears that the genetic contribution of hatchery precocious males on the spawning grounds is related to anadromous fish abundance and those factors that influence the abundance of precocious males on the spawning grounds. Variation in the precocious male contribution suggests that domestication risks may vary among years.

Domestication monitoring program-predation mortality and competitive dominance

Predation Mortality

The wild Yakima River spring Chinook were found to be slightly over two percent more successful at surviving the predation trials than the first generation of the hatchery control population during 2003 and 2004 (Fritts et al. 2007). Beginning in 2005, the supplementation population has generally exhibited an equal to a slight survival advantage over the hatchery control population (Fritts and Stockton 2010). Unfortunately the Naches population has not been available for all years to use as a comparison but the years we have seem to indicate a decreasing trend in survival relative to the other two populations. It is unknown whether the Naches are decreasing in survival or the other two groups are increasing. It is also unknown whether the past few years are the beginning of a trend or just natural variation.

We speculate that it is possible to detect differences in survival between the hatchery, supplementation, and wild Naches origin fry in some years and not to detect differences in other years due to changes in selection pressures between years. For example, assume that the numbers of adults used for broodstock at the CESRF were to remain relatively constant and the numbers of adults spawning naturally fluctuated by several orders of magnitude. It is theoretically possible for the supplementation and Naches populations to express varying degrees of predation vulnerability from year to year due to density dependent selection pressures in the river environment. Offspring from an abundant run of adults may experience less predation pressure per capita from a constant level of predation, thus more juveniles could survive that do not express traits that are advantageous to avoid predation. If these fish survive to spawn, they could produce more offspring that inherit those traits, which may limit our ability to detect a difference between the two origins. Supplementation may initially decrease the per capita predation pressure on fry because it increases the abundance of fry relative to predators. However, it is likely that the predator population will eventually increase in abundance if more prey continues to be available during the “building stage” of supplementation (Pearsons 2002). The opposite would be true for the offspring of a weaker run of adults and there could be greater differences in the two origins that we would be able to detect. Thus, in some years the per capita predation selection could be very similar in natural and hatchery environments (no selection) and in other years the selection differential could be large. In addition, because we do not want to adversely impact the Naches population, we use far fewer adults as broodstock for the study fry than is used for the hatchery and supplementation fry. This greatly increases the chances that individual differences in the Naches adults that we collect will influence the results of the study and therefore may not be representative of the Naches population in some years. This study is still in the early stages and is only in the second generation of hatchery culture. If domestication effects are cumulative over multiple generations (Araki et al. 2008), then it is likely that the years with different predation vulnerability will begin to outnumber the years where there is no difference as the fish are cultured in the hatchery for multiple generations.

Steps were taken to ensure any differences that are detected in survival can be attributed to genetic differences. The great care that is taken to size match the fry is important to ensure the results are not affected by size-influenced predation. Smaller fish may be more vulnerable to predators because of slower swimming speed (Taylor and McPhail 1985) or less likely to be gape limiting to a predator (Pearsons and Fritts 1999). Studies have shown that smaller salmonids are more vulnerable to predators than larger salmonids (Patten 1977; Hargreaves and LeBrasseur 1986), such as was evident all but one year (2012) in the small and large fry that were individually marked during the trials. The sizes of the predators in each net pen were similar in order to decrease the chances of differential size selective predation. Alternating the mark type each origin of fish received between net pens ensured that any marking effect would not influence our conclusions. Although, it is unlikely, we cannot exclude the possibility that these findings were influenced by a maternal effect (Heath and Blouw 1998). However, if maternal effects were occurring one would expect to see significant changes in survival as fish get older because it is believed that maternal effects are most pronounced in young fish (Heath et al. 1999). We did not detect changes in survival through time suggesting that maternal effects were not prevalent in our experiment. Finally, we found very small to no difference in the background mortality of the three groups.

The results of this experiment are also more likely to be representative of the whole population than other studies because we tested the offspring of far more families than any other study of predation and domestication that we are aware of. Only testing a few families increases the chances that any differences would be due to a single adult that had genetic attributes that made them exceptionally good or poor at avoiding predators. Using two types of predators also ensured that the fry would require a more complete suite of predator avoidance tactics. During short observations immediately after introduction of the fry into to net pens, the fry were observed to form a single school and swim along the bottom of the net pens where two or three predatory attacks by the sculpins would be witnessed within the first five minutes. When the fry were recovered on the last day of the trials, they were generally higher in the water column beneath the overhead cover where they were safe from the sculpin but still vulnerable to the trout. Qualitative observations of the stage of decomposition of fry in the stomach contents of the predators showed that both species consumed several fry and that the sculpins consumed most of the fry early during the trial while the trout consumed fry throughout the duration of the trial.

Because the prey fish were treated identically, any differences found should be due to genetic differences and not abnormal behavior that is learned in the hatchery environment. This means that any differences that we find could be expressed in the natural environment. However, because the experiments were conducted in an artificial environment, we do not know how differences will be manifested in the natural environment. For example, in years of low predation pressure, no differences in survival of the offspring may occur. The addition of the Naches line provides a control for baseline predator avoidance ability that will not be influenced by domesticating influences. The Naches control, when available, will be used to compare to the hatchery and supplementation line. If the difference in survival between the Naches and the supplementation line remains similar over generations and the hatchery control line survival diverges compared to the other two lines of fish, then supplementation should be viewed as a success in terms of producing fish that are able to perform in a similar manner as naturally produced fish when encountering piscine predators. Since these returning supplementation fish are expected to spawn and produce viable offspring, any deficiencies expressed in predator vulnerability could limit the success of supplementation.

This is the ninth year of an ongoing study that has shown a diversity of results thus far. Of the peer-reviewed literature that have found predation differences due to domestication selection, those studies have only lasted one or two years and have generally represented a smaller number of families (Table 20). It is important to evaluate behavioral studies for multiple years using high numbers of families because of the annual differences in selection pressures and variability between individuals within populations.

Table 20. A comparison of studies that have tested the effects of domestication on predation vulnerability. Species tested, origins compared, number of generations under hatchery culture, founding stock, rearing environment, years tested, number of families tested, and the metric used to asses vulnerability are compared.

Study	Species	Comparison	H gen	Stock[a]	Rearing[b]	Yrs	Families	Metric[c]
1	Brown trout	wild vs. hatchery	1-2	S?	D	1	5-7	B
2	Steelhead	wild vs. hatchery	1-7	S	S	1	7-10	M
3	Brown trout	wild vs. hatchery	5	S	S	1	9	B
4	Atlantic salmon	wild vs. farmed	7	S	S	1	8	B
5	Steelhead rainbow trout	wild vs. wild/farmed hybrid	5+	D	S	1	11	B
6	Brown trout	wild vs. hatchery/wild hybrid	5	S	S	1	Up to 64 (mixture)	B
7	Atlantic salmon	wild vs. farmed	7	S	S	2	?	B
8	Masu salmon	wild vs. hatchery vs. farmed	7+	D	D	1	?	B
This study	Chinook salmon	Supplemented vs. hatchery w/ wild control	1-2	S	S	8	12-59	M

[1]Alvarez and Nicieza (2003); [2]Berejikian (1995); [3]Ferno and Jarvi (1998); [4]Fleming and Einum (1997); [5]Johnsson and Abrahams (1991); [6]Johnsson et al. (1996); [7]Johnsson et al. (2001); [8]Yamamoto and Reinhardt (2003); *Present study

[a]Same (S) or different (D) founder stock.

[b]Same (S) or different (D) rearing environment.

[c]Behavior (B) or mortality (M).

The first two years of the study (2003-2004) were the last two years where we had the opportunity to use offspring of truly wild spring Chinook from the upper Yakima River because the first adult returns from the Cle Elum Hatchery spawned naturally in 2001. There was a slight chance that a naturally produced jack used for 2003 brood (2004 study population) could have been sired by a hatchery jack in 2000 but we consider that unlikely given the small proportion of hatchery jacks in 2000 relative to the wild population. The hatchery control population began with the spawning of returning hatchery origin fish in 2002, our 2003 study population. We are currently completing the third generation of the hatchery control population and will evaluate our results by generation after the brood year 2013 trials are complete to conclude this study.

Competitive dominance

We have observed the full range of possible outcomes in dominance between supplementation and hatchery fish. Supplementation fish dominated hatchery fish in 2005, opposite results were found in 2006, 2008 and 2013, and neither was dominant in 2007, 2009, 2010, 2011 or 2012. We speculate that the differences that we detected in 2006 and 2008, which was opposite of our 2005 results, was caused by higher aggression of hatchery fish. We think this is likely because the relative dominance between supplementation and Naches fish did not change between 2005 and 2006. Also, the decrease in dominance of supplementation relative to the Naches fish between 2006 and 2008 was similar in magnitude to the decrease in dominance of supplementation relative to hatchery fish during the same years. This assumes that dominance ability in the Naches population will remain relatively constant from year to year because they are not subject varying degrees of hatchery input. Dominance was again equal between supplementation and hatchery fish in 2007, but without the Naches control for that year, there is no way to know whether supplementation dominance increased for one year or hatchery dominance decreased for one year. The difference in relative dominance between years appeared to be driven mainly by the hatchery control fish because their pairings with both supplementation and Naches fish tracked very closely in most years (Figure 14).

At this time we cannot think of any compelling reason why offspring of wild (2003-2004, Pearsons et al. 2007) and the early supplementation population (2005, with minimal natural spawning first generation hatchery influence) appeared to dominate the hatchery population during the first three years of this study. Since that time, there has been no obvious trend of one group becoming more dominant. Lynch and O'Hely (2001) predict that it typically takes 10 to 20 generations for a supplemented population to reach 50% equilibrium in terms of the genetic load from captive breeding depending on strengths of selection in the hatchery and natural environments and proportion of hatchery fish spawning in the wild. If this is the case, then it seems reasonable that the hatchery and supplementation fish could exhibit this flip-flopping of dominance between years for quite some time as deleterious alleles are expressed at different rates depending on environmental pressures and the proportion of hatchery fish on the spawning grounds until they begin reaching equilibrium.

The types and relative frequencies of different types of agonistic behaviors that were observed (e.g., chases, butts, nips) among the three lines were not significantly different, but the frequency of aggression did differ somewhat among the three lines. These findings suggest that stock differences and domestication influences do not affect the types and relative frequencies of behaviors that are used. This is in contrast to a study of hatchery and wild *O. mykiss*, where hatchery fish used more physical contact in aggressive interactions than wild fish and were also more aggressive (McMichael et al. 1999). However, consistent with that study, supplementation and hatchery fish were generally more aggressive than wild fish in this study.

The differences in dominance and aggression that we observed were likely due to an interaction between genetic changes that occurred from fish culture, differences in stocks, and a year effect. However, we cannot exclude the possibility that changes were caused by a maternal rearing environment effect (e.g., not a genetic effect). This might occur if hatchery rearing caused phenotypic differences in females that were passed on to progeny. We believe that this was unlikely to have had much of an effect on our experiments because 1) egg sizes of hatchery and wild fish were not significantly different (Knudsen 2005), and 2) fish were tested approximately 4 months after hatching. Most studies that have reported maternal effects in fish have documented relationships between female size and progeny size (Heath and Blouw 1998). We attempted to control for size effects by size matching our fish. Maternal effects are more likely to occur when fish are very young. In a review of maternal effects in fish, Heath and Blouw (1998) concluded "maternal effects in fishes are usually negligible beyond the early juvenile life stages."

With the exception of this study, annual differences in competitive dominance associated with domestication have generally not been evaluated. Most studies that have evaluated this topic are based on one year of study and none have been longer than two years (Table 21). The study presented in this report combined with the work presented in Pearsons et al. (2007) represent eleven years of study. We have seen considerable annual differences in our results. If we had restricted our study to a single year, then we may have concluded that domestication positively, negatively, or neutrally influenced competitive dominance. This finding suggests that we should use caution when interpreting dominance results that do not evaluate multiple years of study.

In comparison to our observations, juvenile coho salmon reared in hatcheries have been documented to be more aggressive than wild fish (Swain and Riddell 1990; Berejikian et al. 1999) or less aggressive (Berejikian et al. 1996). Furthermore, Einum and Fleming's (2001) meta-analysis of aggression revealed that hatchery fish were more aggressive than wild fish. We suspect that the differences in findings are caused by 1) the duration and type of hatchery practices, and 2) differences in the rearing environment of the fish tested. Most, if not all, of the studies that have previously been conducted outside of the Yakima Basin have used hatchery fish that have been under culture for more than 1 generation and frequently these are of non-local origin (Table 21). If genetic changes or maternal effects are additive, then it is likely that larger differences in aggression will be detected with each additional generation of fish culture. Furthermore, fish that are collected from natural environments and compared to fish reared in hatchery environments are likely to produce differences because of the differences in rearing conditions. For example, in another study, we found that spring Chinook smolts reared in the hatchery dominated salmon smolts that were reared in the Yakima River. Larger fish generally dominated smaller fish, but the size difference did not have to be as great for hatchery fish to dominate as wild fish (Pearsons et al.,WDFW, unpublished data). In short, hatchery fish were dominant over wild fish in contest competition experiments unless wild fish were sufficiently larger than hatchery fish. In a study of coho salmon, Rhodes and Quinn (1998) reported similar findings.

Table 21. Comparison of dominance studies that relate to domestication selection of salmonids of varying origins.

Study	Species	Comparison[a]	Hatch. gener.	Stock	Yrs	Number of families	Trial type[b]	Replicates	Metric[c]
1	Steelhead	W vs. H	4-7	Same	1	13 W; 18H	C	16	A, P, C
2	Coho	W vs. C	1	Different	1	15	C	44	A, P
3[e]	Atlantic	W vs. F	6-10	Different	1	?	C	218	P
4	Grayling	W vs. W H vs. H	2	Same	2	?	?	30?	A
5	Coho	W vs. W H vs. H	5	Different	1	10 & 13 W 11 & 191 H	M	21?	A
6[f]	Chinook	H vs. H	1 vs. 5	Same	1	5	C	40	P, F, A
7[g]	Chinook	W vs. H	1	Same	1	6	C	89	P, F, A
8	Chinook	W vs. H	1	Same	2	54-59	C, S	229, 276	P, F, A
9	Atlantic	W vs. F	7	Different[d]	1	6+ W, 8 F	C	30 stream	P, F, A
9	Atlantic	W vs. F	7	Different[d]	1	6+ W, 8 F	C	15 tank	A
10	Brown trout	W vs. H	10	Same	1	Up to 64 (mixture)	C	12	A
This study	Chinook	S vs. H w/ W control	1-2	Same	8	23-52	C, S	157-299	P, F, A

[1]Berejikian et al. (1996); [2]Berejikian et al. (1999); [3]Metcalfe et al. (2003); [4]Salonen and Peuhkuri (2004); [5]Swain and Riddell (1990); [6]Wessel et al. (2006); [7]Farrell et al. (2003); [8]Pearsons et al. (2007); [9]Fleming and Einum (1997); [10]Johnsson et al. 1996; *Present study.

[a] Offspring of wild (W), supplementation (S), hatchery or sea-ranched (H), farmed (F), and captive brood (C).

[b] Contest (C), mirror image (M), and Scramble (S).

[c] Metrics used to assess dominance are aggression (A), position (P), color (C), and food (F).

[d] Farmed population founded, in part, from wild population.

[e] Subjects were not size matched.

[f] Subjects were within ± 3 mm FL.

[g] Subjects were within 4% FL.

The results presented in this chapter are part of a long-term study that attempts to evaluate if hatchery supplementation alters competitive dominance relative to an unsupplemented reference population and a hatchery population. We will continue to measure the variables described in this study through the third generation of the hatchery control population and determine if there are any changes in dominance that can be attributed to domestication selection.

Spring Chinook reproductive success/spawning channel

Ninety-seven percent successes were achieved at inferring parent-offspring relationships. There is a very uneven pattern of reproductive success among the candidate parents. Based on the subsample of 2,845 fry that were successfully assigned parents, the range of inferred reproductive output among males was 0 - 333 fry; the range for the same period in reproductive output among females was 0 - 165 fry. Some of the dam-sire matings we inferred are well supported (there were a lot of fry assigned to them) and some are weakly supported (not many fry were assigned to them). Caution should be used when interpreting dam-sire-fry combinations that were inferred rarely. Future integration of fecundity estimates for spawners will enrich the interpretation of these estimates of reproductive output.

Interpretation of the inferred parental reproductive output based on parentage assignments by genetic analysis requires the consideration and analysis of individual fish attributes, including fecundity and body size, the closed nature of the experimental environment in which sub-dominant males had a more limited number of alternative females to court than they might have had in an open system, and relative stocking levels and synchronicity of spawning.

Spring Chinook Genetic stock separation-juveniles

Collection of smolts at the Chandler Trap in 2012 utilized a sampling design intended to yield a sample that was proportional to the number of smolts passing the Chandler Trap. Sampling a proportional number of smolts was important to determine an accurate percentage of smolts from each stock that were outmigrating from the basin. Developing the sampling strategy for identifying a “standard” versus “peak” day of smolts that were in the trap and applying a sampling goal for those days allowed for a proportional sample. Subsampling the smolts collected for genetic analysis provided a best fit to the actual passage of smolts for a given day.

Monitoring the relative abundances of Chinook smolts in the Yakima River from the three different populations of spring Chinook (upper Yakima River, American River, and Naches River) and the two populations of fall Chinook (Marion Drain and lower Yakima River) requires the ability to estimate population composition of smolts outmigrating past Chandler trap. Because all five Chinook populations are intermingled when they pass Chandler trap, and the vast majority are unmarked and untagged, the only way to determine population-of-origin is by genetic analysis. This method requires that sufficient genetic differences exist among these populations in the Yakima River basin.

A baseline of 19 individual collections from the five populations in the Yakima River basin was used for the population-of-origin assignments of the outmigrating smolts. The baseline collections as a whole had higher genotyping failure compared to the Chandler smolt samples. Scales were taken from carcasses on spawning grounds for most baseline collections; therefore, DNA quality was presumably poorer than the Chandler smolt collection where tissue was collected from live fish. The upper Yakima

River tissue collections were also taken from live fish at the hatchery and, therefore, genotyping success was higher for this collection than the other baseline collections.

Assessment of spring or fall smolts by morphological and genetic analysis revealed agreement with 75 individuals being identified differently between the two methods. Identification as a spring or fall smolt was the same for 775 smolts collected during the January – February, March, April, May, and June – July time strata.

The majority of the assignments between January and May were from the three spring stocks. The upper Yakima River spring stock accounted for the highest average percentage (56.4%) of smolts present in that period. Rank in abundance of the three spring stocks was the same in the three time strata (January-February, March, April, and May) with upper Yakima River spring stock having the most. The June-July time stratum was predominately composed of the fall Chinook stocks, accounting for over 81.5% of the total number of smolts.

Assessment of DNA Mixture Assignments from 2000 – 2010

Mixed stock analysis has been conducted on Chandler smolts since 2000 (Young 2004, Kassler et al. 2005, Kassler 2006, Kassler and VonBargen 2007, 2008, 2009 and 2010, Kassler and Peterson 2011, Kassler and Bell 2012); however the sampling design for samples collected in 2000 – 2003 was not proportionalized during the run. The yearly assignments are therefore not comparable from those years. Beginning in 2004, staff at the Chandler trap utilized a sampling protocol to provide a number of smolts that was relative to the percentage of smolts passing that day. Samples were then subsampled at WDFW to provide a proportional number of samples that would represent the overall passage to be analyzed.

b. Hatchery RM&E

The performance of the YKFP spring Chinook supplementation program has been documented relative to the project quantitative objectives and has been presented annually in the YKFP M&E project overview (Fritts 2012). Briefly, the project appears to be meeting or is making progress towards achieving the project's objectives (Appendix D).

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Appendix A: Use of Data & Products

Raw electronic data files (Database) are secured on the WDFW Corporate server in Olympia, WA, as well as on WDFW district 8 field office personal computers. Data housed on personal computers are duplicated on the local office server which is in turn backed up on the WDFW corporate server in Olympia, WA nightly.

Appendix B: M&E Project Publication List

The following publication list includes technical reports and peer reviewed publications that have been produced from the work under the Yakima/Klickitat Fisheries Project's monitoring and evaluation program.

Amaral, S. V., F. C. Winchell, and T. N. Pearsons. 2001. Reaction of Chinook salmon, northern pikeminnow, and smallmouth bass to behavioral guidance stimuli. Pages 125-144 in C. C. Coutant, editor. Behavioral technologies for fish guidance. American Fisheries Society, Symposium 26, Bethesda, Maryland.

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Appendix C: Detailed Results

Non-target Taxa of Concern

Changes to non-target fish taxa abundance, size, and distribution in the Yakima River and the relationship to hatchery salmon supplementation

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Abstract

Salmon supplementation and reintroduction programs have the potential to negatively impact other valued fish taxa, which are not the target of enhancement (non-target taxa). We evaluated the impacts of spring Chinook salmon *Oncorhynchus tshawytscha* supplementation and coho salmon *O. kisutch* reintroduction (hereafter supplementation) to non-target fish taxa in the upper Yakima Basin after eleven years of stocking approximately one million yearling smolts annually. Field methods included backpack electrofishing and snorkeling in tributaries, and drift-boat electrofishing in the main stem. We used three sequential steps in our evaluation: First, we determined if spatial overlap in distribution occurred between supplementation fish and non-target taxa. Second, if overlap occurred, we determined if a change in abundance, size, or biomass occurred during supplementation. Lastly, if a change occurred we determined if the change could be reasonably attributed to supplementation. Spatial overlap and changes in abundance, size, or biomass were determined to be significant if they exceeded containment objectives. Salmon rarely overlapped cutthroat trout *O. clarkii* and bull trout *Salvelinus confluentus* in tributaries, but some overlap of cutthroat occurred in relatively high elevations of the main stem, and considerable overlap with rainbow trout occurred in tributaries and the main stem. Salmon overlapped mountain whitefish *Prosopium williamsoni* and sucker species (Catostomidae) in the main stem, and dace (Cyprinidae) and sculpin (Cottidae) species in tributaries. With the exception of steelhead *O. mykiss*, the lower 90% confidence limit of abundance, size, and biomass was above the containment objective for non-target taxa that overlapped significantly with salmon. We used rainbow trout as an analog for steelhead. The lower 90% confidence limit of rainbow trout size in both tributaries and in the main stem, were below our containment objectives for steelhead trout. Comparisons of rainbow trout size in tributaries, and size in main stem sections with relatively high and low salmon abundance revealed that these changes were unlikely to be the result of supplementation (before-after-control-impact-paired site analysis: $P > 0.05$). Our data indicate that early stages of salmon supplementation have not impacted valued species in the upper Yakima Basin beyond predetermined containment objectives. However, the monitoring is insufficient for detecting impacts to some non-target taxa and some localized impacts may have occurred.

Introduction

Despite the long history of stocking hatchery salmon into streams, few large-scale evaluations of impacts to the status of taxa that are not the target of enhancement (non-target taxa; NTT) have been conducted that apply to current stocking practices. Many mechanisms of impacts have been documented (Marnell 1986; McMichael et al. 1999; Hawkins and Tipping 1999), but impacts to NTT growth and abundance have generally not been conclusively demonstrated at scales larger than experimental reaches (Fresh 1997; Weber and Fausch 2003). Most large-scale evaluations of hatchery and wild fish interactions have addressed impacts to naturally produced conspecifics (Nickelson et al. 1986; Chilcote 2003; Nickelson 2003) and/or stocking salmon before the smolt stage (Bjornn 1978; Tripp and McCart 1983). Although these studies are illuminating, most contemporary hatchery salmon programs release smolts, and these smolts are released into areas containing species of concern other than wild conspecifics. Releasing smolts is appealing because they provide the highest adult returns and potentially minimize ecological interactions in the freshwater environment. In order to assess risks of contemporary programs, information about the impacts of smolt releases is needed. This is particularly true in watersheds containing threatened or endangered NTT, such as bull trout *Salvelinus confluentus* and steelhead trout *Oncorhynchus mykiss*.

Although it is assumed that releasing smolts poses less ecological risk than stocking earlier life stages, this assumption has not been tested and the practice is clearly not without risk. It is believed that smolts pose lower risks than earlier life stages because they spend less time interacting with NTT. However, hatchery smolts can interact with wild fish during downstream migration and during periods when they residualize in rearing environments. In addition, some hatchery-released yearlings swim upstream of release locations into areas containing listed species (McMichael and Pearsons 2001). Ecological interactions that can occur during migration include competition, predation, behavioral anomalies, and pathogenic interactions (Pearsons and Hopley 1999). If competition does occur, it may be of short duration because hatchery smolts generally move downstream and feed as they migrate or during brief “resting” periods. It is during these “resting” periods that competition may be most intense (Coutant and Whitney 2006).

Chinook *O. tshawytscha* and coho salmon *O. kisutch* are the most commonly cultured Pacific salmon released as yearlings and are the species of consideration in this paper. Studies have demonstrated the potential for stocked salmon to impact wild target and NTT. Hatchery spring Chinook smolts were observed to behaviorally dominate wild smolts and secure the most food and best habitat in laboratory experiments (Pearsons and Ham 2001). Predation by Chinook and coho salmon smolts on naturally produced salmon has also been demonstrated (Sholes and Hallock 1979; Hawkins and Tipping 1999). The release of large numbers of hatchery smolts can change the functional and numerical response of predators to mixed groups of hatchery and wild fish (Peterman and Gatto 1978; Wood 1987; Collis et al. 1995). Depending upon the predator response, the releases can either benefit or harm naturally produced species. Large numbers of hatchery fish can also alter the behavior of wild fish and influence susceptibility to predators or food acquisition (Hillman and Mullan 1989; McMichael et al. 1999).

Finally, hatchery fish have the potential to directly transmit pathogens or increase the susceptibility to pathogens by wild fish (Goede 1986; Bucke 1993; McVicar 1997). Similar interactions can occur if "smolts" residualize, although the intensity or manifestation of the interaction may differ. For example, competition is likely to be more potent locally when fish residualize because they remain in an area, as opposed to more temporal occupation of areas during downstream migration. Increased natural production of the target taxa translates into potentially increased interactions in the freshwater rearing area (Pearsons 2002).

Naturally produced offspring of hatchery coho and Chinook salmon have the potential to impact trout and other species of concern. Coho salmon dominate cutthroat trout *O. clarki* in pool habitats and many cutthroat trout are displaced to riffle habitats in the presence of coho salmon, particularly at warmer temperatures (Glova 1984, 1986, 1987). Sabo and Pauley (1997) suggested that size was equally important as species identity in determining competitive dominance among stream-dwelling cutthroat trout and coho salmon. Coho salmon displace some steelhead trout from pools or alter habitat use within pools that they would occupy in the absence of coho (Hartman 1965; Allee 1974, 1981). Growth of steelhead was lower in experiments with high coho densities than low ones (Fraser 1969). Despite overlap in several key habitat and food variables (Dolloff and Reeves 1990; Nakano and Kaeriyama 1995), Dolly Varden *S. malma,* who are ecologically similar to bull trout, generally occupy microhabitats close to the bottom whereas coho occupy the water column (Bugert et al. 1991; Nakano and Kaeriyama 1995).

Growth and abundance of rainbow trout in high elevation streams of the upper Yakima Basin were not affected when salmonid densities were doubled with naturally produced Chinook salmon parr (McMichael and Pearsons 1998). These controlled experiments were conducted in small to moderate size enclosures. The current study extends the findings of McMichael and Pearsons (1998) by evaluating production scale supplementation of spring Chinook salmon throughout tributary and main stem waters. In addition, impacts from coho salmon reintroduction were also evaluated. Furthermore, this study includes evaluation of interactions from hatchery produced smolts, residuals, and naturally produced offspring of hatchery fish and extends the findings of Pearsons and Temple (2007). In that study, impacts of the early stages of supplementation (5 years) were within containment objectives for *O. mykiss*, cutthroat trout, and bull trout.

In addition to the aforementioned trout species, several non-trout fish taxa occupy areas in sympatry with both natural and supplementation origin Chinook and coho salmon in the Upper Yakima Basin (Temple and Pearsons 2012). Taxa of interest in this study include mountain whitefish *Prosopium williamsoni*, dace spp. sucker spp. (Catostomidae), and sculpin spp. (Cottidae). These taxa have the potential to be impacted through direct and indirect interactions with salmon, but with the notable exception of Temple and Pearsons (2012), interactions with non-trout fish taxa has received little attention in the literature. The literature that has reported interactions between non-trout fish taxa and salmon has generally focused on impacts to salmon (Fritts and Pearsons 2004; Murdoch et al. 2005; Fritts and Pearsons 2006).

In this paper, we examine the impacts to trout and NTT during the early-middle stages of a spring Chinook supplementation program and the reintroduction of coho salmon in the Yakima Basin, Washington (Figure 1). It was unknown whether impacts would occur from smolts that migrated downstream of acclimation sites, residuals that distributed themselves up- and down-stream of acclimation sites, and/or from an increase in the distribution and abundance of naturally produced offspring of supplemented adults. Thus, NTT that were distributed upstream of acclimation sites were not considered immune from risks because residuals might migrate upstream and overlap with NTT, such as has been observed with hatchery steelhead (McMichael and Pearsons 2001), or an increase in distribution and abundance of naturally produced Chinook salmon could result in overlap that was not observed prior to supplementation. Certainly one of the goals of supplementation is to increase the distribution and abundance of target species.

Concerns about the possibility of hatchery fish having negative impacts on valued NTT in the Yakima Basin prompted the development and implementation of a risk containment monitoring program (Bonneville Power Administration 1996; Busack et al. 1997; Ham and Pearsons 2001). Our primary management interest was to determine if changes in the status of NTT exceeded "specified biological limits" (Regional Assessment of Supplementation Project 1992) or "containment objectives" (Ham and Pearsons 2001) relative to the baseline period in which no stocking occurred. The containment objectives are: 0% impact for bull and steelhead trout; less than 5% impact for mountain sucker *Catostomus platyrhynchus*; less than 10% impact for rainbow and cutthroat trout in the main stem; less than 40% impact for rainbow and cutthroat trout in the tributaries (Pearsons and Hopley 1999; original cutthroat trout containment objective was modified by Yakima/Klickitat Fisheries Project 2004); less than 40% impact for mountain whitefish *Prosopium williamsoni*; 90% impact for sucker species (Catostomidae) in the main stem; 65% and 95% for longnose dace *Rhinichthys cataractae* and speckled dace *R. osculus*, respectively, in tributaries; and 90% impact for sculpin species (Cottidae) in tributaries. These containment objectives were developed based on the status (e.g., abundance) of the NTT, their value to fisheries, and their relative value compared to the expected benefits of supplementation (Pearsons et al. 1998). Containment objectives for NTT are evaluated relative to baseline abundance, size, biomass, and distribution. If containment objectives are exceeded for any one of these population parameters, then further action is warranted.

We believe that it is important to report results within the context of the life-span of a supplementation program. An early stage of supplementation, such as the "broodstock" stage, is less likely to produce impacts than a later stage such as the late "building" stage (Pearsons 2002). However, waiting to report results until a supplementation program has matured can delay the presentation of important information for up to 30 years, and therefore limit information that could be used to help improve current management decisions (Pearsons 2002). Indeed, many scientific reviews about hatchery and wild fish interactions identify the paucity of information that is available to evaluate these risks (Fresh 1997; Weber and Fausch 2003). Therefore, we present the findings of the early to middle stages of supplementation knowing that impacts could change during later stages.

We acknowledge that impacts in areas of overlap cannot be definitively evaluated without adequate controls. Unfortunately, spatial controls for most large river systems are difficult to find and we could not find an adequate spatial control for trout in the upper Yakima River. However, achievement of management objectives can be evaluated relative to fixed standards, such as containment objectives, and performance of NTT relative to containment objectives can be used to prioritize impact evaluations. For example, if the status of an NTT has not changed after stocking, then it would not be a high priority to evaluate farther. In contrast, if the status of an NTT decreases below a certain level then further evaluation should be initiated. We believe that the containment objectives are suitable triggers for prioritizing evaluation. Furthermore, if environmental conditions do not change appreciably before and during supplementation, then impacts can be evaluated using temporal controls. The results presented in this paper are the early stages of risk containment monitoring associated with the Yakima/Klickitat Fisheries Project (YKFP).

Methods

Study Area

The Yakima River Basin is a large river system that drains into the Columbia River near Richland, Washington. The upper Yakima River Basin, which is the subject of this paper, is located upstream of Roza Dam (Figure 1). Historically large numbers of salmon and steelhead returned to the upper Yakima Basin (Bonneville Power Administration 1996). Coho salmon were extirpated by the early 1980s and spring Chinook salmon have been severely depressed (Bosch 2004). Steelhead (mid-Columbia Evolutionarily Significant Unit) and bull trout are currently listed as threatened (National Marine Fisheries Service 1999; U.S. Fish and Wildlife Service 1998). Rainbow trout in the Yakima River provide one of the best wild trout fisheries in Washington (Krause 1991; Probasco 1994) and westslope cutthroat trout are present in many high elevation tributaries. Mountain whitefish and sucker species are widely distributed in main stem areas, although mountain suckers are rarely observed. Sculpin species are widely distributed in tributary areas, while speckled dace inhabit low elevations and longnose dace inhabit mid-to high elevation areas of tributary streams.

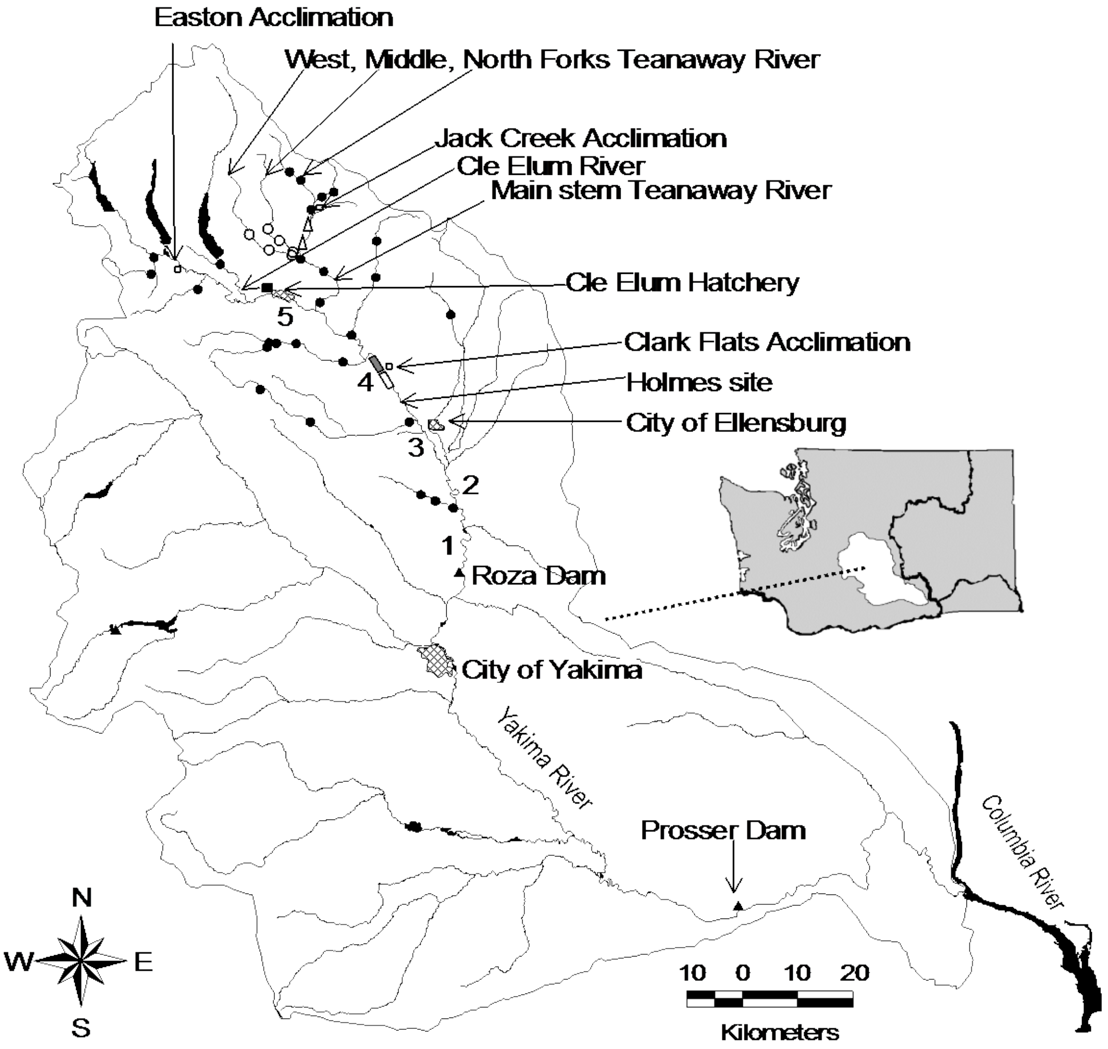

Figure 1. Map of the Yakima River Basin. Darkened circles are tributary NTT distribution sampling sites. Teanaway Basin tributary BACIP control and treatment sites are represented by open circles and triangles, respectively. Bold numbers represent Main stem Yakima River non-target taxa (NTT) monitoring sites where 1, 2, 3, 4, and 5 represent the LCYN, UCYN, EBURG, THORP, and CELUM sections, respectively. Main stem BACIP control and treatment sites are represented by shaded and open rectangles, respectively.

The main stem and tributaries of the Yakima River differ in their physical properties and the way that they are managed. Three dams regulate the flows in the main stem Yakima River. Peak flows during the spring have been truncated and flows are artificially high during the summer. The main stem is heavily fished for trout and a catch-and-release regulation has been in effect since 1990. Tributaries have less flow regulation, but lower portions of tributaries can experience low flows from irrigation withdrawals. The main stem upper Yakima River is currently managed as a catch and release fishery, but anglers are allowed to retain two trout greater than 203 mm in tributary fisheries.

Chinook and coho salmon hatchery programs

Spring Chinook and coho salmon yearlings were released into the upper Yakima Basin for the first time during spring 1999 as part of the YKFP. The goal for both of these species is to increase natural production and to provide harvest opportunity using artificial propagation while keeping adverse impacts within specified biological limits (Bonneville Power Administration 1996; Fast and Craig 1997; Bosch 2004). Approximately 727,090 Chinook salmon smolts have been released annually in the upper Yakima River from 1999 to 2013 (Table 1). Broodstock for the spring Chinook program were natural origin upper Yakima stock collected at a trapping facility at Roza Dam. Chinook were spawned and juveniles were reared at a hatchery facility in Cle Elum, Washington (Knudsen et al. 2006). Spring Chinook salmon yearlings were transported to acclimation sites during January and February and volitionally released into the Yakima River from the Easton and Clark Flats acclimation sites, and from the Jack Creek acclimation on the North Fork of the Teanaway River (Figure 1). Fish were permitted to volitionally migrate between March 15 and May 31 and averaged 120 mm fork length (FL) when released. At the end of May all fish were forced out of the acclimation sites into the river. Mean travel time of migrants from acclimation sites to a detector near Prosser Washington (river kilometer [RKM] 75.6 measured from the confluence with the Columbia River) is about 24.3 days. However, a large number of Chinook salmon residualize in the upper Yakima Basin (Larsen et al. 2004; Pearsons et al. 2005).

Table 1. Numbers and location of yearling spring Chinook salmon released in the upper Yakima River annually.

Brood Year	Release Year	Clark Flats	Easton	Jack Creek	Total
1997	1999	229,290	156,758		386,048
1998	2000	221,460	230,860	137,363	589,683
1999	2001	232,563	269,502	256,724	758,789
2000	2002	285,954	263,061	285,270	834,285
2001	2003	80,782	39,106	250,348	370,236
2002	2004	266,563	290,552	279,789	836,904
2003	2005	273,377	267,711	283,604	824,692
2004	2006	280,598	273,440	231,410	785,448
2005	2007	287,127	281,150	291,725	860,002
2006	2008	209,575	217,932	215,288	642,795
2007	2009	265,907	254,540	250,818	771,265
2008	2010	280,253	287,857	281,195	849,305
2009	2011	279,123	281,395	272,423	832,941
2010	2012	264,420	264,362	265,999	794,781
2011	2013	255,290	248,454	265,438	769,182

The coho salmon reintroduction program releases an average of 386,620 coho in the upper Yakima River annually (Table 2). Release locations have been variable since 1999 as the feasibility of re-establishing extirpated coho runs into Yakima Basin tributaries, main stem areas, and reservoirs is evaluated. Smolt releases have been the primary life-stage released although experimental releases of fry and adults have also been made. Broodstock has generally come from lower Columbia River sources, although one long term goal of the program is to transition to localized broodstock when sufficient numbers of adults return. Mean travel time of migrants from the Holmes release site to a detector near Prosser Washington in 2003 (RKM 75.6) was 41.9 days. Very few coho salmon have been observed to residualize in the upper Yakima River. Coho salmon sizes were not recorded at the time of release, however, two to three months after release, hatchery origin coho smolt fork lengths averaged 157 mm in the migration corridor. Prior to 1999, coho salmon were released in the middle portion of the Yakima River, a considerable distance below Roza Dam. Coho salmon observed in the upper Yakima River prior to 1999 were likely the result of downstream releases. More detail about the study area and background of the supplementation project has been previously described (Busack et al. 1997; Pearsons and Hopley 1999; Ham and Pearsons 2000). Early findings indicate that Chinook and coho hatchery programs are increasing the abundance and distribution of spawners in the upper Yakima Basin (Bosch 2004; Bosch et al. 2007).

Table 2. Numbers of coho salmon released in the upper Yakima River annually.

Brood Year	Release Year	Total Released
1997	1999	498,000
1998	2000	494,676
1999	2001	466,464
2000	2002	314,450
2001	2003	328,000
2002	2004	428,065
2003	2005	332,000
2004	2006	339,791
2005	2007	299,127
2006	2008	382,000
2007	2009	364,412
2008	2010	333,920
2009	2011	419,184
2010	2012	443,030
2011	2013	356,177
Average		386,620

General approach

We used three sequential steps in our evaluation of impacts to trout and NTT. First we determined if distributional overlap between supplemented salmon and NTT occurred. Second, if overlap occurred we determined if a temporal change in abundance, size, or biomass occurred after supplementation. Finally, if a change occurred, we determined if the change could be reasonably attributed to supplementation (Table 3). Increases in distribution of the target species can result in spatial overlap with trout resulting in the potential for impacts. If overlap is less than or equal to containment objectives, then impacts are assumed to be acceptable. However, if distributional overlap exceeds containment objectives, then changes to abundance, size, and biomass were evaluated. Changes were evaluated by comparing the abundance, size, and biomass of trout and abundance and size structure of other NTT before and after salmon were released into the upper Yakima Basin (1999). A change in abundance, size, or biomass does not demonstrate that the hatchery caused the impact. Changes in abundance, size, or biomass can be used to trigger further investigation to identify the causes of changes in monitoring variables. We used a conservative approach to trigger further investigation because of the high inter-annual variability of population parameters (Ham and Pearsons 2000). We used the lower 90% confidence limit (CL) of the response variables (abundance, size, and biomass) as the trigger to initiate further investigation. This ensured that there was a 95% probability that the true value was above the lower CL. If the lower 90% CL was lower than the containment objective, then we attempted to determine causation. Confidence limits associated with parameter estimates were computed based on a *t*-statistic with *n*-1 degrees of freedom (Sokal and Rohlf 1981).

Table 3. Field sampling location (Loc.) and abundance estimation methods used for the following tributary (Trib) and main stem (Main) species; bull trout (B), cutthroat trout (C), rainbow trout (R), dace species (D), sucker species (K), sculpin species (P), mountain whitefish (W), naturally produced spring Chinook and coho salmon (S, O), and hatchery origin spring Chinook salmon (H). Additional abbreviations are as follows: Snork = Snorkeling; BP Efish = backpack electrofishing; DB Efish = drift boat electrofishing; Surrogate Spp. Eff. Exp. = site and size specific surrogate species efficiency expansions; NA = not applicable, MR = mark-recapture; WMI = weighted mean generated from index sites; ORCO = overlap judged relative to containment objectives; 90%CLCO-*t* = 90% confidence limit judged relative to containment objectives; *t* = two-sample student's *t*-test; BACIP = before/after control/impact paired test.

Species	Loc.	Field Method	Spp. Effic. Derived from	Estimation Method	Analysis
			Overlap		
B	Trib	Snork	NA	% Overlap	ORCO
C, R, D, K, P	Trib	BP Efish	NA	% Overlap	ORCO
S, H, O	Trib	Snork / BP Efish	NA	% Overlap	ORCO
R, C, S, H, O	Main	DB Efish	NA	% Overlap	ORCO
			Before / After		
R, S, H, O	Trib	BP Efish	R, S, H, O	Removal Based WMI	90%CLCO-*t*
R, C	Main	DB Efish	R	MR Based WMI	90%CLCO-*t*
S, H, C	Main	DB Efish	R	Surrogate Spp. Eff. Exp.	90%CLCO-*t*
M	Main	DB Efish	-	Visual count	90%CLCO-*t*
K	Main	DB Efish	-	Visual count	90%CLCO-*t*
			Causation		
R	Trib	BP Efish	R	Treatment / Control	BACIP
R	Main	DB Efish	R	Treatment / Reference	BACIP
K	Main	DB Efish	-	Treatment / Reference	BACIP

To determine causation we compared abundance, size, and biomass in control and treatment sites (e.g. tributaries). Where this was not possible (e.g., main stem), we compared abundance, size, and biomass of NTT upstream and downstream of a hatchery acclimation facility. We reasoned that the magnitude of interactions between released salmon and NTT would invariably be larger downstream of a release site.

We used abundance and size of age 1 rainbow trout in the main stem Yakima River and all ages of rainbow trout in the tributaries as an analog for evaluating impacts to steelhead trout. We did this because of the difficulty of monitoring impacts to steelhead in the upper Yakima Basin and the similarities in genetics and pre-smolt ecologies of the two forms of *O. mykiss* (Pearsons et al. 2007). Difficulties of monitoring included; low abundance of steelhead, distinguishing juvenile rainbow and steelhead non-lethally, and sampling upper Yakima steelhead smolts. The spawning populations of rainbow and steelhead trout overlap considerably in space and time, evidence of gene flow has been documented (Pearsons et al. 2007), and it is believed that the juveniles rear together and share similar habitat requirement prior to smoltification. Smoltification in the Yakima Basin generally occurs between ages 1 and 3. Using rainbow trout as an analog does not address impacts that would occur during or after the smolt stage.

Similarly, we used juvenile sized suckers in the main stem Yakima River as an analog for evaluating changes in status of mountain suckers. Mountain suckers are rare in the basin and very few have been observed during field sampling. The low abundance and low containment objective for mountain sucker makes detecting impacts to their status difficult. The use of surrogate species (all subadult suckers) greatly improves our ability to detect impacts, although we must assume mountain suckers and surrogate species respond to supplementation activities similarly. We used subadult suckers because mountain suckers are considerably smaller than the other dominant sucker species, bridgelip *C. columbianus* and largescale *C. macrocheilus* sucker (Wydoski and Whitney 2003).

Environmental variables were compared before and during supplementation to determine if key environmental factors changed between the two periods. In the main stem Yakima River, water temperature and flow were recorded daily at U.S. Bureau of Reclamation (USBOR) gauging stations located throughout the basin (available at http://www.usbr.gov/pn/hydromet/yakima/yakwebarcread.html). We evaluated stream flow and temperature recorded for the months October through September at three gauging stations on the Yakima River including one near the city of Cle Elum (YUMW), one near the city of Ellensburg (ELNW), and one in the Yakima River canyon (UMTW) approximately 22 rkm downstream from the city of Ellensburg, Washington. We calculated mean, maximum and minimum daily flows and temperature for each station annually. We recorded wetted stream width, during the time of trout sampling, at 100 m intervals within our main stem sites and used the standard deviation of the widths as an index of stream morphology. We used two-sample student's t-tests to determine if there were differences in these variables before (1990-1998) and during (1999-2012) the supplementation period.

In upper Yakima Basin tributaries, we measured stream wetted width and stream flow at the time of sampling and computed total stream discharge as described by Gallagher and Stevenson (1999); we excluded 2011 in discharge measurements due to equipment failure. We also generated a longitudinal streambed profile in each site by recording water depth at 1 m intervals along the stream thalweg as described by Kaufmann (1987). Variability of thalweg measurements, calculated as the standard deviation (SD) of thalweg depths, were calculated to index habitat complexity and residual pool volume. Thalweg measurements were discontinued in most tributaries in 2005 but re-initiated in 2008 (2007 in the Teanaway), so before-after comparisons were limited to the period 1993-2004, and 2008-2013. Wetted width and stream flow measurements in tributaries during the years 1990-1992 were not the same as the period 1993-2013 so we excluded this time period in our analysis. To describe mean, minimum and maximum daily tributary stream flow that was of a similar nature as the main stem dataset, we compiled flow data collected at the USBOR Teanaway River (TNAW) gauging station. Direct measures of water temperature were not available for both time periods at this gauging station so we used daily air temperatures to generate an index of tributary water temperature from a SNOTEL gauging station located in the general vicinity of the Yakima River headwaters (Stampede Pass, Washington). The SNOTEL gauging station was operated by the United States Department of Agriculture's Natural Resources Conservation Service both before and during supplementation (available at http://www.wcc.nrcs.usda.gov/snow). In tributaries, daily discharge and temperature was evaluated for the year prior to field sampling (e.g., August through July). Finally, we used two-sample student's t-tests to determine if there were differences in the tributary environmental variables between the baseline and supplementation period.

Field Methods

The spatial overlap between NTT and spring Chinook and coho salmon during supplementation was quantified as the mean annual percent of the NTT distribution in sympatry with salmon (Table 3). Spatial overlap was determined annually at the sites indicated in Figure 1 and Table 4. These sites were used because they consistently had NTT in the years prior to supplementation. Annual NTT distribution was calculated from the sum of the rkm that NTT were present. The annual percent overlap was calculated as the number of rkm used by sympatric salmon divided by the number of rkm used by NTT. The mean percent overlap was the mean of the annual overlaps from 1999-2013 and was compared to the containment objectives for NTT.

Bull trout that had the potential to overlap with salmon in the upper Yakima Basin were primarily located in the North Fork of the Teanaway River. The distribution of bull trout was determined by snorkeling and electrofishing conducted throughout the North Fork of the Teanaway River prior to 1999 (WDFW, unpublished data). The spatial overlap between bull trout and supplemented salmon in the North Fork of the Teanaway River was also inventoried by snorkeling and electrofishing. The entire 9.3 km rearing area of bull trout (1999 only) or a systematic sample of nine 1 km reaches (2000-2008) were snorkeled at night (Thurow et al. 2006) to determine if any salmon were present. The nine index sites sampled during 2000-2008 were 200 m long and were generally situated at 800 m intervals up the stream channel. During September, two divers with underwater lights, moved upstream and counted all fish observed. Additional snorkel surveys were conducted during the spring and summer to determine the maximum upstream distribution of spring Chinook and the presence of other species (see residuals methods). We also supplemented our snorkeling surveys with backpack electrofishing described below.

Our index of abundance for bull trout in the North Fork Teanaway River indicate very few, if any, fish were present in the Teanaway Basin during the period 2006-2013. It is highly probable bull trout have been extirpated in the North Fork of the Teanaway River because they have not been observed for the last several years despite high effort sampling by the Yakima Species Interactions Studies crews as well as several other agencies (Meyer 2009, personal communication). We reprioritized our effort to determine bull trout abundance in 2009 by using our highest elevation electrofishing index site as a trigger to determine if snorkeling should be conducted. We established a linear relationship between our bull trout abundance index (snorkeling) and our electrofishing index site bull trout abundance estimates (electrofishing). The relationship indicates that in years bull trout were observed during our summer sampling in our North Fork Teanaway River index site, they were observed during our fall snorkeling surveys ($P<0.05$). In future years, we will snorkel index sites and establish an annual abundance index if 1) bull trout are encountered during our routine summer sampling, or 2) if spring chinook distribution expands upstream into index areas that contained bull trout in previous years.

Abundance Indices

Population abundance indices of salmonids in upper Yakima tributary sites were generated from single-pass, backpack electrofishing capture efficiency expansions (Temple and Pearsons 2004; Temple and Pearsons 2007; Table 3). In tributary streams, a crew of three to six people electrofished 200 m long index sites during the day with a backpack electrofisher during summer base flows (Table 4). A single upstream electrofishing pass was performed and attempts were made to net all visible fish. Netted fish were held in perforated buckets in the stream margins until they were processed. All fish were anesthetized, identified to species, and the lengths and weights of salmonids were recorded. We assumed, and later verified, that electrofishing efficiencies would be poor for small fish and restricted our evaluation to salmonids greater than 79 mm FL (Reynolds 1983).

An index of salmonid abundance was calculated by expanding the first pass count by a median capture ratio established for each site during the baseline monitoring phase. Median capture ratios were calculated by dividing the number of fish captured on the first electrofishing pass by a multiple-removal maximum likelihood estimate of the number of fish in the site (Zippen 1958). A minimum of two and a maximum of six electrofishing removal passes were used to generate capture efficiencies at each site once annually during the baseline phase. Median efficiencies were based on between four and eight annual baseline replicates depending on the year each site was established. On average, removal based efficiencies indicated that 75% of the trout greater than 79 mm FL were captured during the first electrofishing pass. Approximately 49% of the sites were sampled with multiple removal methods during both periods because insufficient numbers of annual capture efficiency estimates during the baseline period were available to generate expansions.

Table 4. Distribution monitoring site locations in the upper Yakima Basin. A sub-set of distribution sites are used for rainbow trout (R) or cutthroat trout (C) abundance and size before and after supplementation (B-A) comparisons. Before-After-Control-Impact-Paired (BACIP) abundance and size monitoring control (1) and impact (2) site designations are presented as well. Latitude and longitude positions are presented in degrees, minutes, and seconds (D^{o}M'S"). Main stem Yakima River sampling site locations represent the downstream boundary of each site.

Stream / Site	B-A	BACIP	Years	Latitude	Longitude
Yakima River Tributaries					
Cabin Creek 1			90-13	47° 14' 08.72"	121° 13' 8.72"
Domerie Creek A			97-03	47° 14' 12.73"	121° 04' 6.83"
Jungle Creek A			00-09	47° 20' 47.43"	120° 52' 36.08"
Manastash Creek 1			92-13	46° 59' 39.45"	120° 35' 26.81"
Manastash Creek 3			92-13	47° 2' 21.09"	120° 57' 36.41"
Manastash Creek A			98-07[a]	46° 59' 30.35"	120° 50' 57.30"
M.F. Teanaway 1	R	1	90-13	47° 15' 53.54"	120° 53' 53.19"
M.F. Teanaway 2	R	1	90-13	47° 16' 51.06"	120° 55' 50.37"
M.F. Teanaway 3	R	1	90-13	47° 17' 57.47"	120° 57' 42.06"
M.S. Teanaway 1			94-13	47° 10' 58.40"	120° 49' 29.80"
M.S. Teanaway 2			94-13	47° 13' 28.32"	120° 48' 15.61"
M.S. Teanaway 3			94-13	47° 15' 6.65"	120° 52' 27.53"
N.F. Teanaway 1	R	2	90-13	47° 16' 53.10"	120° 51' 53.86"
N.F. Teanaway 1.5			01-13	47° 17' 24.67"	120° 51' 35.38"
N.F. Teanaway 2	R	2	90-13	47° 18' 41.97"	120° 51' 31.40"
N.F. Teanaway 2.5			99-13	47° 19' 36.74"	120° 51" 21.15"
N.F. Teanaway C			02-13	47° 19' 56.28"	120° 51' 22.71"
N.F. Teanaway 3	R		90-13	47° 24' 18.24"	120° 55' 56.68"
N.F. Teanaway A			97-04	47° 22' 51.58"	120° 53' 11.52"
N.F. Teanaway B			98-04[a]	47° 24' 54.67"	120° 56' 20.50"
[b]N.F. Teanaway Rkm 18.2-27.2			97-02	47° 24' 60.24"	120° 52' 48.68"
Stafford Creek A			97-13	47° 21' 20.08"	120° 50' 0.84"
Stafford Creek B			97-04	47° 21' 48.82"	120° 48' 32.18"
Swauk Creek 1	R		92-13	47° 7' 58.30"	120° 44' 51.39"
Swauk Creek 2	R		92-13	47° 13' 45.90"	120° 41' 46.96"
Swauk Creek 3	R		92-13	47° 19' 15.08"	120° 41' 9.65"
Taneum Creek 1	R		90-13	47° 5' 7.71"	120° 46' 8.35"
Taneum Creek 2	R		90-13	47° 6' 46.99"	120° 52' 58.95"
Taneum Creek 3	R		90-13	47° 6' 37.20"	120° 56' 9.09"
Taneum Creek A			97-13	47° 6' 43.34"	120° 55' 45.11"
Taneum Creek B			97-13	47° 6' 30.69"	120° 56' 11.71"
Umtanum Creek 1			92-13	46° 51' 27.63"	120° 29' 49.55"
Umtanum Creek 1.5			92-04	46° 51' 57.13"	120° 32' 4.26"
Umtanum Creek 2			92-04	46° 52' 27.65"	120° 33' 58.07"
W.F. Teanaway 1	R	1	90-13	47° 15' 25.52"	120° 53' 56.00"

W.F. Teanaway 2	R	1	90-13	47° 15' 51.79"	120° 57' 11.25"
W.F. Teanaway 3	R	1	90-13	47° 16' 11.37"	120° 58' 36.13"
Wilson Creek A			97-03	47° 9' 54.93"	120° 30' 38.79"
			Mainstem Yakima River		
Lower Canyon	R		91-13	46° 47' 32.32"	120° 27' 23.94"
Upper Canyon	R		91-13	46° 53' 42.55"	120° 30' 10.93"
Ellensburg	R		91-13	46° 58' 47.39"	120° 34' 9.24"
Thorp	R / C	1 / 2	91-13	47° 5' 58.73"	120° 42' 8.48"
Cle Elum	R / C		91-13	47° 10' 24.96"	120° 51' 36.48"

[a] Intermittent sampling in later years as time allowed.

[b] Latitude and longitude position refers to the lowest reach boundary at river kilometer (RKM) 18.2 (measured from the confluence with the Yakima River).

In the main stem of the upper Yakima River, a crew of two people electrofished 4.2–7.4 km long index sites at night with a drift boat mounted electrofisher as described by Temple and Pearsons (2007). During the electrofishing passes, all fish were identified visually and attempts were made to net all trout. Trout greater than 99 mm fork length were marked with a fin clip and released. One week later, another electrofishing pass was made to determine the proportion of marked and unmarked salmonids. Three types of abundance measures were made based on the abundance of the non-target taxa (Table 3). One type, which was used for rainbow trout, was generated from mark-recapture methods. Main stem rainbow trout were grouped into 25 mm size intervals and mark-recapture abundance estimates for each 25 mm group were generated using the maximum log-likelihood estimator as computed by the computer software program Mark-Recapture for Windows (Mark-Recapture for Windows 1997, Version 5.0 Beta, Montana Fish, Wildlife, and Parks). The general form of the estimator was:

$$\hat{N} = \frac{M}{effic}$$

where $\hat{N}$ was the estimated abundance for each 25 mm size class, *M* was the number fish marked, and *effic* was the log-likelihood model estimated capture efficiency. The sum of the abundance estimates generated for each 25 mm grouping represented total abundance per index section. The assumptions associated with the log-likelihood model included; 1) the proportions of marked and unmarked fish remained equal between mark and recapture sampling, 2) marked and unmarked fish were equally catchable, and 3) fish marks were permanent for the duration of the sampling and were not overlooked during the recapture sample. We used a one-week interval between mark and recapture sampling because; 1) it allowed adequate time to redistribute, and 2) it provided adequate time for marked fish to recover (Mesa and Schreck 1989; Peterson et al. 2004).

The abundance and size of age 1 main stem rainbow trout was calculated for each index site each year using a mixture analysis of the length frequency distributions from each individual site (MacDonald and Pitcher 1979). We used R statistical software (R development core team 2005) and the add in package mixdist (Du 2002) to determine the proportion of age 1 rainbow trout in each index site and their mean fork length. Age 1 rainbow trout abundance was calculated by multiplying the total mark-recapture abundance estimate for each index site by the estimated proportion of age 1 rainbow trout present in each index site. We weighted the age 1 rainbow trout abundance estimates for each index section by the amount of stream each index site represented and then generated a mean abundance estimate of age 1 rainbow trout per km for each year. Confidence limits (95%) for the parameter estimates were calculated by incorporating both the spatial and within site variance components (Bohlin et al. 1989).

To evaluate our age 1 rainbow trout parameter estimates in the main stem, we compared them against known main stem rainbow trout population parameters. In 2003, we systematically collected scales from rainbow trout in our main stem index sites. Scales were mounted on gummed cards in the field and acetate impressions of each scale were made in the lab. Scales were projected with a microfiche reader and were aged by counting annuli (Jearld 1983) by a recognized expert with over 25 years experience. We used a chi square test to compare the known proportion of rainbow trout in each age class in each index site versus the proportions predicted by the mixture analysis. We also used a student's t-test to compare the mean length of known age 1 main stem rainbow trout versus the mean length predicted from the mixture analysis.

Efficiency Expansions

The second type of abundance index, used for main stem cutthroat trout in the THORP and CELUM sections, was based on efficiency expansions. The low abundance of cutthroat trout prevented performing valid mark-recapture estimates due to insufficient recaptures of marked fish. Thus, we calculated an abundance index of cutthroat trout (<250 mm) by expanding the observed number of cutthroat trout captured during electrofishing, by our capture efficiencies for similar sized rainbow trout (Peterson and Zhu 2004). Abundance indices were extrapolated to the reach scale based on reach length.

The next type of abundance index, used for natural origin spring Chinook, was a visual estimate that was expanded by capture efficiencies. The numbers of natural origin spring Chinook visually enumerated during the electrofishing marking runs were expanded by maximum log-likelihood model recapture efficiencies for the smallest sizes of rainbow trout observed (generally between 100 mm and 126 mm). These efficiencies may have been overestimated because naturally produced spring Chinook are slightly smaller than the rainbow trout used to establish the capture efficiencies at this time of year. Thus, the resulting abundance index is likely an underestimate. However, in sites where we could generate capture efficiencies for spring Chinook, our observed rainbow trout electrofishing capture efficiency was within the 95% confidence interval (CI) of the hatchery origin spring Chinook electrofishing efficiency. Thus, we believe that size based efficiencies are reasonable ways of indexing relative abundance because fish size is one of the most important factors that influences electrofishing efficiency (Anderson 1995; Buttiker 1992).

The last type of abundance index, used for mountain whitefish and sucker species (including mountain sucker) in the main stem, was a visual estimate based upon visual counts taken during boat electrofishing surveys. Visual counts appear to provide an adequate index for abundance. Comparisons between mainstem rainbow trout visual counts correlated significantly with rainbow trout abundance estimates generated from mark-recapture electrofishing. Similarly, visual counts of spring Chinook fall parr correlated significantly with smolt counts at Prosser dam the following year. This information was used as support for the use of visual counts to index abundance for mountain whitefish and sucker species.

Five index sites in the main stem Yakima River were used to represent five contiguous study reaches and the index sites comprised approximately 28% of the upper Yakima River between Roza Dam and the Cle Elum River confluence (McMichael et al. 1992). Each index site was selected to be representative of a larger reach. Estimates were generated from abundance and size data collected in the site and were extrapolated to the reach scale based on reach length. The Lower Canyon (LCYN) site was 4.8 km long, the Upper Canyon (UCYN) site was 5.2 km long, the Ellensburg (EBURG) site was 4.2 km long, the Thorp (THORP) site was 5.7 km long, and the Cle Elum (CELUM) site was 7.4 km long. The reach descriptions are as follows: LCYN extends 19.2 km upstream from Roza Dam to Umtanum Creek; UCYN extends 13.4 km upstream from Umtanum Creek to the Ringer Road access; EBURG extends 21.2 km upstream from the Ringer Road access to the Ellensburg Dam; THORP extends 24.1 km upstream from the Ellensburg Dam to the Teanaway River; and CELUM extends 16.2 km upstream from the Teanaway River to the Cle Elum River (Figure 1).

Size of an NTT was quantified using the mean length of fish collected in our main stem and tributary sites. All rainbow trout that were captured were measured to the nearest mm FL. Mean length of rainbow trout in each tributary site was calculated and then the mean of the site means was calculated for each year. Length of age 1 main stem rainbow trout was calculated for each index site each year using a mixture analysis of the length frequency distributions (MacDonald and Pitcher 1979) from each individual site and then weighted by abundance and section length. Biomass estimates were generated from the product of mean estimated abundance and mean weight. Mean weight of rainbow trout in each tributary site was averaged and then the mean of the averages was calculated for each year. The mean annual weight of age 1 main stem rainbow trout was computed for each index site using the log-transformed length/weight regression from mean lengths of fish in individual annual index sites and then weighted by abundance and section length.

Size structure for mountain whitefish, sucker species, and mountain suckers were based upon visual counts of fish in each size class. Since fish were generally not handled while visual counts were performed, an index of size was based upon the proportions of size classes of fish observed during electrofishing. For mountain whitefish, we used the proportion of subadults ($\leq$ 250 mm TL) relative to the total whitefish observed for evaluating changes to size. For sucker species, we used the proportion of adults ($\geq$250 mm TL) relative to the total suckers observed during electrofishing. For our mountain sucker size index, we used the proportion of subadult suckers ($\leq$ 250 mm TL) relative to the total suckers observed during electrofishing. Proportions of fish for the size groupings were arcsine square-root transformed prior to analysis. Estimates of biomass for visually observed species could not be accurately calculated using the data we collected.

Residualization of Hatchery Smolts

Abundance estimates and the presence of residualized hatchery spring Chinook and coho salmon present in the Yakima River from mid-September to mid-October for release years 1999-2013 were calculated using observed abundance and boat electrofishing recapture efficiencies calculated for similar sized rainbow trout. We calculated recapture efficiencies of similar sized rainbow trout utilizing mark-recapture methods in main stem Yakima River electrofishing index sites. The rainbow trout recapture efficiencies were applied to the number of hatchery residuals netted during the mark runs in each index section. A final estimate of hatchery residual abundance was expanded to the reach scale based on reach length.

Distribution and relative abundance of residuals was also estimated by snorkeling the North Fork Teanaway and main stem reaches. The farthest upstream presence of residuals was estimated in the North Fork Teanaway by snorkeling upstream of the acclimation site until no residuals were observed in three sequential pools. Snorkel surveys occurred during the time of maximum distribution from June through September. Snorkeling observations to determine presence or absence were also supplemented by electrofishing.

Natural Production

Spring Chinook salmon natural production occurs primarily in the main stem Yakima River upstream from the city of Ellensburg, Washington (Figure 1). The most intense spawning activity has been observed upstream from the Cle Elum hatchery facility, and in the general vicinity of the Easton acclimation facility (Figure 1). Some spawning activity has also been observed in the Cle Elum and main stem Teanaway Rivers although redd counts indicate these tributaries generally contribute a relatively small proportion of the total redds counted in the upper Yakima River Basin annually.

Abundance indices (fish/km) and 95% CL of naturally produced spring Chinook and coho salmon were generated for the main stem Yakima River and tributary index monitoring sites for the baseline (1990-1998) and during supplementation periods (1999-2013). Spring Chinook and coho salmon visually enumerated during boat electrofishing were expanded by rainbow trout electrofishing efficiencies as previously described. In the tributary index monitoring sites, spring Chinook abundance indices were generated from multiple removal capture efficiencies established during the baseline phase. Due to the low abundance of juvenile coho salmon and their similar size with Chinook salmon, we applied spring Chinook salmon capture efficiencies to the number of coho salmon captured in the first electrofishing pass to obtain an index of coho abundance. We used a two-sample student’s t-test to compare differences in the abundance of salmon before and during the supplementation period.

Before-After and Causation Analysis

Changes in NTT abundance, size, and biomass after stocking began were expressed as percent changes prior to stocking and were judged to warrant further attention if the percent change in before-after samples decreased beyond the containment objectives. We did not confine ourselves to have statistically significant results to trigger further attention because of the chances of committing a type II statistical error from a test with low power (Ham and Pearsons 2000). Impacts to NTT are difficult to detect because of high interannual variation of response variables and the low number of annual surveys available to isolate the impacts that occur during the initial stages of supplementation (Ham and Pearsons 2000; Ham and Pearsons 2001; Pearsons 2002). For example, prospective power analyses indicated that abundance impacts of <19% were not statistically detectable after 5 annual surveys (Ham and Pearsons 2000). Based on these constraints, only large impacts will be statistically detectable. However, one-tailed student’s t-tests were used to determine if the results were statistically significant and 90% CL were calculated to aid with interpreting the magnitude of the results and triggering the next phase of evaluation.

We used linear models to test the hypothesis that the log length-log weight relationships of main stem age 1 rainbow trout in the main stem, and rainbow trout in tributaries did not differ between the baseline (1990-1998) and supplementation (1999-2013) periods. For both main stem and tributary rainbow trout, a homogeneity of slopes test was first performed to determine if the slopes of the log transformed length/weight relationships were significantly different between periods. The log length-log weight relationships for both main stem and tributary rainbow trout before vs. during the supplementation period did not meet the assumptions of equal slopes required for an analysis of co-variance statistical test (ANCOVA), so we tested the relationship using a similar test (separate slopes model) that does not require this assumption.

Although age 1 rainbow trout are used for evaluating impacts to rainbow trout and serve as an analog for steelhead trout in the main stem, we also evaluated changes in the response variables of catchable sized main stem rainbow trout. Since large trout are valued as a fishery resource, we calculated mean size, abundance, and biomass of all main stem rainbow trout greater than 249 mm FL. The field methods and calculations for these larger fish were the same as was described for age 1 rainbow trout in the main stem.

When NTT exhibited decreases in abundance, size, or biomass below the lower 90% CL, we attempted to determine if the change was caused by supplementation. In the Teanaway Basin and in the main stem near Thorp, we used a before-after-control-impact-paired site analysis (BACIP; Stewart-Oaten et al. 1986) to evaluate changes to the abundance, size, or biomass of NTT. This test evaluates the annual paired differences in control and impact sites before and after stocking. Paired differences before and after stocking were compared with a Mann-Whitney U-test. We used a subset of sites and field methods for the BACIP (Table 3). The treatment sites were based on their proximity to acclimation sites and the control sites were selected based on their geographic proximity to treatment sites. Two sites located downstream from the Jack Creek acclimation facility on the North Fork Teanaway River and three sites in the main stem Teanaway River were used as treatment sites and three sites on each of the Middle and West Forks of the Teanaway River and one upstream site in the North Fork Teanaway River were the paired controls (Figure 1). The “before” period was from 1990-1998 and the “after” period was from 1999-2013. When BACIP results were significant, we investigated relationships between core variables using simple linear regression.

The data from the Thorp main stem site was divided into control and impact sites after 1992 and the “before” and “after” periods were the same as for the Teanaway River. The area downstream from the Clark Flats acclimation site was the impact site and the area upstream from the acclimation site was the control. Abundance estimates and associated 95% CL generated for the Thorp site were partitioned by the proportions of fish marked in either the treatment or control section during the electrofishing marking runs. Rainbow trout located immediately downstream from the Clark Flats acclimation facility had the potential to interact with all upstream spring Chinook and coho smolt releases as well as residual salmon that did not migrate to the ocean. Rainbow trout immediately upstream from the Clark Flats acclimation facility had the potential to interact with migrating smolts and residualized Chinook salmon from the other upstream release locations in the upper Yakima Basin. In essence, we tested if the fish released from the Clark Flats acclimation site caused additional impact to rainbow trout beyond what occurred from releases farther upstream. No controls were available for cutthroat trout in the main stem.

Results

General approach

Comparisons of environmental variables measured in main stem and tributary areas before and during supplementation indicated that there were generally no significant differences in the variables evaluated (Table 5). We did observe a significant increase (P = 0.01) in the minimum daily stream discharge in the main stem Yakima River during the supplementation period. We hypothesize that increased minimum daily stream discharge would benefit NTT and would not confound our evaluation because the increase was not significantly correlated with our monitoring variables (e.g., age 1 rainbow trout size, P = 0.24; cutthroat trout size, P = 0.63).

Table 5. Environmental variables and results from two-sample student's t-tests of before versus during supplementation periods in the Yakima Basin. Main stem variables were based on water year (October through September of the following year as recorded at three gauging stations) and tributary mean, minimum (Min.), and maximum (Max.) discharge and mean air temperature (based on August through July of the following year) recorded at a single tributary gauging station or weather station. Main stem standard deviation of stream width was recorded at the time of sampling. Tributary mean summer discharge, mean wetted width, mean thalweg depth, and standard deviation of thalweg depth were recorded at the time of sampling. Degrees of freedom (df) were 22 for all comparisons unless otherwise noted.

Environmental Variable	Before Mean	During Mean	*t*	P
Main stem				
Mean Daily Discharge (m^3/s)	61.20	56.41	0.78	0.45
Min. Daily Discharge (m^3/s)	13.42	17.21	-2.72	0.01
Max. Daily Discharge (m^3/s)	239.39	207.16	0.65	0.52
Mean Daily Water Temperature (^oC)	8.69	8.86	-0.71	0.48
Min. Daily Water Temperature (^oC)	0.30	0.67	-1.06	0.30
Max. Daily Water Temperature (^oC)	17.81	18.36	-1.23	0.23
Standard Deviation of Stream Width (m)[a]	12.28	12.81	-0.66	0.52
Tributary				
Mean Daily Discharge (m^3/s)	10.13	9.50	0.41	0.68
Min. Daily Discharge (m^3/s)	0.29	0.38	-1.80	0.08
Max. Daily Discharge (m^3/s)	100.62	80.77	0.91	0.37
Mean Summer Discharge (m^3/s)[a]	0.22	0.30	-1.03	0.32
Mean Daily Air Temperature (^oC)	3.93	4.20	-0.94	0.36
Mean Wetted Width (m)[b]	6.18	6.99	-1.72	0.10
Mean Thalweg Depth (m)[b]	0.30	0.32	-1.25	0.23
Standard Deviation of Thalweg Depth (m)[b]	0.15	0.13	1.38	0.19

[a] Degrees of freedom (df) was 19 for these tests.

[b] Degrees of freedom (df) was 18 for these tests.

Field Methods

Results from statistical tests indicated that using the mixture analysis to determine the parameter estimates from the length frequencies of main stem age 1 rainbow trout were valid. Chi squared tests of known versus predicted proportions of main stem rainbow trout in the population were not significantly different in the LCYN (X^2 = 0.14; 4 df; P>0.99), UCYN (X^2 = 0.03; 2 df; P>0.99), EBURG (X^2 = 0.10; 4 df; P>0.98), THORP (X^2 =0.12; 4 df; P>0.99), or CELUM index sites (X^2 = 0.10; 4 df; P>0.99) during 2003. In addition, student's t-tests of the known versus model predicted mean length of age 1 main stem rainbow trout were not significantly different in the LCYN (t = -2.96; 404 df; P>0.99), UCYN (t = -0.85; 270 df; P>0.60), EBURG (t = -0.44; 262 df; P>0.34), THORP (t = -0.89; 174 df; P>0.63), or CELUM (t = -0.16; 200 df; P>0.13) index sites.

Residualization of Hatchery Smolts

Many spring Chinook salmon did not migrate to the ocean after release (residuals) and may have interacted with trout. In contrast, very few coho salmon residuals were observed (Table 6). Residuals were most concentrated below acclimation sites during the spring and summer, but were found in all reaches of the main stem that we sampled. Residuals were observed approximately 1-2 km upstream of the acclimation site in the North Fork Teanaway during most years and also migrated upstream into the Cle Elum River. Many residuals were observed in the main stem as late as September and October.

Table 6. Estimated annual abundance (fish/km) of spring Chinook and coho salmon residuals in the main stem Yakima River sampling sections. The LCYN section is the Lower Canyon, UCYN is the Upper Canyon, EBURG is Ellensburg, THORP is Thorp, and CELUM is Cle Elum section.

Year	LCYN	UCYN	EBURG	THORP	CELUM	TOTAL
			Spring Chinook Residuals			
1999	12	5	3	5	0	25
2000	12	5	1	25	3	46
2001	196	71	85	71	0	423
2002	6	0	7	2	0	15
2003	23	8	3	5	0	39
2004	80	21	29	9	0	139
2005	34	29	3	0	0	66
2006	1	1	0	0	0	2
2007	5	9	6	8	0	28
2008	8	10	4	9	0	31
2009	102	43	22	23	0	190
2010	25	19	5	2	0	51
2011	8	10	4	9	0	30
2012	25	34	17	35	0	110
2013	29	36	22	64	0	150
			Coho Salmon Residuals			
1999	7	0	0	0	4	11
2000	0	0	0	0	0	0
2001	3	0	0	0	0	3
2002	0	0	0	0	0	0
2003	0	0	0	0	0	0
2004	0	0	11	0	7	18
2005	0	0	25	0	0	25
2006	0	0	5	0	0	5
2007	0	0	20	0	0	20
2008	0	0	0	0	0	0
2009	0	0	0	0	0	0
2010	0	0	0	0	0	0
2011	0	0	0	0	0	0
2012	0	0	0	0	0	0

2013	0	0	0	0	0	0

Natural Production

We did not observe coho salmon in our tributary index monitoring sites before the supplementation period (0 coho/33 sites). During the supplementation period (1999-2012) we observed coho in 8 of our index sites (between 25 and 36 sites sampled annually) averaging 29 ± 23 per km (mean ± 95%). This increase was statistically significant (t = -2.14; 22 df; P=0.04). Our coho abundance index calculated for the main stem Yakima River averaged 0.10 ± 0.25 (mean ± 95%) coho per river km before supplementation releases. During supplementation, we estimated 2.5 ± 1.4 coho per km (mean ± 95%). The increase in main stem coho salmon abundance was nearly statistically significant (t = -2.07, 19 df, P= 0.05). Since 2009, snorkelers have observed increasing numbers of coho in mixed pods with Chinook salmon in the Yakima River upstream from our monitoring sites relative to previous years (WDFW unpublished data).

Before supplementation, our abundance index was 23 ± 17 (mean ± 95%) spring Chinook salmon per km in our tributary monitoring sites and during the supplementation period it was 62 ± 31 per km (mean ± 95%). The observed increase during this period was not statistically significant (t=-1.95; 22 df; P=0.06) with the addition of 2013 data due to the increased variation associated with the large abundance in 2013 relative to previous years. In the main stem Yakima River, our abundance index for spring Chinook salmon indicated there were approximately 1,987 ± 843 fish per km during the baseline period while there were 2,403 ± 769 fish per km during the supplementation period. The observed difference was not significant (t=-0.68; 19 df; P = 0.50).

Overlap

The degree of trout overlap with salmon was highest in main stem areas, intermediate for cutthroat and rainbow trout in tributaries, and absent for bull trout (Figure 2). There was no overlap of salmon and bull trout in our index sites. In fact, the shortest distance between the uppermost distribution of Chinook salmon and the lowermost distribution of bull trout was approximately 8 km. Cutthroat trout and supplemented spring Chinook overlapped in distribution in both tributary and main stem Yakima River areas. The distributional overlap in tributary streams was approximately 11%, confined to relatively moderate elevations, and was less than the 40% containment objective (Figure 2). Salmon overlapped 100% of the main stem distribution of cutthroat trout (Figure 2). In tributaries, salmon overlapped 50% of the distribution of rainbow trout. Overlap was predominately confined to lower portions of tributaries (e.g., Swauk Creek 1 and Umtanum Creek 1) and farther upstream in the North Fork Teanaway River. However, salmon did not overlap rainbow trout in high elevation portions of tributaries.

There was also extensive overlap between rainbow trout, sucker species, and mountain whitefish and salmon in the main stem (100%; Figure 2). Salmon overlapped in distribution with longnose dace (58%) and speckled dace (72%) in tributaries, although mean overlap was less than the containment objectives for both species. Salmon overlapped sculpin species 19% in tributaries but this was less than the containment objective. Finally, there was 23% overlap in distribution between sucker species and salmon in tributary streams, although this was also less than the containment objective.

Data that were collected at similar times and sites by snorkeling and electrofishing methods were consistent with each other. For example, in areas that we found salmon, rainbow trout, cutthroat trout or bull trout, they were detected with both electrofishing and snorkeling methods. In addition, we did not capture any salmon when we electrofished areas where bull trout were present.

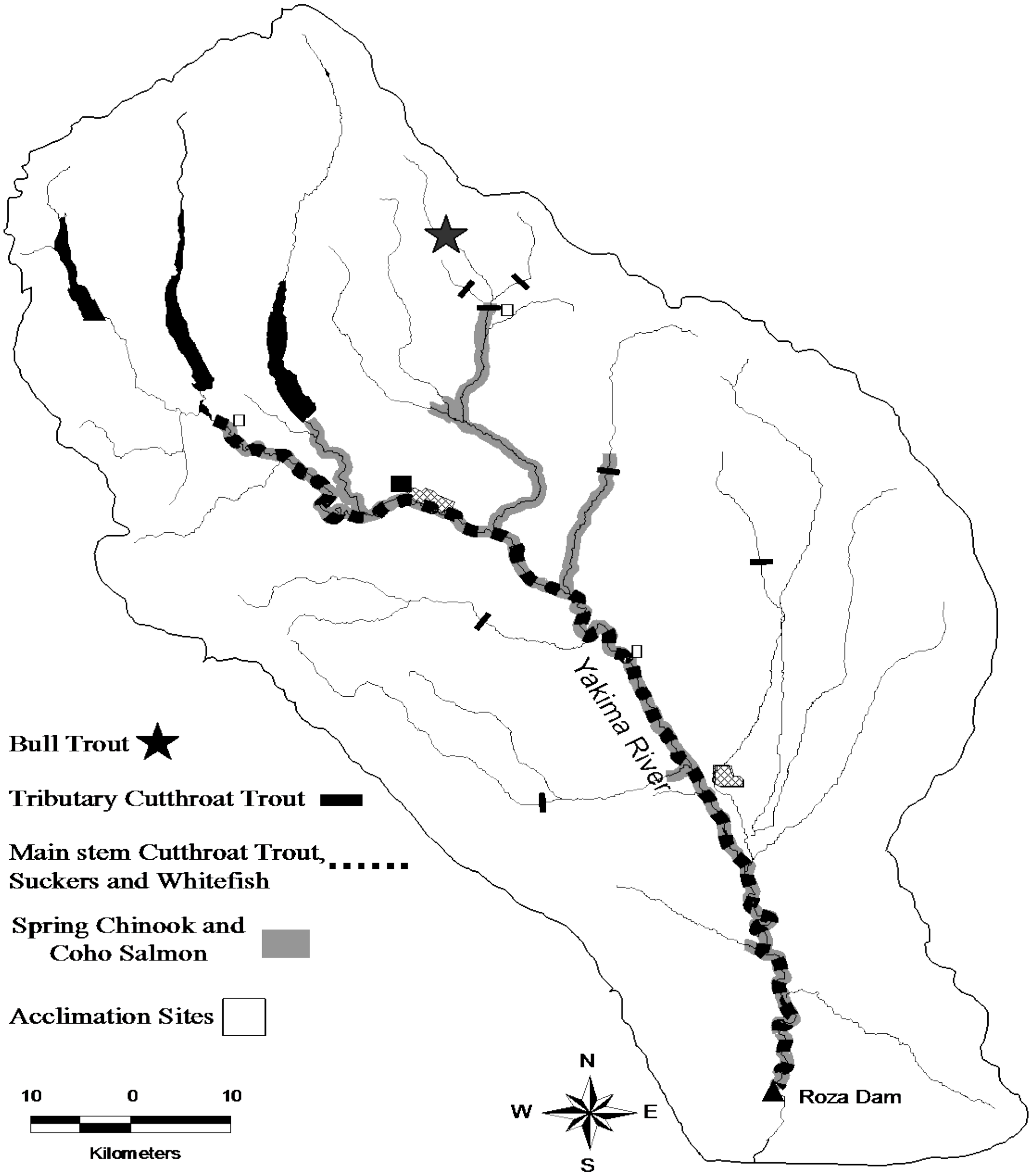

Figure 2. Map of species distributions in the upper Yakima Basin. Spring Chinook and coho salmon distributions are shaded grey. The lowest elevation observations of bull trout and cutthroat trout in tributary streams are marked with stars and bars, respectively. Cutthroat trout, suckers and mountain whitefish distribution in the main stem is marked as a dashed line. The Cle Elum hatchery facility is marked with a black square and

hatchery acclimation sites are marked with open squares. Rainbow trout are widely distributed throughout the basin and are not marked on the map.

Before-After Analysis

Rainbow (age 1) and cutthroat trout (<250mm), mountain whitefish, and sucker species in the main stem, and rainbow trout in tributaries (all ages; analog for steelhead) exhibited distributional overlap with salmon that were outside the containment objectives and therefore we compared their abundance, size, and biomass (salmonids) before and after stocking began. The mean abundance and 90% CL of sympatric rainbow trout (all ages) was 34 ± 17% higher in the tributaries and 33 ± 16% higher in the main stem (age 1) in the years when supplementation occurred than during the baseline phase (Tables 7 and 8; Figure 3). The mean abundance of cutthroat trout (<250 mm) was 446 ± 361% CL higher in the main stem during supplementation than during the baseline phase (Table 7; Figure 3). The mean abundance of sub-adult mountain whitefish increased 109 ± 43% CL during supplementation period, while the mean abundance of sucker species adults decreased 44 ± 8% CL and the decrease was significant (P=0.001), although it was within our containment objectives (Figure 4). Finally, we observed a 27 ± 24% CL increase in sub-adult sized sucker abundance (analog for mountain sucker) during supplementation and the lower 90% CL did not exceed our containment objective (Figure 4).

During the supplementation period, the mean and 90% CL of rainbow trout size (age 1) in the main stem indicated that size decreased by 5 ± 2% (Table 7; Figure 3). Slopes between log length-log weight of age 1 rainbow trout in the main stem were not significantly different before and during supplementation (P=0.79). An ANCOVA revealed the average weight of fish for a given length was significantly greater during the supplementation period (P=0.004, Figure 5). In addition, biomass increased by 13 ± 17% CL. Similarly, the mean and 90% CL of cutthroat trout size (<250 mm) in the main stem indicated a 0 ± 3% CL decrease, and an increase in biomass of 713 ± 858% CL (Table 7; Figure 3). The size of rainbow trout in the tributaries (all ages) was similar during both periods (1 ± 2% CL; Table 8; Figure 3). Slopes between log length-log weight for rainbow trout in tributaries (all ages) were not significantly different before and during supplementation (P=0.30). An ANCOVA indicated the mean weights at each length were significantly greater during the supplementation period than the before period (P<0.001; Figure 5). Additionally, tributary rainbow trout biomass (all ages) increased by 28 ± 12% CL (Table 8; Figure 3). Our index of mountain whitefish size indicated that the proportions of subadults observed increased 10 ± 2% CL during the supplementation period (Figure 4). Our index of sucker species size indicated that the proportion of adults decreased 41 ± 10% during supplementation, and although the decrease was significant (P<0.001), it was still well within our containment objectives (Figure 4). Our index of mountain sucker size indicated a 28 ± 10% CL increase in the proportion of subadults during the supplementation period (Figure 4).

The mean abundance, size, and biomass of catchable sized main stem rainbow trout (>249 mm) did not decrease during supplementation. The mean abundance of rainbow trout greater than 249 mm increased by 15 ± 14% (mean ± 90% CL), mean size increased by 2 ± 1%, and biomass increased by 42 ± 17% during supplementation when compared to baseline conditions.

The only NTT with parameter estimates outside of the containment objectives was steelhead, which uses rainbow trout as an analog. The lower 90% CL for age 1 rainbow trout size in the main stem and rainbow trout size (all ages) in the tributaries were exceeded, so we tested whether the decrease was caused by supplementation.

Table 7. Annual abundance (fish/km), size (mm, FL), and biomass (kg/km) estimates and associated 95% confidence intervals of age 1 rainbow trout and cutthroat trout less than 250 mm fork length in the main stem Yakima River.

	Abundance		Size		Biomass	
Year	RBT	CUT	RBT	CUT	RBT	CUT
1990			210 ± 33	237 ± 5		
1991	189 ± 67	11 ± 14	205 ± 27	237 ± 11	19 ± 14	1.6 ± 3.2
1992	151 ± 28	1	217 ± 31	242	18 ± 7	0.1
1993	193 ± 48	6 ± 17	232 ± 36	238 ± 3	27 ± 11	0.8 ± 3.5
1994	180 ± 33	2 ± 1	217 ± 32	225 ± 17	21 ± 8	0.3 ± 1.4
1995	190 ± 54	6 ± 17	235 ± 34	239 ± 6	28 ± 12	0.9 ± 3.5
1996	182 ± 27	5 ± 11	217 ± 32	239 ± 10	22 ± 7	0.7 ± 2.4
1997	272 ± 49	10 ± 44	203 ± 35	239 ± 5	27 ± 10	1.4 ± 8.9
1998	130 ± 20	16 ± 84	212 ± 34	230 ± 5	15 ± 6	2.0 ± 16.8
1999	182 ± 25	12 ± 25	217 ± 33	236 ± 5	22 ± 7	1.8 ± 5.1
2000	214 ± 40	13 ± 1	210 ± 36	227 ± 13	24 ± 10	1.8 ± 1.4
2001	384 ± 81	18 ± 85	206 ± 32	238 ± 7	41 ± 16	2.5 ± 17.1
2002	207 ± 39	7 ± 42	203 ± 31	232 ± 6	20 ± 9	0.9 ± 8.4
2003	230 ± 41	10 ± 34	207 ± 30	234 ± 7	24 ± 9	1.3 ± 7.0
2004	275 ± 19	16 ± 34	223 ± 32	234 ± 5	35 ± 15	2.3 ± 6.9
2005	272 ± 20	28 ± 142	213 ± 32	229 ± 5	30 ± 9	3.4 ± 28.6
2006	150 ± 12	16 ± 11	216 ± 34	235 ± 5	17 ± 7	2.1 ± 2.5
2007	233 ± 17	22 ± 35	210 ± 33	233 ± 5	26 ± 8	3.1 ± 7.1
2008	264 ± 26	24 ± 61	204 ± 33	229 ± 7	26 ± 9	3.0 ± 12.3
2009	156 ± 29	44 ± 138	188 ± 29	231 ± 3	12 ± 3	5.8 ± 27.8
2010	233 ± 48	32 ± 111	197 ± 36	230 ± 5	21 ± 7	4.1 + 22.3
2011	273 ± 23	39 ± 63	199 ± 34	227 ± 4	26 ± 9	5.0 ± 12.8
2012	270 ± 30	70 ± 250	192 ± 33	226 ± 5	23 ± 8	8.7 ± 50.3
2013	359 + 38	237 ± 335	196 ± 27	290 ± 9	32 ± 10	75.6 ± 68.0

Table 8. Annual abundance (fish/km), size (mm, FL), and biomass (kg/km) estimates and associated 95% confidence intervals for rainbow trout in Yakima River Basin tributary streams.

Year	Abundance	Size	Biomass
1990	241 ± 129	136 ± 8	8 ± 13
1991	204 ± 102	131 ± 8	6 ± 8
1992	375 ± 240	130 ± 5	11 ± 24
1993	317 ± 158	131 ± 7	9 ± 17
1994	328 ± 129	132 ± 8	11 ± 15
1995	213 ± 118	139 ± 8	7 ± 14
1996	165 ± 109	133 ± 8	5 ± 11
1997	294 ± 119	132 ± 5	8 ± 11
1998	442 ± 174	138 ± 7	15 ± 25
1999	288 ± 175	135 ± 8	12 ± 27
2000	318 ± 135	144 ± 8	11 ± 21
2001	464 ± 178	129 ± 3	12 ± 17
2002	321 ± 131	132 ± 6	10 ± 15
2003	291 ± 142	132 ± 5	8 ± 14
2004	243 ± 135	142 ± 5	9 ± 15
2005	349 ± 163	127 ± 5	9 ± 16
2006	434 ± 171	134 ± 5	13 ± 20
2007	368 ± 153	138 ± 4	12 ± 18
2008	331 ± 166	138 ± 7	11 ± 19
2009	256 ± 123	138 ± 12	9 ± 19
2010	548 ± 243	127 ± 5	15 ± 25
2011	486 ± 215	124 ± 7	12 ± 20
2012	490 ± 163	124 ± 4	13 ± 15
2013	571 ± 232	129 ± 5	16 ± 24

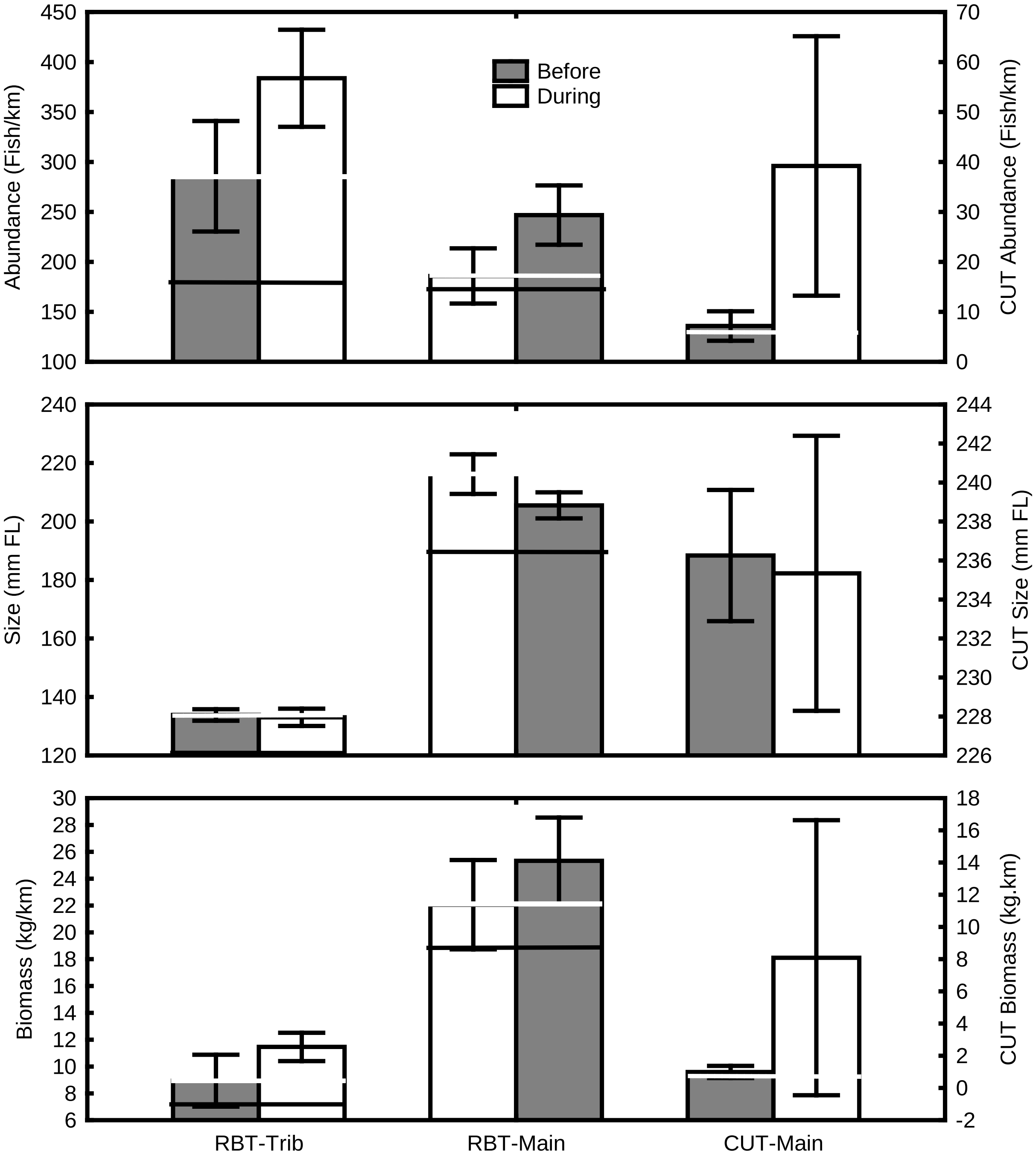

Figure 3. Abundance (n/km), size (FL mm), and biomass (kg/km) of tributary rainbow trout, main stem Yakima River rainbow trout (age 1) and cutthroat trout (<250 mm) before and during supplementation. Main stem cutthroat trout abundance, size, and biomass are associated with the right *y*-axis. The horizontal dashed line represents the 0% containment objective (CO) for steelhead in the main stem and tributaries, and the 10% CO for mainstem cutthroat trout. The solid horizontal line represents the 10% CO

for main stem rainbow trout and 40% CO for tributary rainbow trout. Error bars represent 90% confidence intervals.

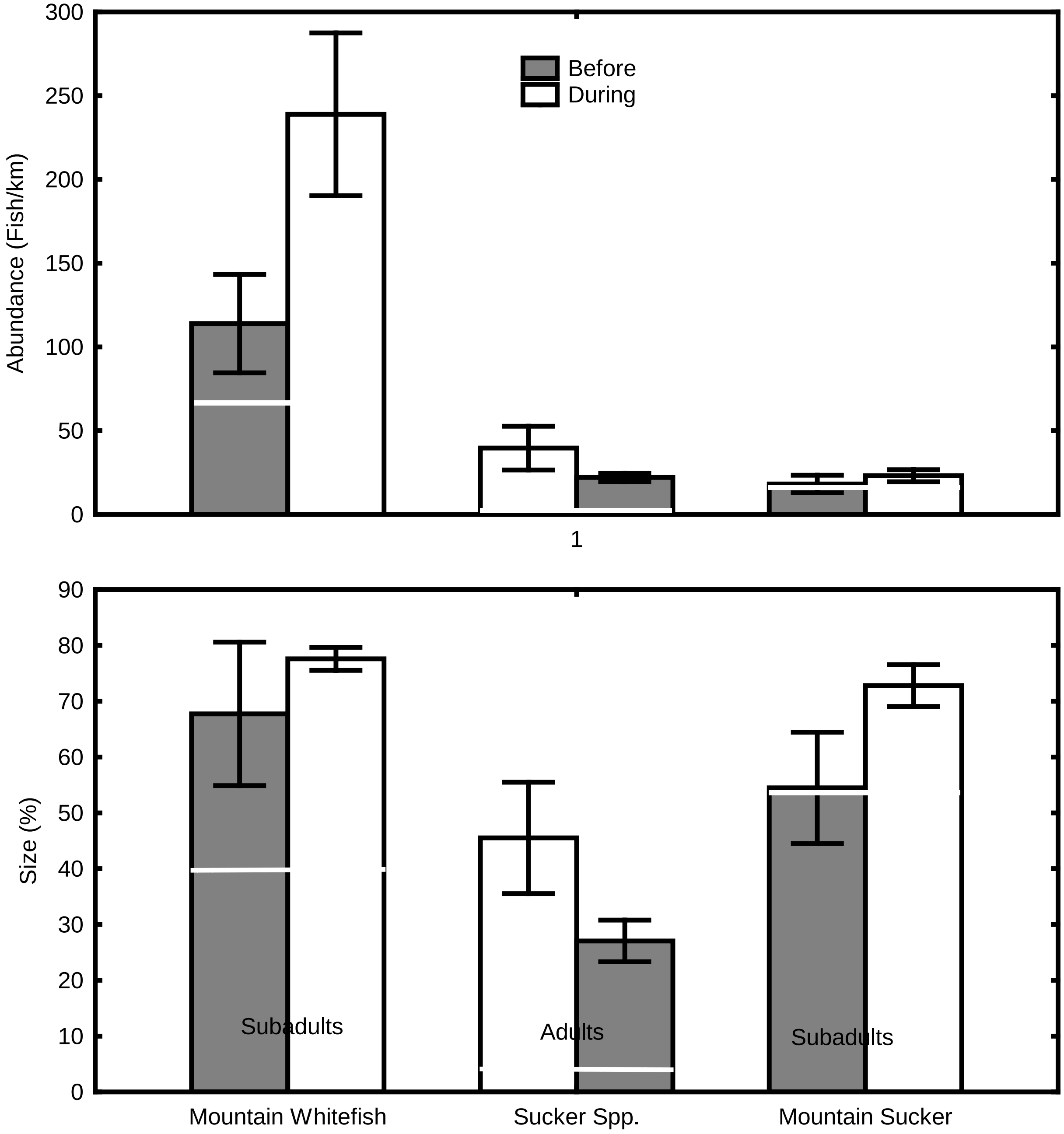

Figure 4. Abundance (fish/km) and size (percent by size class) of mountain whitefish, suckers, and mountain suckers before and during supplementation. Error bars represent the 90% confidence interval. Dashed lines represent the 40% containment objectives for mountain whitefish, 90% for sucker species (Spp), and 5% for mountain suckers.

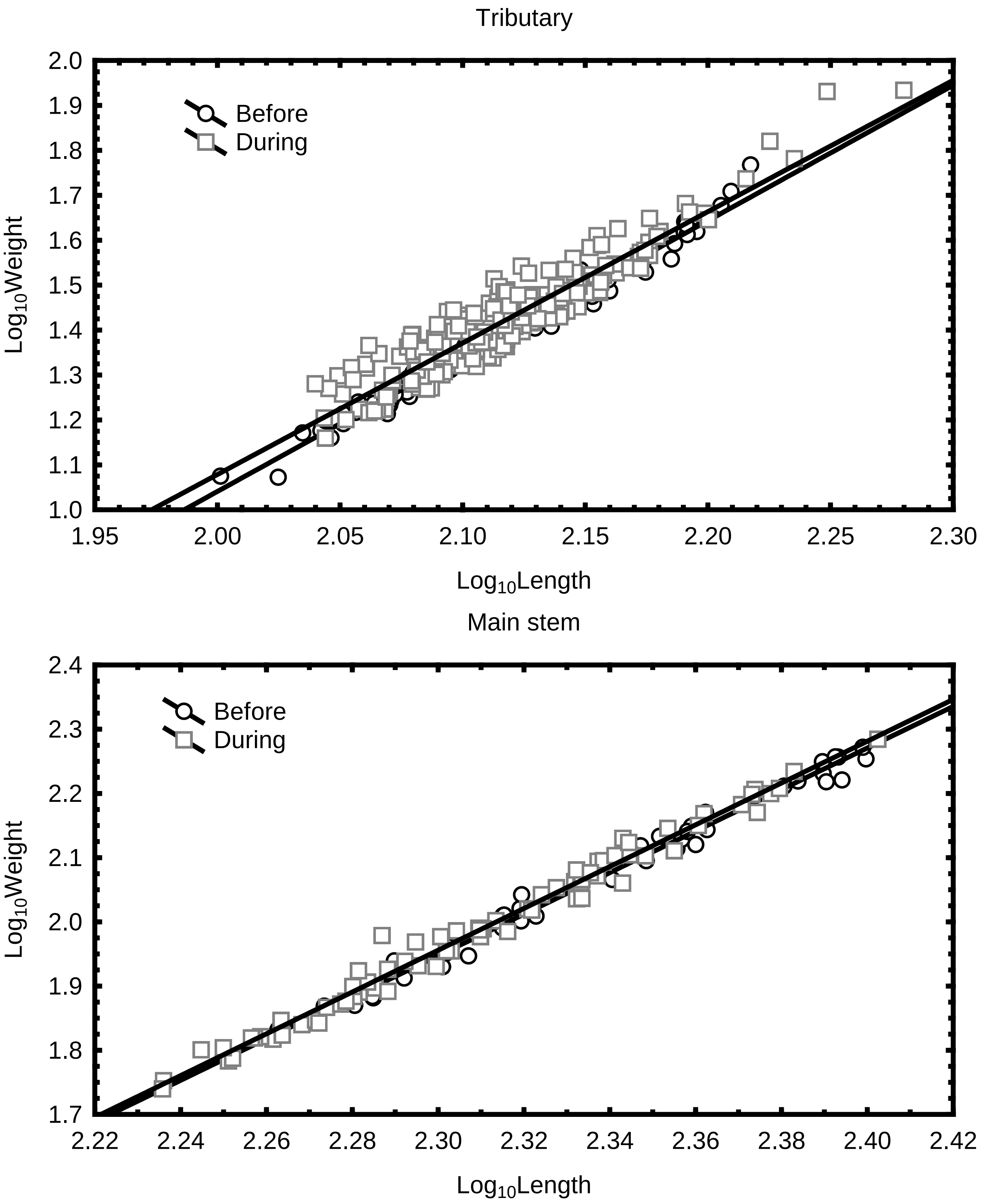

Figure 5. Mean length-weight relationships of tributary and age 1 main stem Yakima River rainbow trout before (1990-1998) and during (1999-2013) the supplementation period. Each data point represents the mean from a sample site.

Causation

Since the lower 90% confidence limit for our steelhead size index was exceeded in both the Yakima River main stem (age 1 rainbow trout) and Yakima Basin tributaries (all ages of rainbow trout), we tested if the changes could be reasonably attributed to supplementation. We did not detect a statistically significant decrease in our steelhead size index (age 1 rainbow trout; BACIP; P = 0.79) or our steelhead biomass index (age 1 rainbow trout; BACIP; P = 0.50) in the main stem downstream from the Clark Flats acclimation facility. Interestingly, we did not detect a significant relationship between our steelhead abundance and size index relationship ($R^2 = 0.11$; P = 0.12) suggesting density dependence was probably not influencing our steelhead size index. For tributary comparisons, we did not detect significant differences in our steelhead size index in comparisons between the North Fork Teanaway River down stream from the Jack Creek acclimation facility (treatment sites) and the West (BACIP; P=0.14) and Middle Fork (BACIP; P=0.41) Teanaway River reference sites. Additional comparisons of our steelhead size index in the main stem Teanaway River relative to the West and Middle Fork Teanaway River reference sites were not consistent with an impact (i.e. all changes were positive). Thus, at this time, the weight-of-evidence suggests declines in our steelhead size index are not likely the result of salmon supplementation activities in the basin.

Although the before vs. after comparisons of rainbow trout abundance did not indicate declines warranting a refined analysis of abundance, we erred on the side of caution and conducted the analysis given our concerns related to the depressed steelhead size index. A comparison of rainbow trout abundance in index monitoring sites located downstream from the Jack Creek Acclimation Facility (e.g., North Fork and Main stem Teanaway Rivers) relative to reference sites in the Middle and West Fork Teanaway Rivers revealed significant reductions in the abundance of rainbow trout relative to the control streams (BACIP). We attempted to account for factors that may influence abundance such as movement and angler induced mortality. Motion activated cameras mounted in both treatment and reference sites during the open angling season in 2011 indicated that the reduction in abundance was probably not angler induced. In addition, we have not detected large scale movements of tagged rainbow trout between treatment and reference streams that would be consistent with a largescale displacement of trout. However, we do have evidence that the North Fork of the Teanaway River produces a higher proportion of anadromous steelhead smolt migrants than the reference streams and significant migrant production may contribute to reduced resident trout abundance. We will continue this investigation in the coming year in cooperation with the Yakima Steelhead VSP project.

Discussion

We failed to reject the hypothesis that early-middle stages of salmon supplementation have impacted valued trout species in the upper Yakima Basin beyond predetermined containment objectives. There were no impacts of supplementation activities on bull and cutthroat trout that inhabited tributary streams because limited or no overlap with hatchery or naturally produced salmon occurred. However, the potential existed for much overlap between salmon and bull and cutthroat trout in the tributaries of the upper Yakima Basin. For example, hatchery steelhead that were released in 1994 very close to the release site in the North Fork of the Teanaway River, migrated upstream into areas containing bull trout and cutthroat trout (McMichael and Pearsons 2001). Hatchery spring Chinook also migrated upstream of the acclimation site in the North Fork of the Teanaway River, but not nearly as far as hatchery steelhead. This finding is consistent with our earlier work and extends the findings into later stages of supplementation (Pearsons and Temple 2007).

It is possible that some overlap occurred at times and places when/where we did not sample. However, substantial overlap was unlikely because we sampled at times and places that overlap was most likely. There are certainly areas outside the upper Yakima watershed where overlap occurs at the times that we sampled. Furthermore, overlap has been detected using the methods we used (e.g. snorkeling). Salmon and bull and cutthroat trout overlap during the summer in another large tributary in the Yakima Basin that parallels the upper Yakima River. In the Naches Basin, which merges with the upper Yakima River near the city of Yakima, substantial overlap exists between bull and cutthroat trout and naturally produced Chinook salmon (T. Pearsons, unpublished data). Hatchery coho salmon are released into that basin and undoubtedly overlap with bull and cutthroat trout. Other studies have also documented overlap between salmon and cutthroat and bull/Dolly Varden trout (Glova 1984; Bisson et al. 1988; Nakano and Kaeriyama 1995; Thurow et al. 1997).

There are a variety of possible reasons why overlap was not detected in tributaries of the upper Yakima River. First, all but one of the acclimation sites for salmon were located in the main stem and the acclimation site in the tributary was located downstream of bull and cutthroat trout. Risks to bull and cutthroat trout were one of many factors that contributed to acclimation site placement. Second, the distribution of juvenile salmon has not increased substantially even though the abundance of adult salmon has increased. We had expected that the distribution of juvenile salmon would have increased with increasing abundance of spawners. Third, high abundance of rainbow trout in lower elevation portions of tributaries may competitively exclude cutthroat and bull trout to higher elevations that salmon do not occupy. Relaxation of competition could result in broader distributions of bull and cutthroat trout and the possibility of greater overlap with salmon. Fourth, salmon, bull trout, and cutthroat trout have different habitat preferences. Salmon typically occupy streams of lower gradient, lower elevation, and warmer water temperatures than cutthroat and bull trout (Glova 1987; Dunham and Rieman 1999). Glova (1987) concluded that impacts to cutthroat trout could be reduced by stocking coho in areas with gradients greater than 1% and ample fast water habitats. Faster water velocities allow for more resource partitioning and competitive dominance by trout. Most of the tributaries in the upper Yakima Basin met these criteria. We did observe overlap between salmon and cutthroat trout in the main stem, where water temperatures were more suitable for both of these species.

Contrary to our previous findings (Pearsons and Temple 2007), we did detect a significant difference in the abundance of rainbow trout in treatment areas in the North Fork Teanaway River and main stem Teanaway River relative to our control sites (Pearsons and Temple 2010). With each additional year of sampling we will have increased power to detect smaller differences (Ham and Pearsons 2000). However, it is important to note that our “Before-After” detection plan would not have triggered the “Causation” analysis that was used to detect the decline and the decline was isolated to a small area and was small relative to the total population size. Furthermore, we do not yet have evidence to support the decline was due to mortality of fish in the treatment area. Other possibilities may include displacement, and perhaps angler harvest, both of which we are currently evaluating.

Although we observed decreases in the size of rainbow trout during the post-supplementation period, the decline is unlikely to have been caused by supplementation. If supplementation had changed the size structure or growth of the steelhead size index, we would expect to detect this change in areas with high densities of salmon. We did not detect a reduction in the size of rainbow trout in the high-density areas of the target taxa below the Clark Flats acclimation site or below the release site in the North Fork Teanaway River. These areas are likely to have the greatest potential of detecting an impact. One potential explanation for the observed decrease in main stem rainbow trout size is that intraspecific density dependent mechanisms have altered the size of main stem Yakima River rainbow trout. The abundance of rainbow trout increased by approximately 30% (30% increase of age 1 fish, and 29% increase of fish greater than 249 mm) after stocking began. This information and results from small-scale enclosure experiments (McMichael et al. 1997) leads us to believe that the decline in rainbow trout lengths is most likely the result of intraspecific competition.

With the exception of the BACIP results from the Teanaway basin, the lack of detectable impacts to rainbow trout were consistent with results that were derived from smaller scale enclosure experiments between naturally produced spring Chinook salmon and rainbow trout in high elevation tributaries (McMichael and Pearsons 1998). In these experiments, growth and abundance of rainbow trout were not impacted when the density of salmonids was doubled by the addition of naturally produced spring Chinook salmon parr. However, growth of rainbow trout was suppressed when the density was doubled with rainbow trout (McMichael et al. 1997), which supports the previously mentioned idea of intraspecific impacts to rainbow trout growth in the main stem. The current results extend the findings of McMichael and Pearsons (1998) to smolts, residuals, coho salmon, and to lower elevation waters such as the main stem. Our ability to detect impacts with the BACI design and the longer experimental period in this study (higher statistical power) may explain the differences among the studies. Opportunities for cumulative impacts to manifest and larger sample sizes may be necessary to detect impacts where high natural variation occurs.

It is possible that our abundance estimates in the main stem and tributaries and the size estimates in the tributaries were influenced by the size breaks that we used in our analysis. The lower size breaks were necessary (e.g., 80 mm in tributaries and 100 mm in the main stem), because we capture very few of these fish due to our low electrofishing efficiencies on small fish and hence cannot calculate valid estimates on these fish. This could result in varying proportions of age 0 and 1 fish in our estimate if the length at age varied across years or sites. However, we do not believe that length truncations significantly affected our conclusions. For example, if fish length was negatively impacted then the distribution of fish size would have become smaller, and more age 1 fish could have been pushed below 100 mm. Regardless of how many fish may have been shifted below 100 mm, if the impact occurred to the whole age class then we should have detected a decrease in size for fish above 100 mm (e.g., the whole length frequency curve would be shifted to smaller sizes). Similarly, if many fish were impacted so that they were less than 100 mm then the abundance of age 1 fish would have been negatively biased. In other words, we would expect to detect less fish than we did prior to supplementation. If we had concluded that impacts had occurred, then our length truncations would be a more serious issue.

We did not detect impacts to non-trout NTT that could be attributed to supplementation. In the tributaries, this was because none of the non-trout NTT overlapped salmon at high enough levels to exceed the CO. All non-trout NTT in the main-stem overlapped completely, but none exceeded the containment objectives.

With the exception of minimum daily stream discharge in the main stem Yakima River, we did not detect changes in the environmental variables that were measured. We hypothesize the increased minimum daily stream discharge observed would benefit NTT. However, the increased minimum daily discharge was not significantly correlated with our NTT monitoring variables suggesting that it did not confound our results. Average and maximum stream discharge and temperature were heavily regulated by upstream irrigation reservoirs providing a relatively stable environment to conduct risk containment monitoring. Although discharge in tributaries is unregulated, summer baseflows have not differed drastically during the time of sampling from year to year. The relatively stable environmental conditions observed in both tributary and main stem areas supports the use of time as a control in our evaluation.

Management Implications

There are a number of management decisions that likely contributed to the small number of impacts in our study. For example, acclimation site placement and the release of fish at the smolt stage reduced the potential spatial and temporal overlap with NTT. Implementation of strategies to limit the number of precociously mature male salmon entering the natural environment would further decrease the risk of failing to meet containment objectives in the future. By reducing the number of these precociously mature salmon, both direct and indirect undesirable interactions with NTT will be reduced. Strategies to reduce the production and release of precociously mature salmon have been implemented in the past (Larsen et al. 2006).

We are implementing the approach described by Ham and Pearsons (2001) to contain risks to NTT throughout the life span of salmon supplementation programs in the Yakima Basin (Pearsons 2002). According to this risk containment approach, if we detect a decrease in NTT status that is greater than a containment objective, then we attempt to determine if the decrease was caused by the supplementation program. Only changes that are due to supplementation warrant risk containment action specific to the supplementation program. The only NTT that was outside of its containment objective was our steelhead index. Steelhead trout were monitored using age 1 rainbow trout as an analog of juvenile steelhead in the main stem and all rainbow trout in the tributaries as analogs. The declines in mean steelhead trout size and the surpassing of the lower 90% CL of the containment objective for steelhead were unlikely to be due to supplementation and therefore do not require risk containment actions. However, the isolated decline in rainbow trout abundance in the North Fork Teanaway is cause for concern because it exceeds the CO for steelhead in this locality. It is important to note, however, rainbow trout abundance in all other tributaries monitored generally increased during supplementation (e.g., population level increase). Finally, we are now monitoring the response of NTT during the building stage of supplementation and this stage is likely to be the one where the risk of impacts is the highest because the number of salmon is potentially very high due to a combination of hatchery and naturally produced fish (Pearsons 2002).

We recommend that our results be placed into context of supplementation dynamics and the ecological context of the Yakima Basin. Our evaluation addressed impacts that occur during the early-middle stages of supplementation. These stages correspond to the Broodstock stage and the Building stage described by Pearsons (2002). The Building stage provides the greatest opportunity for interactions between hatchery fish, naturally produced offspring of hatchery fish, and NTT. Large numbers of hatchery smolts, residuals, and the offspring from returning hatchery adults increases interaction potentials between hatchery and wild fish in the freshwater migration corridor and freshwater rearing area. The Yakima main stem is a highly altered system, and this alteration could change the outcomes of ecological interactions. For example, the flows in the upper Yakima main stem are heavily regulated by the United States Bureau of Reclamation's water storage and delivery programs and are highly unnatural. Artificially high flows during the summer might decrease interspecific interactions among species because of the large volume of water and ability for species to partition resources. In contrast, flows in the tributaries are more normative and might be more reflective of tributaries in other regions. Application of our results should be tempered within these important contexts.

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DNA-Based Parentage Assignments of Chinook Salmon from the Cle Elum Spawning Channel in 2012

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Abstract

We used a maximum likelihood parentage assignment procedure to estimate the reproductive output of Chinook salmon spawners from the hatchery-control line (two generations of hatchery influence) and the supplementation hatchery line (SH – one generation of hatchery influence) in the Cle Elum experimental spawning channel for the 2011 brood year. The assignments were based on offspring genotypes at 14 microsatellite loci. The probabilities of exclusion (inferring non-parentage by randomly picked adults) assuming neither parent was known were estimated to be 0.999999. Two thousand eight hundred and forty-five of 2,925 fry from the 2011 brood that were genotyped at eight or more loci were assigned to a parental pair with 95% confidence. The number of progeny attributed to individual potential parents was quite variable, ranging from 0 to 396 for all males and from 0 to 222 for females. The sum of progeny attributed to the hatchery-control line males and females was 1,281, while the sum of progeny attributed to supplementation hatchery line males and females was 1,564.

Introduction

Although hatcheries have been extensively utilized in Chinook salmon management for over 100 years, only recently have rigorous experiments been developed to measure the relative reproductive success of hatchery- and natural-origin spawners in a shared natural setting. Some of the difficulty in designing informative studies has stemmed from the challenges of controlling entry to natural spawning areas and collecting representative samples of recently hatched fry. Furthermore, if control could be established over the potential spawners in the spawning area, the measurement of individual reproductive output still would require a means of associating individual fish captured in one year with individuals that spawned in a previous year. The spawning behavior of Chinook salmon adds to the complexity of quantifying individual reproductive output through behavioral observations: at a redd site, a female might be courted by several males that compete for access to the female, providing opportunities for multiple paternity in a single redd. In areas with moderate to high spawning densities, males might attend females on several adjacent redds. Microsatellites, a class of highly polymorphic, codominant DNA markers, provide a means to quantify individual spawners' reproductive output. A suite of 10 to 15 highly variable microsatellites can resolve individual identity in a moderate to large population, and through a simple inheritance model, can illuminate parent-offspring relationships.

Washington Department of Fish and Wildlife (WDFW) and the Yakama Nation (YN) are cooperating on a study of Chinook salmon reproductive success in a presumably closed access spawning observation channel at the Cle Elum Hatchery. Viewing blinds line the channel, allowing researchers to observe spawning activities.

Chinook salmon carrying visible external marks were released into the spawning channel in September 2011. Hatchery-control line (two generations of hatchery influence) males and females were released into three of six shared spawning areas and supplementation hatchery line (one generation of hatchery influence) males and females were released into the other three shared spawning areas to select and compete for mates. Prior to the release of the potential spawners, researchers collected and preserved samples of fin tissue to enable genetic characterization of the potential spawners and to allow subsequent inference of parent/offspring relationships after juveniles were collected and genotyped. One group of researchers examined morphological characteristics of these potential parents and observed and recorded spawning area behaviors and interactions. The results of the morphological and behavioral work are described in a separate report.

The potential parents' fin tissue samples and the collected progeny (fry) were delivered to the WDFW Molecular Genetics Laboratory in Olympia, Washington for genetic screening and parentage analysis following the same protocols that have been used from 2002 – 2007, 2009 – 2012 (Young and Kassler 2005, Kassler 2005, Kassler 2006, Kassler and Von Bargen 2007, 2008, and 2010, Kassler et al. 2011; Kassler and Peterson 2012). The genetic analyses provide direct, quantitative estimates of fry production by individual spawning Chinook salmon. This report presents the parentage results for the 2011 – 2012 Cle Elum spawning channel experiments.

Materials and Methods

Collection of potential spawners – 2011

Fin tissue was collected from a total of 48 adult females and 48 adult males (Table 1) prior to their release into each of six sections in the spawning channel during September 2011. The genetic analysis program CERVUS (version 3.0; Marshall et al. 1998) was used to check for identical multilocus genotypes among the potential parents. Data recorded for each released fish included gender, and whether it was of hatchery-control line origin or supplementation hatchery line origin (Table 1).

Collection of Fry

Fry collections occurred from December 1, 2011 to May 2, 2012. Fry samples were collected from each section daily when fry were present. During that period a total of 3,385 fry were collected.

DNA Extraction Methods

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.

PCR Methods

Potential spawners and offspring from 2012 were genotyped at 14 loci (Table 2). Potential spawners were screened twice and scored independently at all 14 loci by two biologists to minimize potential genotyping error of the parents.

The polymerase chain reaction mixture contained the following for a 10 µl reaction: approximately 25 ng template DNA, 1X Promega buffer, 1.5 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP, and dTTP, approx. 0.1 µM of each oligonucleotide primer, and 0.05 units Go*Taq* Flexi DNA polymerase (Promega). Amplification was performed using MJ Research PTC-200 and AB 9700 thermocyclers. The thermal profile was as follows: an initial denaturation step of 2 minutes at 94oC; 40 cycles of 15 seconds at 94oC, 30 seconds at 49-58oC, and 1 minute at 72oC; plus a final extension step at 72oC for 10 minutes, followed by a final indefinite holding step at 4oC.
Microsatellite DNA loci (Table 2) were amplified via the polymerase chain reaction (PCR) using fluorescently labeled primers (obtained from Applied Biosystems or Integrated DNA Technologies). Loci were combined into multiplexes to increase efficiency and decrease costs.

Data were collected using an AB-3730 Genetic Analyzer. Applied Biosystems GENEMAPPER v.3.7 software was used to collect and analyze the raw data and to determine genotypes at each locus (based on estimated allele sizes in base pairs using an internal size standard). Alleles were binned in GENEMAPPER using the standardized allele sizes established for the Chinook coastwide standardization efforts (Seeb et. al. 2007).

Parentage Assignments

The dataset included 40,962 single-locus genotypes. A genotyping error rate in that dataset of 1.0% would result in 410 incorrect single-locus genotypes. Our error rate is unknown, but possibly greater than 1%. Since parentage analyses involve comparing genotypes of candidate parental pairs with offspring genotypes, genotyping errors can produce parent-offspring genotype mismatches and suggest exclusion of true parent-offspring pairings from consideration. Alternatively, genotyping errors can lead to failure to exclude parent-offspring pairings that are incorrect. We used a maximum likelihood procedure, implemented in CERVUS (version 3.0; Marshall et al. 1998) to infer parent-offspring relationships. The procedure uses allele frequency data to assign likelihoods to parent-offspring combinations, and allows mismatching genotypic data to be evaluated concurrently with matching genotype data.

Genotyping error is not the only potential source of mismatches between the genotypes of fry and their putative parents. We would expect allele misidentification to be randomly distributed throughout the genotype dataset and not to occur in clusters. Parent-offspring mismatches can result also from germ-line mutation in which a parent passes a changed allele to its offspring or from the inadvertent exclusion of one or more contributing parents from the parental dataset. These mismatches are due to correctly assigned but unexpected genotypes, and we expect that those genotypes should cluster in families. Distinguishing between mutation-based mismatches and mismatches that result from reproductive participation by un-genotyped parents is difficult. Assuming that all dams in the experimental channel are represented in the parental data set, we might suspect reproductive participation by one or more unrepresented sires if groups of fry that are assigned to a dam-offspring relationship with no mismatching loci, have multiple locus mismatches with all candidate sires, and no more than four alleles at a locus within the group. The data set was carefully examined for evidence of reproductive contributions by such un-genotyped parents (because evidence of ungenotyped parents had been observed in previous years).

Results

Parents

Genetic analysis revealed that all 96 fish released or found in the spawning channel had unique genotypes. There were a total of 24 hatchery control line (HC) adult males, 24 HC adult females, 24 supplementation hatchery line (SH) adult males, and 24 SH adult females. Four HC males and four HC females were released into three of the six sections and four SH males and four SH females were released into the other three sections (Table 1).

Loci Screened

A total of 14 loci were screened and all 14 were used in the analysis (Table 2). Number of alleles ranged from 5 - 32 (*Ots-9* and *Omm-1080* respectively) and observed heterozygosity ranged from 0.330 – 0.958 (*Ots-G474* and *Omm-1080* respectively). Individual exclusionary power was below 46.3% for five loci (*Ogo-2, Ogo-4, Ots-G474, Ots-3M,* and *Ots-9*) and above 60.5% for the remaining loci when neither parent was known. Exclusionary power was below 40.6% for three loci (*Ots-G474*, *Ots-3M* and *Ots-9*) and above 59.2% for the remaining loci when one parent was known. Cumulative exclusionary power was 1.000000 for analysis using all loci when one parent was known.

Parentage Assignments

Parentage assignments were made when genotype data was available for nine or more loci. All 96 parents were genotyped at 8 or more loci while 2,925 of the 3,000 offspring were successfully genotyped at eight or more loci (Table 3).

Parentage analysis was conducted independently for each of the six sections using all 96 adults as possible parents. Each fry was assigned a dam-sire-fry combinations (trios) based on the most likely candidate male parents (sires) and female parents (dams). Those assignments yielded possible. Any fry-sire assignments with more than two mismatching loci were excluded from further consideration.

Of the total 2,925 fry included in the analysis a total of 2,845 fry were assigned to a single male and female parent (2,845/2,925 = 97.3.0%).

Discussion

Ninety-seven percent successes were achieved at inferring parent-offspring relationships. Examination of Table 4 reveals a very uneven pattern of reproductive success among the candidate parents. Based on the subsample of 2,845 fry that were successfully assigned parents, the range of inferred reproductive output among males was 0 - 333 fry; the range for the same period in reproductive output among females was 0 - 165 fry. Some of the dam-sire matings we inferred are well supported (there were a lot of fry assigned to them) and some are weakly supported (not many fry were assigned to them). Caution should be used when interpreting dam-sire-fry combinations that were inferred rarely. Future integration of fecundity estimates for spawners will enrich the interpretation of these estimates of reproductive output.

Interpretation of the inferred parental reproductive output based on parentage assignments by genetic analysis requires the consideration and analysis of individual fish attributes, including fecundity and body size, the closed nature of the experimental environment in which sub-dominant males had a more limited number of alternative females to court than they might have had in an open system, and relative stocking levels and synchronicity of spawning.

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Table 1. Potential Chinook salmon spawners in the six section of the Cle Elum experimental spawning channel in 2011. Origin is identified as hatchery-control (HC) or supplementation hatchery (SH).

	Section 1 – 1A	Section 1 – 2A	Section 1 – 3A	Section 2 – 1A	Section 2 – 2A	Section 2 – 3A
Origin	**Females**	**Females**	**Females**	**Females**	**Females**	**Females**
HC	4	--	4	--	4	--
SH	--	4	--	4	--	4
	Males	**Males**	**Males**	**Males**	**Males**	**Males**
HC	4	--	4	--	4	--
SH	--	4	--	4	--	4

	Section 1 – 1B	Section 1 – 2B	Section 1 – 3B	Section 2 – 1B	Section 2 – 2B	Section 2 – 3B
Origin	**Females**	**Females**	**Females**	**Females**	**Females**	**Females**
HC	4	--	4	--	4	--
SH	--	4	--	4	--	4
	Males	**Males**	**Males**	**Males**	**Males**	**Males**
HC	4	--	4	--	4	--
SH	--	4	--	4	--	4

Table 2. Locus summary.

					Exclusionary power	
Locus	# alleles	# parents genotyped	H_O (observed)	HE (expected)	neither parent	one parent
Oki-100	20	96	0.948	0.916	0.698	0.822
Ots-201b	19	96	0.918	0.878	0.605	0.755
Ots-208b	26	96	0.938	0.936	0.757	0.861
Ssa-408	18	96	0.674	0.918	0.701	0.825
Ogo-2	9	96	0.753	0.820	0.463	0.638
Ssa-197	18	96	0.866	0.909	0.674	0.806
Ogo-4	10	96	0.773	0.785	0.412	0.592
Ots-213	20	96	0.938	0.919	0.706	0.828
Ots-G474	6	96	0.330	0.327	0.055	0.180
Omm-1080	32	96	0.958	0.954	0.815	0.898
Ots-3M	7	96	0.639	0.630	0.230	0.406
Ots-211	23	96	0.907	0.932	0.743	0.853
Ots-212	19	96	0.928	0.884	0.610	0.758
Ots-9	5	96	0.711	0.665	0.240	0.396

Table 3. Summary of genotyping efficiency in potential parents and offspring.

Loci genotyped	Parents (11IM)	Offspring (12MU)
14	70	2,080
13	13	439
12	4	243
11	4	101
10	10	34
9	1	13
8	2	15
7	0	5
6	0	9
5	0	14
4	0	10
3	0	7
2	0	9
1	0	7
0	0	14
	96	3,000

Table 4. Total number of offspring assigned to females and males from each of the six sections in the spawning channel and the life stage (HC - hatchery control line; SH - supplementation hatchery line) for each fish.

Females	Section	HC or SH	Total Offspring	Males	Section	HC or SH	Total Offspring
11IM0050	1-1A	HC	11	11IM0002	1-1B	HC	0
11IM0054	1-1A	HC	33	11IM0004	1-1B	HC	53
11IM0055	1-1A	HC	40	11IM0005	1-1B	HC	270
11IM0056	1-1A	HC	34	11IM0007	1-1B	HC	0
11IM0001	1-1B	HC	0	11IM0009	1-2B	SH	0
11IM0003	1-1B	HC	112	11IM0010	1-2B	SH	306
11IM0006	1-1B	HC	0	11IM0011	1-2B	SH	15
11IM0008	1-1B	HC	209	11IM0015	1-2B	SH	0
11IM0059	1-2A	SH	117	11IM0017	1-3B	HC	33
11IM0060	1-2A	SH	59	11IM0018	1-3B	HC	0
11IM0061	1-2A	SH	20	11IM0020	1-3B	HC	11
11IM0063	1-2A	SH	63	11IM0023	1-3B	HC	0
11IM0012	1-2B	SH	0	11IM0025	2-1B	SH	0
11IM0013	1-2B	SH	27	11IM0026	2-1B	SH	70
11IM0014	1-2B	SH	82	11IM0030	2-1B	SH	0
11IM0016	1-2B	SH	212	11IM0031	2-1B	SH	0
11IM0066	1-3A	HC	0	11IM0033	2-2B	HC	26
11IM0069	1-3A	HC	166	11IM0034	2-2B	HC	396
11IM0070	1-3A	HC	28	11IM0039	2-2B	HC	0
11IM0071	1-3A	HC	14	11IM0043	2-2B	HC	7
11IM0019	1-3B	HC	11	11IM0040	2-3B	SH	170
11IM0021	1-3B	HC	0	11IM0044	2-3B	SH	4
11IM0022	1-3B	HC	33	11IM0045	2-3B	SH	247
11IM0024	1-3B	HC	0	11IM0047	2-3B	SH	0
11IM0074	2-1A	SH	154	11IM0049	1-1A	HC	0
11IM0076	2-1A	SH	30	11IM0051	1-1A	HC	8
11IM0078	2-1A	SH	86	11IM0052	1-1A	HC	0
11IM0080	2-1A	SH	90	11IM0053	1-1A	HC	110
11IM0027	2-1B	SH	0	11IM0057	1-2A	SH	33
11IM0028	2-1B	SH	0	11IM0058	1-2A	SH	104
11IM0029	2-1B	SH	70	11IM0062	1-2A	SH	122
11IM0032	2-1B	SH	0	11IM0064	1-2A	SH	0
11IM0083	2-2A	HC	0	11IM0065	1-3A	HC	0
11IM0084	2-2A	HC	141	11IM0067	1-3A	HC	207
11IM0087	2-2A	HC	0	11IM0068	1-3A	HC	0
11IM0088	2-2A	HC	21	11IM0072	1-3A	HC	0
11IM0035	2-2B	HC	86	11IM0073	2-1A	SH	0
11IM0036	2-2B	HC	0	11IM0075	2-1A	SH	272
11IM0037	2-2B	HC	222	11IM0077	2-1A	SH	54
11IM0038	2-2B	HC	120	11IM0079	2-1A	SH	34
11IM0091	2-3A	SH	65	11IM0081	2-2A	HC	144
11IM0092	2-3A	SH	0	11IM0082	2-2A	HC	17
11IM0095	2-3A	SH	67	11IM0085	2-2A	HC	0
11IM0096	2-3A	SH	0	11IM0086	2-2A	HC	0
11IM0041	2-3B	SH	25	11IM0089	2-3A	SH	130
11IM0042	2-3B	SH	0	11IM0090	2-3A	SH	2
11IM0046	2-3B	SH	194	11IM0093	2-3A	SH	0
11IM0048	2-3B	SH	203	11IM0094	2-3A	SH	0
			2845				2845

DNA-Based Population-of-Origin Assignments of Chinook Salmon Smolts Outmigrating Past Chandler Trap at Prosser Dam (Yakima River) in 2012

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Abstract

A population-of-origin assignment procedure was used to estimate the percentages of unknown-origin smolts from each of five stock groups outmigrating past Chandler Trap (Yakima River) from January – July 2012. Mixture analysis was conducted on a proportional subsample of 1,112 smolts collected during the outmigration at Chandler Trap. Assignment of each individual to a population-of-origin was determined if the posterior probability of the assignment was greater than 90.0%. The largest percentage of outmigrating smolts in the January/February, March, April, and May time strata was from the upper Yakima River stock while the June – July time stratum was dominated by the fall stocks with 81.5% of the total assignments. Comparison of morphological assessment and genetic assignment as a spring or fall Chinook smolt conducted for all time strata indicated agreement for 775/850 (91.2%) of the smolts.

Introduction

Production and survival of the Yakima River basin spring Chinook stocks (American River, Naches River, and upper Yakima River) are monitored, as part of the Yakima/Klickitat Fishery Project supplementation evaluation program. However, in the lower Yakima River, where the best facilities to collect samples exist, the three spring Chinook stocks are mixed with one another and with the Marion Drain and Yakima River fall Chinook stocks, during downstream juvenile migration. Thus, methodologies for discriminating stocks in an admixture are vital for development of stock-specific estimates. Domestication monitoring plans require discrimination of the three spring Chinook salmon stocks in the basin, and a complete analysis of migration timing and stock abundance for all Chinook requires discrimination of the two fall stocks as well. Accurate assignments of Chinook smolts captured at the Chandler fish passage facility to population-of-origin will allow researchers and managers to estimate production by the three spring Chinook stocks, assess smolt-to-smolt survival of the three spring Chinook stocks, and could be utilized to evaluate stock-specific environmental condition factors.

The methodology used in this study to estimate the population-of-origin for individual fish in a mixture followed a Bayesian approach by Rannala and Mountain (1997). This approach assumes linkage equilibrium among loci and uses the multilocus genotype of an individual to compute the probability of that genotype belonging to a population in the baseline. Others have used the methodology developed by Rannala and Mountain (1997) to provide robust population-of-origin assignments of unknown individuals (Hauser et al. 2006, Taylor and Costello 2006, and Waples and Gaggiotti 2006).

Calculation of population-of-origin for Chinook smolts trapped at Chandler trap throughout the entire outmigration (January through July) was hindered in the first few years of analysis for several reasons: non-representative temporal sampling of the downstream migration, past omission of the Marion Drain fall and lower Yakima River mainstem fall Chinook stocks from the DNA baseline, and by maintenance and other shutdowns of trap operations in December and January in many years. In the analyses of samples from 2004 - 2010, attempts were made to eliminate the problems present in previous analyses. A new sampling design was initiated to provide a proportional sample of smolts outmigrating past Chandler trap and a larger number of smolts were analyzed. Repeated multi-year samples of all five baseline stocks were used to characterize the potential sources of smolts in the Yakima River basin.

This report presents the population-of-origin assignments for outmigrating smolts collected at the Chandler trap during 2012.

Materials and Methods

Collections

There were no collections added to the Yakima River baseline this year. Since 1989, sampling crews from the Yakama Nation and WDFW have collected adult spawning ground tissue samples to be included in the baseline. The tissue samples consisted of dry-mounted scales or fin tissue preserved in 100% ethanol from five baseline stocks collected across multiple years (American River spring, Naches River spring, upper Yakima River spring, Marion Drain fall, and lower Yakima River fall; Table 1 and Figure 1).

An estimated total of 627,514 smolts passed the lower Yakima River at Chandler from January 1 – July 26, 2012. This estimate was based on expansion of the total number of smolts counted at the Chandler trap (38,377) to account for trap efficiency, etc. Unknown-origin smolts were collected at Prosser Dam (Chandler Trap) following a sampling design that would identify a proportional number of smolt samples that represents the entire smolt outmigration. The following five time strata (January – February, March, April, May, and June – July) were used for analysis. Samples were collected from January 4 – July 27, 2012. These samples were genetically analyzed to get reliable estimates of population proportions. Each day, the total number of smolts at the trap was visually estimated before any processing occurred. If that number was below a predetermined threshold then a “standard” day’s sample was taken (e.g. 10 fish). If the number of smolts was above the threshold then a “peak” day’s sample was taken (e.g. 30 fish). The threshold for “standard” and “peak” days and the numbers of samples to be taken on each day varied for each of the time strata. These values were determined by analyzing the number of “peak” and “standard” days counted during four years of smolt outmigration monitoring. Based on this sampling design, 2,679 Chinook smolt samples were collected for genetic analysis.

The total estimated numbers of smolts passing the Chandler Trap each day were plotted with the total number of genetic samples that had been collected. A process was then employed to proportionalize the available genetic samples with the daily counts to provide a representative number of smolts that were outmigrating from January – July. A total of 1,112 smolts were identified for analysis.

DNA Extraction Methods

Genomic DNA was extracted by digesting a small piece of fin tissue (all smolt and some adult baseline collections) or scales (most adult baseline collections) 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.

PCR Methods

The polymerase chain reaction mixture contained the following for a 10 µL reaction: approximately 25 ng template DNA, 1X Promega buffer, 1.5 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP, and dTTP, approx. 0.1 µM of each oligonucleotide primer, and 0.05 units Go*Taq* Flexi DNA polymerase (Promega). Amplification was performed using MJ Research

PTC-200 and Applied Biosystems 9700 thermocyclers. The thermal profile was as follows: an initial denaturation step of 2 minutes at 94°C; 40 cycles of 15 seconds at 94°C, 30 seconds at 50-60°C, and 1 minute at 72°C; plus a final extension step at 72°C for 10 minutes, followed by a final indefinite holding step at 10°C.

Eleven microsatellite DNA loci (Table 2) were amplified via the polymerase chain reaction (PCR) using fluorescently labeled primers (obtained from Applied Biosystems or Integrated DNA Technologies). Loci were combined in multiplexes to increase efficiency and decrease costs.

Data were collected using an AB-3730 Genetic Analyzer. Applied Biosystems GENEMAPPER v.3.7 software was used to collect and analyze the raw data and to determine genotypes at each locus (based on estimated allele sizes in base pairs using an internal size standard). Alleles were binned in GENEMAPPER using the standardized allele sizes established for the Chinook coastwide standardization efforts (Seeb et. al., 2007).

Population-of-origin Analysis

The program ONCOR (Kalinowski et al. 2008) was used to assign each individual to one of the baseline collections. ONCOR uses conditional maximum likelihood to estimate mixture proportions (Millar 1987) and genotype probabilities are calculated using a partial Bayesian procedure method of Rannala and Mountain (1997). This Rannala and Mountain (1997) method uses the expectation-maximization (EM) algorithm to calculate the population-source probabilities (posterior probabilities) for each sample. All assignments with a posterior probability greater than or equal to 90% were accepted.

Comparison of Morphological ID and Genetic Assignment

Smolts were categorized as spring or fall Chinook when they were intercepted at the Chandler Trap based on morphological characteristics. Three morphological features (length, size of the eye, and snout shape) were used to identify smolts as spring or fall (Mark Johnston, Yakama Nation; pers. comm.).

Results

Collections

A total of 1,112 unknown Chinook smolts were selected and analyzed from those collected at Chandler Trap. Smolt samples that were missing data for six or more loci (N = 27) were dropped from statistical analyses.

Population-of-origin Analysis

The mixture composition estimates for the entire 2012 smolt outmigration indicated that the largest overall percentage of spring smolts was from the upper Yakima River followed by the

Naches River and American River in the first four strata. During the migration from January – May, the proportion of the upper Yakima River stocks was between 38.8 and 65.1% while the American River and Naches River spring stocks was between 5.3 and 31.6%. The proportion of the two fall stocks was between 0.0 – 17.0% for the first four time strata and 81.5% in the June - July time stratum (Table 3).

Comparison of Morphological ID and Genetic Assignment

A comparison of the morphological assessment to genetic assignment was conducted for all five time strata. A total of 39 smolts in January/February, 54 smolts in March, 164 smolts in April, 214 smolts in May, and 379 in the June/July time strata were scored, and therefore included in the analysis. Results for the time strata were as follows: January/February time stratum – all 39 smolts were assigned identically using morphological and genetic methods (39 spring); March stratum – all 54 smolts were assigned identically using morphological and genetic methods (54 spring); April time stratum – 164 smolts were assigned identically using morphological and genetic methods (164 spring); May time stratum – 199 out of 214 smolts were assigned identically using morphological and genetic methods (168 spring – 31 fall), eight of the 15 discrepancies were identified as a fall by the genetic analysis and spring with morphological identification and the remaining seven were identified as a spring by the genetic analysis and fall with morphological identification ; June/July time stratum – 319 out of 379 smolts were assigned identically using morphological and genetic methods (40 spring and 279 fall), 33 discrepancies were assigned as fall by the genetic analyses while morphological identification was spring, the remaining 27 discrepancies were identified as a spring by the genetic analysis and fall with morphological identification.

Discussion

Collection of smolts at the Chandler Trap in 2012 utilized a sampling design intended to yield a sample that was proportional to the number of smolts passing the Chandler Trap. Sampling a proportional number of smolts was important to determine an accurate percentage of smolts from each stock that were outmigrating from the basin. Developing the sampling strategy for identifying a “standard” versus “peak” day of smolts that were in the trap and applying a sampling goal for those days allowed for a proportional sample. Subsampling the smolts collected for genetic analysis provided a best fit to the actual passage of smolts for a given day.

Monitoring the relative abundances of Chinook smolts in the Yakima River from the three different populations of spring Chinook (upper Yakima River, American River, and Naches River) and the two populations of fall Chinook (Marion Drain and lower Yakima River) requires the ability to estimate population composition of smolts outmigrating past Chandler trap. Because all five Chinook populations are intermingled when they pass Chandler trap, and the vast majority are unmarked and untagged, the only way to determine population-of-origin is by genetic analysis. This method requires that sufficient genetic differences exist among these populations in the Yakima River basin.

A baseline of 19 individual collections from the five populations in the Yakima River basin was used for the population-of-origin assignments of the outmigrating smolts. The baseline collections as a whole had higher genotyping failure compared to the Chandler smolt samples.

Scales were taken from carcasses on spawning grounds for most baseline collections; therefore, DNA quality was presumably poorer than the Chandler smolt collection where tissue was collected from live fish. The upper Yakima River tissue collections were also taken from live fish at the hatchery and, therefore, genotyping success was higher for this collection than the other baseline collections.

Assessment of spring or fall smolts by morphological and genetic analysis revealed agreement with 75 individuals being identified differently between the two methods. Identification as a spring or fall smolt was the same for 775 smolts collected during the January – February, March, April, May, and June – July time strata.

The majority of the assignments between January and May were from the three spring stocks. The upper Yakima River spring stock accounted for the highest average percentage (56.4%) of smolts present in that period. Rank in abundance of the three spring stocks was the same in the three time strata (January-February, March, April, and May) with upper Yakima River spring stock having the most. The June-July time stratum was predominately composed of the fall Chinook stocks, accounting for over 81.5% of the total number of smolts.

Assessment of DNA Mixture Assignments from 2000 – 2010

Mixed stock analysis has been conducted on Chandler smolts since 2000 (Young 2004, Kassler et al. 2005, Kassler 2006, Kassler and VonBargen 2007, 2008, 2009 and 2010, Kassler and Peterson 2011, Kassler and Bell 2012); however the sampling design for samples collected in 2000 – 2003 was not proportionalized during the run. The yearly assignments are therefore not comparable from those years. Beginning in 2004, staff at the Chandler trap utilized a sampling protocol to provide a number of smolts that was relative to the percentage of smolts passing that day. Samples were then subsampled at WDFW to provide a proportional number of samples that would represent the overall passage to be analyzed.

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Table 1. Nineteen Chinook salmon collections assembled into a baseline and used for the analysis of the known-origin and unknown-origin smolts. "*" the 05LU collection from Marion Drain was not used in the baseline, but is listed here as a collection from Marion Drain. The percentage of single locus genotypes missing are shown for each collection.

Baseline Collections	Collection Code	# Processed	# Analyzed	% Single Locus Genotypes Missing
American River - spring	89AG	80	77	10.4%
	91DQ	102	87	9.8%
	93DO	18	17	3.2%
	03EH	100	70	6.6%
		300	251	8.6%
Naches River - spring	89AC	76	74	11.4%
	89AI	26	22	7.0%
	93DQ	50	45	6.3%
	93DR	32	25	7.3%
little Naches River - spring	04BI	42	41	2.2%
	04EM	56	45	9.9%
		282	252	7.9%
upper Yakima River - spring	92DN	24	23	5.9%
	97DA	123	115	3.9%
	03GO	99	99	1.4%
		246	237	3.0%
Marion Drain - fall	89BX	100	92	8.3%
	92FQ	92	92	5.4%
	93DY	8	8	8.0%
	05LU*	65	47	15.3%
		265	239	8.6%
lower Yakima River - fall	90DF	109	104	12.6%
	93DW	82	80	9.8%
	98FB	61	50	8.7%
		252	234	10.8%
Chandler Trap Smolts - 2012	12AY	1,112	1,085	0.5%

Table 2. Microsatellite locus information (number alleles/locus and allele size range) for multiplexed loci used in the analysis of Chinook from five stocks in the Yakima River Basin. Also included are the percent missing genotypes for both baseline and smolts collections and heterozygosity (observed (H_o) and expected (H_e)) for each locus.

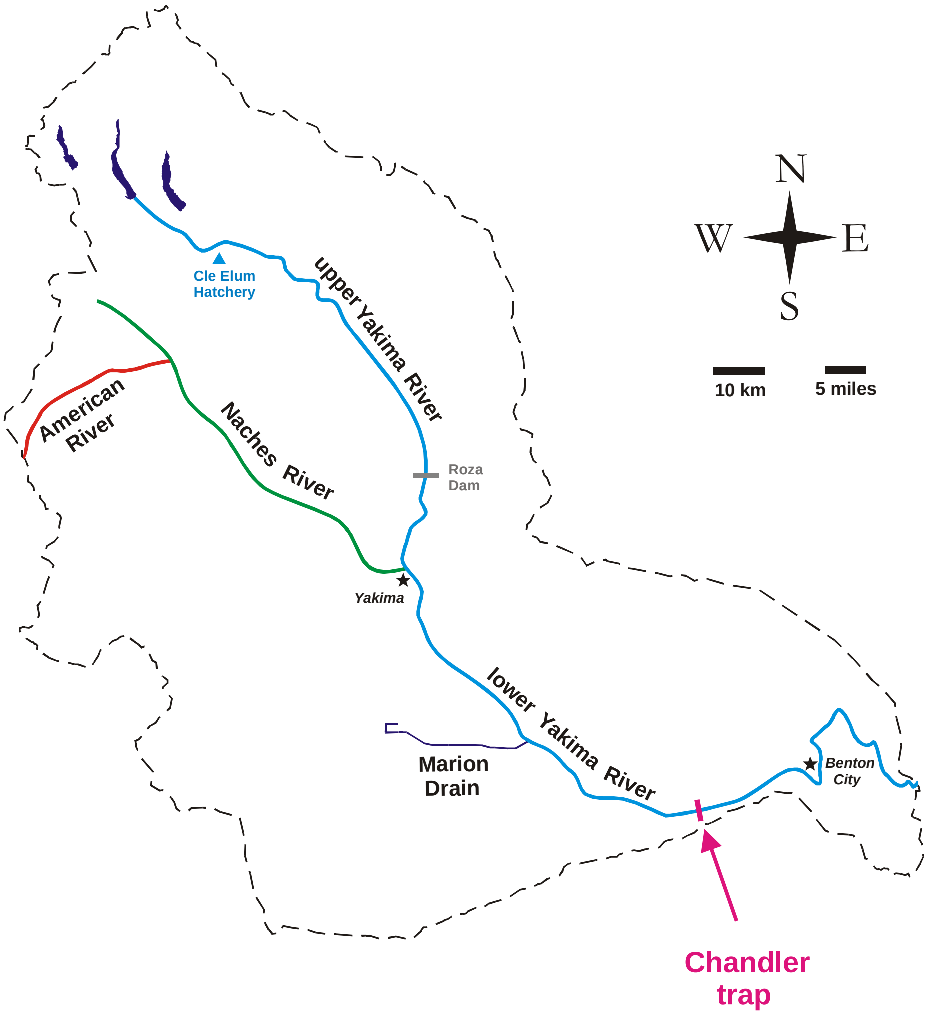
							Heterozygosity	
Multiplex	Locus	Annealing temp °C	# Alleles/ Locus	Allele Size Range (bp)	% missing genotypes baseline N = 1,213	% missing genotypes smolts N = 933	H_o	H_e
Ots-M	*Oki-100*[a]	50	41	164 - 365	11.4	5.7	0.913	0.940
	Ots-201b[a]	50	42	137 - 310	7.3	2.1	0.916	0.936
	Ots-208b[b]	50	52	158 - 342	9.9	5.5	0.943	0.954
	Ssa-408[c]	50	32	184 - 308	4.0	3.1	0.827	0.934
Ots-N	*Ogo-2*[d]	60	19	202 - 256	4.5	0.4	0.756	0.854
	Ssa-197[e]	60	38	181 - 318	11.9	0.4	0.915	0.940
Ots-O	*Ogo-4*[d]	56	17	132 - 164	15.6	1.6	0.776	0.884
	Ots-213[b]	56	40	182 - 362	9.4	1.8	0.908	0.940
	Ots-G474[f]	56	15	152 - 212	3.8	1.5	0.507	0.697
Ots-R	*Ots-3M*[g]	53	15	128 - 158	2.9	1.4	0.601	0.672
Ots-S	*Ots-9*[g]	60	8	99 - 113	5.0	1.0	0.668	0.709

[a] = Unpublished
[b] = Greig et al. 2003
[c] = Cairney et al. 2000
[d] = Olsen et al. 1998
[e] = Oreilly et al. 1996
[f] = Williamson et al. 2002
[g] = Banks et al. 1999

Table 3. Stock-of-origin assignments for five stocks of Chinook in the Yakima River Basin using ONCOR.

	American R.	Naches R.	upper Yakima R.	Marion Drain	lower Yakima R.
Jan - Feb	11.0%	31.6%	57.4%	0.0%	0.0%
March	5.3%	29.6%	65.1%	0.0%	0.0%
April	6.2%	29.3%	64.5%	0.0%	0.0%
May	11.1%	31.2%	38.8%	2.0%	17.0%
June-July	4.4%	5.5%	8.7%	8.0%	73.5%

Figure 1. Geographic location of the Chandler trap on the Yakima River, Washington and the primary streams in the basin.



Appendix D. Performance measures relative to project quantitative objectives

Performance Measure	Goal	Performance	Comments
Natural Production of Target Species	Increase while maintaining the long-term fitness of the target population (see quantitative objectives; Pearsons et al. 2006)	Quantitative objectives for adults and smolts are being achieved. Differences in traits of hatchery and natural origin fish are a concern	- Too early to evaluate conclusively, but strategies to reduce genetic risk are being implemented. - Hatchery has increased the number and distribution of adult spawners on the spawning grounds. Quantitative management objectives for natural production of upper Yakima and basin total spring Chinook adults and smolts are being achieved. - Significant changes in many demographic and reproductive success traits indicate cause for concern. Recent data suggest significant genetic contribution to many of these changes. - Predation and competition may be limiting natural production objectives and may constrain the benefits of supplementation.
Harvest	Increase (see quantitative objectives; Pearsons et al. 2006)	Increased, and objectives are being met	- Tribal subsistence fisheries occurred on both hatchery and naturally produced fish in all years. Sport fisheries on hatchery fish have also occurred in the Yakima River in 8 of the 12 years since 2001. - Quantitative harvest objectives for the upper Yakima stock and all Yakima basin stocks

			combined are being met for the Columbia or Yakima Rivers
Genetics	Minimize genetic impacts to non-target taxa	Achieved to date	Stray rates are very low
Ecology	Keep impacts to non-target taxa within containment objectives (see Pearsons et al. 2006)	Achieved for most taxa to date	Impacts for most species are within containment objectives or are currently not attributable to supplementation.
Habitat	Protect the most productive stream reaches and increase productivity/capacity of freshwater environment so that quantitative objectives can be achieved.	Progress	Habitat protection, restoration, and tributary passage efforts are ongoing, with incremental progress each year. - Habitat actions should enhance the benefits of supplementation, especially over the long-term.
Science	Disseminate important findings for use by other researchers and managers.	Achieved to date	Numerous annual reports were submitted to BPA, all tasks were reported on at annual conferences, and manuscripts have been prepared and published.