# Reproductive Ecology of Yakima River Hatchery and Wild Spring Chinook and Juvenile-to-Adult PIT-tag Retention 

Yakima/Klickitat Fisheries Project Monitoring and Evaluation

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# Reproductive Ecology of <br> Yakima River Hatchery and Wild Spring Chinook 

and

# Juvenile-to-Adult PIT Tag Retention 

Annual Report 2001

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## Executive Summary

This is the first of a series of annual and progress reports that address reproductive ecological research and comparisons of hatchery and wild origin spring chinook in the Yakima River basin. Data have been collected prior to supplementation to characterize the baseline reproductive ecology, demographics and phenotypic traits of the unsupplemented upper Yakima population, however this report focuses on data collected on hatchery and wild spring chinook returning in 2001; the first year of hatchery adult returns. This report is organized into three chapters, with a general introduction preceding the first chapter and summarizes data collected between January 1, 2000 and December 31, 2000 in the Yakima basin. Summaries of each of the chapters included in this report are described below.

- Adult traits: Size-at-Age - Naturally spawning age-3 and age-4 hatchery fish were significantly smaller than wild fish of the same age: 2.9 cm smaller in 3-year olds and 2.1 cm smaller in 4-year olds. Both 3- and 4-year old hatchery origin fish weighed approximately 0.3 kg less than wild fish of the same age. There was no significant Treatment effect (OCT vs SNT) in body size. Age-4 fish from the American River (male 67.6 cm ; female 64.3 cm ) were larger than Naches fish (male 63.3 cm ; female 63.3 cm ). American River fish (male 83.3 cm ; female 76.4 cm ) were also larger at age- 5 than Naches fish (male 71.9 cm ; female 74.2 cm ) of the same age. These populational differences in size-at-age are likely local adaptations developed in response to selection pressures such as migration difficulty, spawn timing, and intra-sexual competition. Sex Ratio - The female:male (F:M) ratios of upper Yakima River wild and hatchery origin fish collected at either Roza Adult Monitoring Facility (RAMF) or on the spawning grounds as carcass samples were not significantly different. The F:M ratios of American (1.1) and Naches (2.0) spawning ground carcass samples were significantly different from the upper Yakima population (3.2) and may partially reflect between population differences in rates of nonanadromous precocial male development. Age Composition - As in previous years, the overwhelming majority of upper Yakima River wild fish returned as 4 -year olds ( $85 \%$ ) with $5 \%$ returning at age-5. Age-4 fish made up $86 \%$ of the total hatchery origin returns. Age-3 jacks made up 10 and $14 \%$ of the total wild and hatchery populations, respectively. Using linear discriminant function analysis, we classified wild fish into 3 -, 4- and 5-year-old ages with 100,89 , and $94 \%$ classification accuracy, respectively. Based on scale sampled carcass recoveries, age composition of the American River was 0,67 and $33 \%$ age-3, -4 and -5 , respectively. Naches system fish were 1, 65 and $35 \%$ age- $3,-4$ and -5 , respectively. Historically, American River fish return at older mean age than Naches fish, but in 2001 age compositions were essentially equivalent due in large part to the very strong 1997 (age-4) broodyear return across all three populations. Sexual Dimorphism - There were no significant Sex (Male vs. Female) effects detected in body size of wild and hatchery age-4 carcass recoveries in the Naches and upper Yakima River populations. Upper Yakima River wild age-5 fish did demonstrate significant sexual dimorphism in body size, as did the American River population age-4 and -5 year olds. In paired length and weight samples collected from the same fish, first at RAMF and later at CESRF, fork length increased 4.4\% in males and 2.6\% in females on average, while male and female body weights decreased by $16.3 \%$ and $13.4 \%$, respectively. Run/Spawn Timing- Mean and median passage timing at RAMF and mean
spawn timing (Sept. 26) of both upper Yakima River hatchery and wild fish was not significantly different. Mean and median spawn timing was August 19 and 21, respectively, for the American River and September 14 and 17, respectively, for the Naches population based on carcass recoveries. The earlier American River and Naches, to a lesser degree, spawn timing is a local adaptation to cooler incubation water temperatures experienced by the higher elevation populations that require more time to accumulate sufficient temperature units to fry emergence, which is generally synchronized across all Yakima basin spring chinook populations. Carcass Recovery Bias - The F:M ratio at RAMF was significantly different from the F:M ratio of spawning ground carcass recoveries, indicating that sex ratios estimated from carcass recoveries are biased. This is because female carcasses are recovered at higher rates than male carcasses. In addition, a comparison of the proportion of age-3, -4 and -5 old fish in the RAMF sample and the carcasses recovery sample indicated that older, larger fish were recovered as carcasses at significantly higher rates than younger, smaller fish. Within age classes, the mean POHP of wild origin age- 4 and age- 5 carcass recoveries did not differ significantly from fish sampled at RAMF. Thus, carcass recovery length distributions do appear to accurately represent size-at-age.
- Gametic traits: Naturally spawning age-4 hatchery females (3,820 eggs) were $9 \%$ less fecund ( 340 eggs) than wild origin females ( 4,160 eggs) based on the observation in Chapter 1 that age- 4 hatchery females were on average 2.1 cm smaller in POHP length than age-4 wild females and common length/fecundity slopes (see below). Age-5 wild origin females ( 5,101 eggs) were significantly more fecund on average than age- 4 wild females. Fecundity and female body size were positively correlated in both hatchery and wild origin age-4 females. The fecundity/length and fecundity/weight slopes of age-4 hatchery and wild origin females were not significantly different (common slopes $=863$ eggs $\bullet[\mathrm{kg} \text { body weight }]^{-1}$ and 165 eggs $\bullet[\mathrm{cm} \mathrm{POHP}]^{-1}$ ). Wild age- 5 females also had significant, but weaker, positive correlations between female body size and fecundity and had significantly shallower linear relationships, producing less than half as many eggs per kg increase in body weight (389 eggs $\bullet[\mathrm{kg} \text { body weight }]^{-1}$ ) and almost $1 / 3$ fewer eggs per cm increase in POHP length (113 eggs• $[\mathrm{cm} \mathrm{POHP}]^{-1}$ ). Including body weight, mean egg weight and POHP in a multi-variate fecundity regression equation significantly increased the amount of variation explained and improved the precision of fecundity estimates. There was no significant difference between age- 4 hatchery $(0.195 \mathrm{~g})$ and wild ( 0.192 g ) origin mean egg weights. Age- 5 wild origin females had significantly heavier eggs ( 0.216 g ). There were weak positive correlations between egg weight and female POHP and body weight. The relationship between egg size and fecundity was negative, weak and significant only in wild age-4's. Female Reproductive Effort (RE), the ratio of the weight of gametes to total body weight, of hatchery females (mean $=0.207$ ) was greater than age-4 (mean $=0.201$ ) and age- 5 (mean= $=0.193$ ) wild females. The difference between hatchery and age- 5 wild females was statistically significant. There was no significant difference in viability of eggs of hatchery (mean viability $=0.87$ ) and wild (mean viability $=0.89$ ) origin females. Both hatchery (mean $=0.009$ ) and wild (mean=0.004) origin females had very low proportions of abnormally developing fry, but the hatchery proportion was significantly greater than the wild proportion. There was no significant difference between wild and hatchery origin fry fork lengths or body weights at the "button up" stage. There were strong positive relationships between fry size and egg weight for both wild and hatchery origin females. The fry fork length/egg weight relationship explained 73
and $62 \%$ of the total variation and the fry body weight/egg weight relationship explained 93 to $82 \%$ of the total variation in wild and hatchery fish, respectively. There were weak positive correlations between fry size and adult female size, although the total variation explained ranged from only 5 to $10 \%$.
- PIT tag Loss: We estimated tag loss in spring chinook returns 1-3 years after release. Annually from 1998 to 2001, approximately 40,000 0-age juveniles were tagged with a PIT tag, Coded-wire tag (CWT) injected into the snout, and an adipose fin clip to estimate downstream juvenile survival through the Yakima and Columbia rivers. Upon return, fish fell into one of four categories based on tag retention: 1) PIT tagged/CWT/Ad clipped (all tags and marks retained), 2) CWT/Ad clipped (lost PIT tag), 3) PIT tagged/Ad clipped (lost CWT), or 4) Ad clipped only (lost both PIT and CWT). Returning age-2, -3 and -4 hatchery origin fish were monitored at RAMF in 2001 for the presence of each tag and mark, and tag loss estimates were calculated. We found that PIT tags were lost in increasing proportions as return age increased. Age-2,-3 and -4 fish were estimated to have total loses of 3, 10 and $16 \%$ of their PIT tags, respectively. PIT tag loss also increased over time within a year. Adult female PIT tag loss estimates were 15, 17 and $23 \%$ in May, June and July-September, respectively. Adult males PIT tag loss estimates were 11, 10 and $24 \%$ in May, June and July-September, respectively. Spring chinook pass RAMF 1-5 months prior to spawning, while still not fully mature and our data likely underestimate actual loss at the time of spawning. This study utilized $400-\mathrm{kHz}$ PIT tags and some of our tag "loss" may actually be due to missed, undetected PIT tags. While this might result in overestimated loss, it would not explain the increase in PIT tag loss over time. Juvenile-to-adult survival studies using PIT tags will underestimate actual survival rates when tag loss occurs. However, comparisons between similar groups of PIT tagged fish that lose tags at comparable rates are valid relative survival comparisons. Care should be taken before extrapolating adult survival estimates from PIT tagged fish to untagged populations.

All findings in this report should be considered preliminary and subject to further revision unless they have been published in a peer-reviewed technical journal.

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## General Introduction

This report is intended to satisfy two concurrent needs: 1) provide a contract deliverable from Oncorh Consulting to the Washington Department of Fish and Wildlife (WDFW), with emphasis on identification of salient results of value to ongoing Yakima/Klickitat Fisheries Project (YKFP) planning, and 2) summarize results of research that have broader scientific relevance. This is the first in an anticipated series of reports that address reproductive ecological research and monitoring of spring chinook in the Yakima River basin. In addition to within-year comparisons, between-year comparisons will be made to determine if traits of the wild Naches basin control population, the naturally spawning population in the upper Yakima River and the hatchery control population are diverging over time. This annual report summarizes data collected between April 1, 2001 and March 31, 2002. In the future, these data will be compared to previous years to identify general trends and make preliminary comparisons.

Supplementation success in the Yakima Klickitat Fishery Project's (YKFP) spring chinook (Oncorhynchus tshawytscha) program is defined as increasing natural production and harvest opportunities, while keeping adverse ecological interactions and genetic impacts within acceptable bounds (Busack et al. 1997). Within this context demographics, phenotypic traits, and reproductive ecology have significance because they directly affect natural productivity. In addition, significant changes in locally adapted traits due to hatchery influence, i.e. domestication, would likely be maladaptive resulting in reduced population productivity and fitness (Taylor 1993). Thus, there is a need to study demographic and phenotypic traits in the YKFP in order to understand hatchery and wild population productivity, reproductive ecology, and the effects of domestication (Busack et al. 1997). Tracking trends in these traits over time is also a critical aspect of domestication monitoring (Busack et al. 2002) to determine whether trait changes have a genetic component and, if so, are they within acceptable limits. The first two chapters of this report deal with monitoring phenotypic and demographic traits of Yakima River basin spring chinook comparing hatchery and wild returns in 2001; the first year of adult hatchery returns. The first chapter deals specifically with adult traits of Naches basin and upper Yakima River spring chinook, excluding gametes. The second chapter examines the gametic traits and progeny produced by upper Yakima River wild and hatchery origin fish. Another facet of the YKFP monitoring effort involves comparing hatchery and wild origin reproductive ecology of naturally spawning wild and hatchery origin fish. As part of that effort, hatchery and wild fish are allowed to compete for mates and spawning sites within an experimental channel located at CESRF, their behavioral interactions recorded, and reproductive success determined from their progeny using DNA microsatellite pedigree analysis (Schroder et al. 2002; Young and Shaklee 2002). In addition, wild and hatchery spawning behavior and redd characteristics in the upper Yakima River will be monitored in situ and reported on in future reports.

In the third chapter of this report, we analyze tag recovery data from hatchery spring chinook PIT tagged as juveniles and returning in 2001. The large scale PIT
tagging of juveniles each year in the YKFP affords us an opportunity to assess retention rates of PIT tags up to the time fish are passed upstream at Roza Adult Monitoring Facility. There has been a lack of rigorous testing of PIT tag retention since Prentice et al. (1994) first estimated PIT tag loss as high as $60 \%$ in female coho salmon. Given the degree to which we utilize and depend on PIT tags in YKFP studies, it seemed prudent to estimate the loss of PIT tags from the juvenile-to-adult stage. This analysis will be repeated annually over the next 5 years as PIT tagged fish from the 1998-2001 broodyears return. We will evaluate the performance of a number of other marking/tagging technologies utilized in the YKFP in future reports, as well.

The chapters in this report are in various stages of development and should be considered preliminary unless they have been published in a peer-reviewed journal. Additional fieldwork and/or analysis is in progress for topics covered in this report. Readers are cautioned that any preliminary conclusions are subject to future revision as more data and analytical results become available.

## Acknowledgments

We would like to thank Bonneville Power Administration for financially supporting this work. In addition, we could not have completed this work without the help and support of many individuals during 2001. We have tried to recognize each of them either on title pages or in acknowledgments within each chapter of this report.

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## Chapter 1

# Monitoring Phenotypic and Demographic <br> Traits of Yakima River 

# Hatchery and Wild Spring Chinook: Spawner Traits 

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#### Abstract

A major component of determining supplementation success in the Yakima Klickitat Fishery Project's spring chinook (Oncorhynchus tshawytscha) program is an increase in natural production. Within this context, comparing upper Yakima River hatchery and wild origin fish across traits such as sex ratio, age composition, size-at-age, fecundity, and run timing is important because these traits directly affect population productivity and individual fish fitness which determine a population's productivity. In addition, comparisons of these traits across the three putative wild populations in the Yakima River basin: American River, Naches, and upper Yakima River, help us identify and understand how local adaptation has uniquely shaped each population.


Sex Ratio - The female:male (F:M) ratios of upper Yakima River wild (1.6) and hatchery (2.0) origin fish collected at Roza Adult Monitoring Facility (RAMF) were not significantly different. The F:M ratios of wild and hatchery origin fish in the spawning ground carcass sample were 3.5 and 3.1, respectively, and were also not significantly different. The F:M ratios of American (1.1) and Naches (2.0) spawning ground carcass samples were significantly different from the upper Yakima population and may reflect between population differences in rates of nonanadromous precocial male development. Visual sexing of fish at RAMF identified females more accurately ( $96 \%$ correct) than males ( $66 \%$ correct) resulting in a significant overestimate of the proportion of returning females

Age Composition - The overwhelming majority of upper Yakima wild fish returned as 4year olds ( $85 \%$ ) with $5 \%$ returning at age-5. Age-4 fish made up $86 \%$ of the total hatchery origin returns. Age-3 jacks made up 10 and $14 \%$ of the total wild and hatchery populations, respectively. Linear discriminant function analysis was used to classify wild upper Yakima fish into 3-, 4- and 5-year-old age classes with 100, 89 , and $94 \%$ classification accuracy, respectively. Based on scale sampled carcass recoveries, age composition of the American River was 67 and $33 \%$ age- 4 and -5 , respectively. Naches system fish were 1, 65 and $35 \%$ age- $3,-4$ and -5 , respectively. In 2001, age compositions of all populations were strongly influenced by the very strong 1997 (age-4) broodyear return.

Sexual Dimorphism - In general, there were no significant Sex (Male vs. Female) effect detected in body size of wild and hatchery age-4 carcass recoveries in the Naches and upper Yakima River populations. Upper Yakima River wild age- 5 fish did demonstrate significant sexual dimorphism in both POHP length and body weight, as did the American River population age- 4 and -5 year olds. In both populations, mean male POHP length was significantly greater than female length. Paired length and weight samples from fish sampled at RAMF and then later at CESRF were compared. Fork length increased $4.4 \%$ in males and $2.6 \%$ in females and male and female body weights decreased by $16.3 \%$ and $13.4 \%$, respectively.

Size-at-Age - Naturally spawning hatchery fish were significantly smaller than wild fish of the same age. Four-year old hatchery fish (mean $=59.3 \mathrm{~cm}$ ) were 2.1 cm smaller than wild origin fish (mean $=61.4 \mathrm{~cm})$. Three-year old hatchery fish (mean $=40.0 \mathrm{~cm}$ ) were 2.9 cm smaller than wild fish (mean $=42.9 \mathrm{~cm}$ ). Both 3- and 4-year old hatchery origin fish weighed approximately 0.3 kg less than wild fish of the same age. The reduction in hatchery body weight resulted in 2.7 mt ( 5,800 pounds) less biomass returned to the upper Yakima system in 2001 than would have occurred in the same number of wild fish had returned. There was no significant difference in body size between OCT and SNT treatment groups of either age- 3 or -4 returns. Age- 4 and -5 fish from the American River were larger than the Naches and upper Yakima populations. These wild origin populational differences in size-at-age are likely due in large part to local adaptations developed in response to selection pressures such as migration difficulty, spawn timing, and intra-sexual competition.

Run/Spawn Timing - Mean and median passage timing of hatchery and wild fish at RAMF differed by 2 and 4 days, respectively, with wild fish passing earlier than hatchery fish. Mean spawn timing of both upper Yakima River hatchery and wild fish, based on in-river carcass recoveries, was Sept. 26 and there was no significant difference in temporal distributions of recoveries. Wild origin fish did exhibit a significant, though very weak, positive linear relationship between passage date at RAMF and date of spawning at CESRF in 2001, which explained only $1 \%$ of the total variation in spawn timing. Hatchery origin fish showed no correlation between date spawned at CESRF and passage date at RAMF. Mean and median spawn timing was August 19 and 21, respectively, for the American River and September 14 and 17, respectively, for the Naches population based on carcass recoveries. The earlier spawn timing of each population is likely a local adaptation to cooler incubation water temperatures experienced by the higher elevation populations requiring more time to accumulate sufficient temperature units to fry emergence.

Carcass Recovery Bias - The F:M ratio at RAMF was significantly different from the $\mathrm{F}: \mathrm{M}$ ratio of spawning ground carcass recoveries, indicating that sex ratios estimated from carcass recoveries are biased. This is because during the period of active spawning female carcasses are recovered at higher rates than male carcasses. A comparison of the proportion of age-3, -4 and -5 old fish in the RAMF sample and the carcasses recovery sample also indicated that older, larger fish were recovered as carcasses at significantly higher rates than younger, smaller fish. Within age classes, the mean POHP of carcass recoveries did not differ significantly from fish sampled at RAMF. Thus, carcass recovery length distributions do appear to accurately represent size-at-age.

All findings in this report should be considered preliminary and subject to further revision unless previously published in a peer-reviewed technical journal.

## Introduction

Supplementation success in the Yakima Klickitat Fishery Project's (YKFP) spring chinook (Oncorhynchus tshawytscha) program has been defined as an increase in natural production and harvest opportunities, while keeping adverse ecological interactions and genetic impacts within acceptable bounds (Busack et al. 1997). Within this context, monitoring phenotypic and demographic traits is important because these traits bear directly on a population's productivity and fitness.

Changes in phenotypic and demographic traits due to hatchery influences (domestication) can have a genetic or environmental cause or be a complex combination of both (Hard 1995; Kinnison et al 2001; Quinn et al. 2001; Su et al. 2002). Significant changes in locally adapted traits due to domestication would likely be maladaptive in the wild, reducing reproductive success resulting in lower population productivity and fitness (Taylor 1991; Fleming and Gross 1993; Hard 1995; Fleming and Petersson 2001; Lynch and O'Hely 2001). A non-selective fitness reduction, that is where lower reproductive output is random with respect to any heritable trait's distribution, will not generate a genetic response in subsequent generations. Consequently, population productivity is reduced for a single generation. For example, significant differences in the body morphology of cultured (captive brood) and wild coho salmon derived from a common gene pool can be caused by the culture environment (Hard et al. 2000) and contribute to reduced fitness of cultured fish naturally reproducing (Berijikian et al. 1997). Irrespective of the underlying causes, body size affects a female's ability to compete in the wild for nest sites and construct and guard redds (Schroder 1982; van den Berghe and Gross 1984; van den Berghe and Gross 1989; Foote 1990), influences redd vulnerability to scour during flood events (van den Berghe and Gross 1989; Steen and Quinn 1999) and directly influences fecundity (Fleming and Gross 1990; Beacham and Murray 1993; Chapter 2 of this report). Body size can also influence spawning distribution by affecting the ability of fish to colonize more distant or higher elevation spawning areas (Beacham and Murray 1993; Kinnison et al. 2001) and larger portions of river systems (Rogers 1987; Blair et al. 1993; Hendry and Quinn 1997). Lower mean body weight reduces the average carcass biomass returning to the natal basin, reducing exogenous nutrients utilized by rearing juveniles (Bilby et al. 1996). Changes in demographic/life history traits, such as a reduction in age classes or sex ratio, also have direct impacts reducing a population's phenotypic variation, total annual egg production and effective size (Nunney 1991). In addition, significant changes in spawn timing can shift fry emergence timing outside the locally adapted temporal window resulting in reduced fry survival (Brannon 1987; Beacham and Murray 1993; Quinn et al. 1995; Hendry et al. 1998; Smoker et al. 1998; Beer and Anderson 2001; Quinn et al. 2002).

Hatchery origin Pacific salmon have been shown to exhibit lower reproductive success than wild fish in some studies (Resenbichler and McIntyre 1977; Chilcote et al. 1986; Leider et al. 1990; Fleming and Gross 1992, 1993). Documenting changes in traits related to productivity and fitness, whether genetically or environmentally driven, contributes to our understanding of the immediate impacts of supplementation. In
addition, tracking trends in these traits over time is an important aspect of the YKFP's domestication selection monitoring effort (Busack et al. 2002) designed to determine whether there is a significant genetic component in observed trait changes.

We begin this report by describing three sets of biological data collected from hatchery and wild origin spring chinook returning to the upper Yakima River and describe changes in length and body weight due to secondary sexual development between passage at RAMF and spawning, analyze sexing accuracy at RAMF and compare estimated sex ratios. Next, we compare hatchery and wild origin fish returning in 2001 over the following traits: age composition, size-at-age, passage timing at RAMF, and spawning timing as represented by the temporal distributions in carcass recoveries. We also make comparisons of age composition, size-at-age, and spawning timing between upper Yakima River, Naches, and American River wild spring chinook populations. Finally, we examined bias in carcass recovery samples. In the second chapter of this report we compare the reproductive traits, gametes, and progeny produced from hatchery and wild origin upper Yakima River spring chinook returning in 2001. A future report will compare wild origin samples representing the historical baseline years 1997-2000 to hatchery and wild origin samples from supplemented return years.

## Methods and Materials

## Study Populations

Three genetically distinct wild spring chinook substocks have been identified in the Yakima River basin (Busack and Marshall 1991): the upper Yakima River, Naches system (including the Naches River, Little Naches River, Rattlesnake River and Bumping River) and the American River (Fig. 1). These three populations have also been shown to differ significantly in life history and demographic traits (Major and Mighell 1969; Fast et al. 1991; Knudsen 1991). The following analyses focus primarily on the upper Yakima River population spawning upstream of RAMF, the population targeted for supplementation under the YKFP (Busack et al. 1997), but also includes comparisons between the upper Yakima population and the American and Naches populations. The age- 4 component of the Naches population will serve in the future as a wild control population for the YKFP domestication monitoring program (Busack et al. 2002).

## Data Sets

There are three distinct sets of biological data collected from spring chinook returning to the upper Yakima River above RAMF. The first set represents hatchery origin fish sampled as they pass upstream through RAMF. After sampling, these fish are immediately released back into the river to complete their spawning migration. The second set represents both hatchery and wild origin fish collected at RAMF for use at the Cle Elum Supplementation Research Facility (CESRF) as either broodstock or experimental subjects in reproductive success studies. These fish are referred to collectively as the CESRF sample and are initially sampled at RAMF and then held to
maturity at CESRF where they were again sampled at spawning. The third dataset represents in-river carcass recoveries of hatchery and wild origin fish collected on the spawning grounds over the course of the spawning season. All data representing the American River and Naches populations come from spawning ground carcass recoveries.


Figure 1. Yakima River basin showing the upper Yakima River, Roza Adult Monitoring Facility (RAMF), the Cle Elum Supplementation Research Facility (CESRF), Naches system and American River.

## Hatchery Fish at RAMF

The largest and most comprehensive sampling of upper Yakima hatchery spring chinook occurs at RAMF as fish move upstream between April and September (Sampson and Fast 2001). Data from these hatchery origin fish are used to represent the population's age composition, size-at-age, and run timing at RAMF. Hatchery origin fish are identified as they pass through RAMF either by the presence of a Coded-Wire Tag (CWT), which activates a sensor tripping a hydraulic gate, or by the visual identification of the missing clipped adipose fin. Prior to release in the spring of 1999, all broodyear 1997 hatchery fish were adipose fin clipped and tagged with at least one CWT. During 2001, $98 \%$ of the hatchery origin fish passing RAMF (7,009 of 7,170 total hatchery fish age-3 or older) were sampled for length (Fork Length [FL], Post-Orbital-to-Hypural Plate [POHP], and mid-eye-to-hypural plate), body weight, and mark type. Passage date was
recorded and each fish classified to sex by visual inspection. After being sampled, 98\% of hatchery fish were released back into the river to spawn naturally. Those not released ( $\mathrm{n}=123$ ) were transported to CESRF for use in reproductive success studies comparing hatchery and wild origin fish (see Chapter 2 of this report; Schroder et al. 2002). In addition, 747 age- 2 hatchery origin precocial males were length and weight sampled at RAMF, but are not included in these analyses.

## Hatchery and Wild Origin Fish Held at CESRF

From 1997 to 2001, only wild origin fish were collected at RAMF for broodstock. Data collected from these wild origin fish are used to represent the wild population's age composition, size-at-age, sex ratio, run timing at RAMF and spawn timing. The estimated total number of broodstock needed for full production is based on the mean historical sex ratio, age composition, age-specific fecundity, adult pre-spawning mortality, BKD infection rates, and in-culture egg-to-smolt survival. No attempt is made at RAMF to select broodstock based on sex. A fixed proportion of the total number of broodstock is collected each week over the entire run based on weekly mean historical passage proportions at RAMF with the first week beginning on the day the first fish passes RAMF (B. Bosch, YN, pers. comm.). For example, if historically $6 \%$ of the run passes RAMF during the third week, then $6 \%$ of the broodstock are collected during the third week after the first fish passes RAMF. Using this methodology, broodstock take is a fixed number spread out over the entire run weighted by historical passage timing. This ensures that significant over- and under-collecting of broodstock does not occur, as can happen when the broodstock collection goal is a fixed percentage of the predicted run and actual run size significantly deviates from the prediction. Weekly broodstock collections in 2001 were evenly spread out over 3-4 days within each week when 10 or more fish were collected in a week. When less than 10 fish were scheduled for collection in a week, they were taken over 1-4 days. As long as the returning population does not deviate significantly from historical run timing trends, broodstock will be proportionately collected over the entire run. The broodstock collection profile for 2001 and mean historical passage profile are shown in Figure 2.

Collection of wild origin jacks for broodstock is handled differently than adults. The proportion of jacks collected is based on the historical geometric mean proportion of jacks returning within a cohort or brood (approximately 6\% of a total cohort from 19832000). This is done to minimize the interannual variation in jack proportions within a return year due to either very strong or very weak cohorts. For example, in 1999 because of the very strong 1996 cohort and relatively weak 1994 and 1995 cohorts, jacks made up $48 \%$ of the total returning wild population and under a representative sampling design should have made up $48 \%$ of the broodstock collected. However, under the current YKFP broodstock collection protocols, jacks would represent only $6 \%$, rather than $48 \%$, of the total broodstock collected in a return year like 1999. Since there are significant differences in size between age-3 and age-4 fish, it is possible to identify jacks at RAMF based on length (see Size-at-age results below). The estimated proportion of wild jacks returning is based on length criteria visually estimated as fish pass through RAMF. There is some unknown amount of error involved in this methodology due to the short


Figure 2. Weekly broodstock sampling in 2001. The "Predicted Run" values (■) are the predicted weekly proportion of the run passing RAMF based on mean historical run size over time. The "Actual Run" values ( $\uparrow$ ) are weekly proportions of fish passing RAMF in 2001. The "Proportion BS Taken" values ( $\triangle$ ) are the proportion of the total broodstock collected during that week in 2001.
time fish can be observed as they pass down an inclined chute and the fact that there is some small overlap in the length distributions of age-3 and -4 fish. Additional jacks beyond those needed for broodstock were collected in 2001 for use in reproductive success studies.

Biological sampling of the wild origin CESRF sample at RAMF included length (FL and POHP), body weight, scale samples, passage date and a provisional visual sex classification. In addition, all fish transported to CESRF (both hatchery and wild) are tagged intramuscularly in the pelvic girdle with an 18 mm Passive Integrated Transponder (PIT) tag so that their history from the time of capture through spawning can be tracked (Johnston and McCutcheon in prep.). At spawning, length (FL and POHP), body weight, and sex of each fish are again recorded. This data, along with gametic traits collected at spawning, can then be linked back to that fish's biological data collected at RAMF.

Artificial spawning at CESRF occurs over a five-week period from early September through early October. The additional wild and hatchery origin fish collected at RAMF for use in reproductive success studies are sampled at the same time and in the same manner as broodstock and are included in the age composition, sex ratio and size-at-age analyses below, as appropriate. In 2001, there were 596 wild origin fish collected for broodstock and reproductive success studies and 123 hatchery origin fish.

Hatchery and Wild In-river Carcass Recoveries
The third dataset is made up of hatchery and wild origin carcasses recovered by YN personnel during the course of weekly spawning ground surveys made throughout the Yakima River basin between July and October (Sampson and Fast 2001). Sex, origin
(hatchery/wild based on the presence of marks), recovery date and stream reach are recorded for each carcass. Length (FL and POHP) and age (scale samples) are collected on a subsample of carcasses. In 2001, carcasses were recovered in the American River between July 17 and September 5, in the Naches system between August 10 and September 21, and between September 11 and October 12 in the upper Yakima River.

## Traits

Sex Ratio

Estimates of Female:Male (F:M) ratios were calculated for fish collected at RAMF (excluding jacks) and held at the CESRF facility and for in-river carcass recoveries (wild origin $n=160$; hatchery origin $n=145$ ). The sex of these fish could be identified unambiguously by post mortem inspection of the body cavity and residual gametes. In addition, the accuracy of RAMF visual sex classifications of live fish made 1-5 months prior to spawning were determined by comparing them to the CESRF post mortem sexing of the same fish identified by the PIT tag codes.

Age Composition
Ageable scale samples were collected from 37.0\% (2,861 fish) of the returns age3 or greater passing RAMF in 2001. This represented approximately every third fish plus all fish selected for broodstock or reproductive ecology studies. Scales were placed on gummed cards and labeled so that the PIT tag number and other biological data collected could be linked to the fish's age. Ages are designated as the number of years from the year of conception (broodyear) to return year. Thus, a fish produced by parents spawning in the fall of 1997 and returning in 2001 is designated an age- 4 fish. Under this convention, precocial males (nonanadromous males maturing in the first [wild only] or second [wild and hatchery] year) are designated age-1 and age-2, respectively. Age composition of the wild adult ( $\geq$ age-4) population was estimated from fish held at CESRF ( $\mathrm{n}=563$ of which 534 had ageable, non-regenerated scales) and the wild age-3 jack proportion was estimated based on visual estimations of length as fish passed RAMF. Acetate impressions were made from the scale cards and ages determined by examining the impressions using a microfiche reader. Two scale analysts: T. Swan, YN, and J. Sneva, WDFW, independently aged all scales. In-river wild origin carcass samples, in which the sex of fish was confirmed by examining residual gonads, were compared to wild origin samples collected at RAMF to determine whether there was bias due to unequal carcass recovery rates of different age classes and sexes. In addition, age compositions of Naches system ( $\mathrm{n}=178$ ) and American River ( $\mathrm{n}=197$ ) populations were estimated from scale sampled carcass recoveries.

Sexual Dimorphism in Body Size and Development of Secondary Sex Characteristics
Sexual dimorphism in body size is common in Pacific salmon (e.g. Quinn and Foote 1994; Knapp and Vrendenburg 1996; Knudsen et al. in prep.) and can be an
indicator of the intensity of sexual selection, particularly in males (Fleming and Gross 1994). However, it can also be strongly affected by selection from size and sex selective fisheries (Beaty 1996; Knudsen et al. in prep.). We examined the 2001 CESRF data set (sex confirmed by post mortem inspection) and compared length (POHP) and body weight differences between upper Yakima River age-4 wild adults due to Sex (Male vs. Female) effects using a 1 -way ANOVA. The age- 5 wild CESRF data were tested using the same 1-way ANOVA. We analyzed the in-river carcass dataset using a 2 -way ANOVA testing for Sex (Male vs. Female), Origin (Hatchery vs. Wild), and Interaction effects. If no significant Sex or Interaction effects were found in these analyses, then we pooled body size data across sexes. This allowed use of the larger RAMF dataset in subsequent analyses comparing hatchery and wild origin size-at-age. We also analyzed age- 4 and -5 carcass recoveries from Naches system ( $\mathrm{n}=177$ ) and American River ( $\mathrm{n}=197$ ) wild populations for sexual dimorphism. In these analyses, we used a 2 -way ANOVA to estimate Age (4 vs. 5), Sex (Male vs. Female) and Interaction effects.

After entering the Columbia River spring chinook stop feeding and must rely on endogenous energy stores to sustain themselves. This, along with development of secondary sexual traits and gametes, can cause morphological changes in fish over time. We estimated how much upper Yakima River spring chinook body size changes between the time they pass RAMF and spawn by comparing the length and body weight of fish sampled first as they entered RAMF and then again, 1 to 5 months later, when spawned at CESRF using a paired-sample $t$-test.

## Size-at-Age

The size-at-age of hatchery and wild origin fish were compared within two datasets: the RAMF and in-river carcass samples, using ANOVA. In addition, a linear discriminant function analysis was performed on age 3-, 4- and 5-year old wild origin fish, using log transformed POHP length and body weight collected at RAMF as classification variables. Classification accuracy was estimated using a jackknife classification procedure (Efron 1982).

Length distributions of American, Naches and upper Yakima wild population carcass recoveries were compared. Differences in age- 4 and -5 's and betweenpopulation differences were examined.
Run/Spawn Timing
We examined the linear relationship between the date wild origin fish were collected at RAMF and the date they were subsequently spawned at CESRF by regressing passage date at RAMF against spawning date at CESRF in 2001. The RAMF cumulative passage timing distributions of hatchery and wild origin fish were compared using a KolmogorovSmirnoff goodness-of-fit test (K-S test; Zar 1984). Within hatchery fish, passage timing of the OCT and SNT treatment groups at RAMF were also compared with a K-S test. We estimated median and mean initial redd count dates and carcass recovery dates for hatchery and wild origin fish and compared their cumulative distributions using a K-S
test. Median and mean initial redd count dates and carcass recovery dates for American and Naches populations were also estimated.

## Carcass Recovery Bias

Since the RAMF samples are collected from throughout the run and in proportion to how the run has historically progressed (Fig. 2), they should be representative of hatchery and wild origin fish naturally spawning above RAMF. If naturally spawning fish of different ages, sizes and sex are equally likely to be recovered as carcasses, then the proportion of fish in each sex/age class observed at RAMF should be equal to the proportions observed in the carcass recovery sample. However, Peterson (1954), Clutter and Whitesel (1956), Ward (1959), Eames and Hino (1981), Boechler and Jacobs (1984), Knudsen (1992) and Zhou (2002) found that carcass recovery rates between ages and sexes can differ, leading to bias in age composition and sex ratios estimates. In general, each of these studies found that larger fish are recovered at higher rates than smaller fish, and females are recovered at higher rates than males. We estimated whether bias occurred in 2001 upper Yakima spring chinook carcass recoveries by comparing size-atage, age composition and the F:M ratio of fish passed upstream at RAMF to estimates generated from in-river carcasses recoveries made 1 to 5 months later.

## Results

## Sex Ratio

## Accuracy of Visual Sexing at RAMF

Table 1 shows the overall classification accuracy of visually sexing fish at RAMF in 2001. Females are more accurately identified ( $96 \%$ correctly identified) than males ( $66 \%$ correctly identified). This creates bias in sex ratios by over estimating the

Table 1. Classification matrix showing the accuracy of sex identifications at RAMF in 2001 based on visual classification of fish. Each cell shows the number of fish of known sex ("Correct sex" determined from carcasses) that were classified as male or female (Classification result). The percentage of fish classified into each category is in parentheses. Overall Mean Accuracy is the mean of the Percentage Correctly Classified as male and female.

| Correct sex | Classification result |  | Percentage correctly <br> classified |
| :---: | :---: | :---: | :---: |
|  | Male | Female |  |
| Male | $101(65.6 \%)$ | $53(34.4 \%)$ | $96.1 \%$ |
| Female | $10(3.9 \%)$ | $248(96.1 \%)$ | $80.9 \%$ |

proportion of females. Results from classifications made in 1997 to 2000 were similar; the major difference being male classification accuracy was $5-10 \%$ higher in the earlier years. The CESRF (1.6) and visually based (2.9) F:M estimates were significantly
different ( $X^{2}=20.35$ with Yates correction; $\mathrm{df}=1 ; \mathrm{p}<0.001$ ). Inaccurate sexing also creates problems when analyzing traits in which sex can be an important covariate, such as age composition, size by sex, and potential egg deposition. Potential egg deposition based on the F:M ratio of 2.9 from visual sexing would be $21 \%$ higher than an estimate based on a F:M ratio of 1.6 from the CESRF broodstock sample. However, for traits that are independent of sex it can be advantageous to increase sample size by using the much larger, more comprehensive RAMF dataset (over an order of magnitude larger than the CESRF hatchery origin dataset) and pooling over sexes.

CESRF and Spawning Ground Sex Ratios
The F:M ratios of wild (1.6) and hatchery (2.0) origin fish in the CESRF sample (hatchery $\mathrm{n}=123$; wild $\mathrm{n}=583$ ) were not significantly different ( $X^{2}=0.915$ with Yates correction; $\mathrm{df}=1 ; \mathrm{p}=0.339$ ). Percentages by sex are given in Table 2. In the spawning ground sample, the F:M ratios of wild and hatchery origin fish were 3.3 and 3.2, respectively, and were not significantly different ( $X^{2}<0.001$ with Yates correction; $\mathrm{df}=1$; $\mathrm{p}=1.00$ ). Between population spawning ground samples had significantly different $\mathrm{F}: \mathrm{M}$ ratios: American (1.16), Naches (2.02) and upper Yakima (hatchery and wild combined 3.20) ( $X^{2}=27.54 ; \mathrm{df}=2 ; \mathrm{p}<0.001$ ), with the upper Yakima having 2.7 times more female carcasses recovered per male than in the American River.

Table 2. Sex ratios estimated for upper Yakima River hatchery and wild populations in 2001 from carcass recoveries and from fish collected at RAMF and held at CESRF. Sex was identified by post mortem inspections.

| Carcass recoveries |  | Male $-\mathrm{n}(\%)$ | Female $-\mathrm{n} \mathrm{( } \mathrm{\%)}$ |
| ---: | ---: | :---: | :---: |
|  | Hatchery | $38(23.8)$ | $122(76.2)$ |
|  | Wild | $34(23.4)$ | $111(76.6)$ |
| RAMF Samples | Hatchery | $41(33.3)$ | $82(66.7)$ |
|  | Wild | $224(38.4)$ | $359(61.6)$ |

## Age Composition

## Upper Yakima River Wild Origin

All sampled wild and hatchery origin spring chinook returning in 2001 were aged as yearling outmigrants (stream type) having spent one full year in freshwater prior to outmigrating as smolts. Age composition of adult (age-4 and older) wild origin fish was estimated from fish selected at RAMF and held until mature at CESRF ( $\mathrm{n}=565$ ). This includes fish selected for broodstock and reproductive ecology studies as well as onstation mortalities. These fish were sexed post mortem and parsed into age classes by sex (Table 3). As in previous years, the overwhelming majority of wild origin fish (85\%) returned as 4 -year olds with $9 \%$ returning at age- 5 . Jacks made up the remaining $6 \%$ of the wild population based on visual length classifications made at RAMF. Wild origin spawning ground carcass recoveries ( $\mathrm{n}=160$ aged fish) were also used to generate age composition estimates by sex (Table 4). Age-3 fish made up only $0.6 \%$, age- 4 's $86.3 \%$
and age-5's $13.1 \%$ of the total sample. Thirty-two wild jacks were held at CESRF and all were determined to be males.

Table 3. Age composition of 2001 upper Yakima River wild and hatchery origin spring chinook based on scale samples collected at RAMF.

|  | Sex | Age | N | Percent by sex ${ }^{\text {d }}$ | Overall percent |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Upper <br> Yakima Wild | Males | 3 | N/ $\mathrm{A}^{\text {a }}$ | 16.2 | 6.3 |
|  |  | 4 | 169 | 73.8 | 28.7 |
|  |  | 5 | 23 | 10.0 | 3.9 |
|  | Females | 3 | 0 | 0.0 | 0.0 |
|  |  | 4 | 330 | 91.9 | 56.1 |
|  |  | 5 | 29 | 8.1 | 4.9 |
| Upper Yakima Hatchery | Males | 3 | 305 | 31.5 | 13.3 |
|  |  | 4 | 663 | 68.5 | 28.9 |
|  |  | 5 | $0^{\text {b }}$ | 0.0 | 0.0 |
|  | Females | 3 | $0^{\text {c }}$ | 0.0 | 0.0 |
|  |  | 4 | 1,325 | 100.0 | 57.8 |
|  |  | 5 | $0^{\text {b }}$ | 0.0 | 0.0 |

${ }^{\text {a }}$ Wild jack proportion based on visual estimates of length as fish pass RAMF (336 estimated jacks of 5,346 total wild fish) and assumes all jacks are male. Other wild age percentages are adjusted to include jacks.
${ }^{\mathrm{b}}$ There are no hatchery 5-year olds returning until 2002.
c All hatchery jacks were assumed to be male. There is a small, likely $\ll 1 \%$, proportion of females that return as age- 3 's (see Table x below), but we do not have an accurate estimate since these fish were not sexed.
${ }^{\mathrm{d}}$ The sex of individual wild fish was confirmed post mortem. The proportion of female and male hatchery origin fish is based on a subsample of fish taken to CESRF and held to maturity.

Linear discriminant function analysis was used to estimate the accuracy with which wild origin fish of known age (based on scales) can be classified. Log transformed POHP length and body weight of fish collected at RAMF were used to classify fish into $3-$, 4 - and 5 -year-old age classes. Males and females were pooled within age classes (see Sexual Dimorphism section below). Jackknifed classification accuracies were 100, 89, and $94 \%$ for 3-, 4- and 5-year olds, respectively (Table 5; Fig. 3), with the majority of errors occurring between age- 4 and -5 classes. Variation in body size, particularly body weight, increased with age causing heteroscedasticity. Linear discriminant function analysis assumes between-group variance-covariance matrices are equal and the $\log$ transformations were used to correct for unequal between-group variances.

## Upper Yakima River Hatchery Origin

Since 2001 was the first year of adult (age-4) hatchery returns from the initial 1999 smolt releases, hatchery age composition in 2001 was limited to fish age- 4 and younger. Age composition of hatchery origin fish was estimated from the 2,293 scale

Table 4. Age composition of 2001 American River and Naches wild and upper Yakima River hatchery and wild origin spring chinook based on scale samples collected from inriver carcass recoveries.

| Population | Sex | Age | N | Percent by sex | Overall percent |
| :---: | :---: | :---: | :---: | :---: | :---: |
| American River | Male | 3 | 0 | 0.0 | 0.0 |
|  |  | 4 | 79 | 85.9 | 39.9 |
|  |  | 5 | 13 | 14.1 | 6.6 |
|  | Female | 3 | 0 | 0.0 | 0.0 |
|  |  | 4 | 86 | 81.1 | 43.4 |
|  |  | 5 | 20 | 18.9 | 10.1 |
| Naches system | Male | 3 | 1 | 1.7 | 0.6 |
|  |  | 4 | 50 | 84.7 | 28.1 |
|  |  | 5 | 8 | 13.6 | 4.5 |
|  | Female | 3 | 0 | 0.0 | 0.0 |
|  |  | 4 | 80 | 67.2 | 44.9 |
|  |  | 5 | 39 | 32.8 | 21.9 |
| Upper <br> Yakima Wild | Male | 3 | 1 | 2.6 | 0.6 |
|  |  | 4 | 34 | 89.5 | 21.3 |
|  |  | 5 | 3 | 7.9 | 1.9 |
|  | Female | 3 | 0 | 0.0 | 0.0 |
|  |  | 4 | 104 | 85.2 | 65.0 |
|  |  | 5 | 18 | 14.8 | 11.3 |
| Upper Yakima Hatchery | Male | 3 | 8 | 23.5 | 5.5 |
|  |  | 4 | 26 | 76.5 | 17.9 |
|  |  | 5 | $0^{\text {a }}$ | 0.0 | 0.0 |
|  | Female | 3 | 1 | 0.9 | 0.7 |
|  |  | 4 | 110 | 99.1 | 75.9 |
|  |  | 5 | $0^{\text {a }}$ | 0.0 | 0.0 |

${ }^{\text {a }}$ There are no hatchery 5 -year olds returning until 2002.

Table 5. Jackknifed classification matrix from a linear discriminant function analysis estimating the ageing accuracy of wild origin spring chinook based on RAMF log transformed POHP length and body weight in 2001. "Age classification" cells show the number of fish of known age that were classified as age- $3,-4$ or -5 . The percentage classified into each category is in parentheses. Overall mean accuracy is the mean of the "Percent correctly classified" values.

| Known age | Age classification - N (\%) |  |  | Percent correctly <br> classified |
| :---: | ---: | ---: | ---: | :---: |
|  | Age 3 | Age 4 | Age 5 |  |
| Age 3 | $32(100.0 \%)$ | $0(0.0 \%)$ | $0(0.0 \%)$ | $100.0 \%$ |
| Age 4 | $7(1.5 \%)$ | $427(89.3 \%)$ | $44(9.2 \%)$ | $89.3 \%$ |
| Age 5 | $0(0.0 \%)$ | $3(5.8 \%)$ | $46(94.2 \%)$ | $94.2 \%$ |
| Overall mean accuracy |  |  |  | $94.5 \%$ |

samples collected at RAMF. The estimated proportion of hatchery origin jacks was $13 \%$ based on scales samples and $14 \%$ based on visual length classifications at RAMF and age-4's made up the remaining $86-87 \%$ of the returns (Table 3). A total of 118 age- 4 adult hatchery fish were held at CESRF and the proportion of each sex in this sample was used to partition the RAMF age-4 class by sex (Table 3). Only 6 hatchery jacks were collected and held at CESRF and all were identified as male. Of nine hatchery origin jack carcasses recovered, one was identified as a female. A total of 716 age- 2 hatchery fish (based on body size and mark recoveries) were observed at RAMF as upstream migrants in the latter portion of the run.

Canonical Scores Plot


Figure 3. Canonical scores from a discriminant function analysis of age based on log transformed POHP length and body weight as classification variables.

American River and Naches

Based on scale sampled carcass recoveries, age composition from the American River was 83 and $17 \%$ age- 4 and -5 , respectively. Naches system fish were 1, 73 and $26 \%$ age-3, -4 and -5 , respectively (Table 4). Historically, American River fish return at older mean age than Naches fish (Major and Mighell 1969; Fast et al. 1991; Knudsen 1991), but in 2001 the populations' age compositions were essentially equal and only slightly older than the upper Yakima River carcass age composition. This was due to the extremely strong 1997 brood (age-4's in 2001) that returned at exceptionally high rates across all three Yakima basin spring chinook populations and over all Columbia River basin spring chinook populations in general. For this reason it is better to look at age composition within cohorts across years, rather than within a return year, when trying to characterize a population's age composition. Tracking a single cohort over time allows the effects of adjacent strong or weak cohorts to be removed. In future reports we will estimate age composition by cohort (broodyear).

Table 6. POHP length and body weight of upper Yakima wild origin spring chinook sampled at as they passed RAMF or at spawning at CESRF.

| Population | Sex | Age | POHP (cm) | Weight (kg) | N |
| :---: | :---: | :---: | :---: | :---: | :---: |
| RAMF | Male | 4 | 61.4 | 4.6 | 169 |
|  |  | 5 | 74.4 | 7.7 | 23 |
|  | Female | 4 | 61.4 | 4.6 | 330 |
|  |  | 5 | 70.7 | 6.9 | 29 |
| CESRF | Male | 4 | 59.4 | 3.8 | 119 |
|  |  | 5 | 74.1 | 7.1 | 14 |
|  | Female | 4 | 60.5 | 3.9 | 199 |
|  |  | 5 | 69.8 | 6.0 | 14 |

Table 7. Tukey test p-values from 1-way ANOVA's of POHP length and body weight measured at both RAMF and CESRF. Cell values indicate the probability pairs of samples are not significantly different. Bonferoni adjustments were made to the $\alpha$ levels to correct for multiple comparisons. Bolded values are greater than 0.05 (nonsignificant).

| Trait | Wild female 4 | Wild female 5 | Wild male 4 |
| ---: | :---: | :---: | :---: |
| POHP at RAMF |  |  |  |
| Wild female 5 | 0.000 |  | 0.000 |
| Wild male 4 | $\mathbf{0 . 8 6 2}$ | 0.000 | 0.010 |
| Wild male 5 | 0.000 |  |  |
| Body weight at RAMF |  |  | 0.000 |
| Wild female 5 | 0.000 | 0.000 |  |
| Wild male 4 | $\mathbf{0 . 8 2 8}$ | 0.006 |  |
| Wild male 5 | 0.000 |  | 0.000 |
| POHP at CESRF |  | 0.000 |  |
| Wild female 5 | 0.000 | 0.005 | 0.000 |
| Wild male 4 | 0.053 |  |  |
| Wild male 5 | 0.000 |  |  |
| Body weight at CESRF |  | 0.000 | 0.000 |
| Wild female 5 | 0.000 |  |  |
| Wild male 4 | $\mathbf{0 . 2 2 7}$ |  |  |
| Wild male 5 | 0.000 |  |  |

## Sexual Dimorphism and Development of Secondary Sex Characteristics

Sexual Dimorphism
We began by using a 2-way ANOVA to analyze POHP length and Body Weight of wild origin age-4 and -5 fish. Sex, Age and Interaction effects were estimated for two datasets separately: RAMF and CESRF. Datasets were analyzed separately because the time between sampling may have been long enough for secondary sexual characteristics to develop (Table 6). However, in every 2-way ANOVA there was a significant

Sex*Age interaction term ( $\mathrm{p}<0.01$ ), because the difference in size between sexes was negligible in age-4's and relatively large in age-5's. We reanalyzed the data using a 1way ANOVA and Tukey multiple comparisons test to determine which sex/age groups were significant different treating each of the four age/sex classes as a separate group. In the Tukey tests there were no significant differences detected in POHP length ( $\mathrm{p}=0.86$ ) or body weight ( $\mathrm{p}=0.83$ ) of wild age- 4 males and females measured at RAMF (Table 7). Comparisons of body size at CESRF also showed no significant difference in body weight ( $\mathrm{p}=0.23$ ), but there was a significant difference in POHP ( $\mathrm{p}=0.05$ ); age- 4 females were 1 cm larger than males. In contrast to age-4's, age- 5 wild origin males and females were significantly different from each other in every comparison of POHP and body weight at both RAMF and CESRF ( $\mathrm{p}<0.01$; Table 7).

Table 8. Size-at-age of American, Naches and upper Yakima wild and hatchery origin spring chinook based on scale sampled carcass recoveries.

| Population | Sex | Age | POHP (cm) | sd | N |
| :---: | :---: | :---: | :---: | :---: | :---: |
| American River | Male | 3 |  |  | 0 |
|  |  | 4 | 67.6 | 6.0 | 78 |
|  |  | 5 | 83.3 | 5.5 | 13 |
|  | Female | 3 |  |  | 0 |
|  |  | 4 | 64.3 | 4.4 | 86 |
|  |  | 5 | 76.4 | 4.3 | 20 |
| Naches system | Male | 3 | 45.0 | 0 | 1 |
|  |  | 4 | 63.3 | 6.6 | 50 |
|  |  | 5 | 71.9 | 4.6 | 8 |
|  | Female | 3 |  |  | 0 |
|  |  | 4 | 63.3 | 5.8 | 80 |
|  |  | 5 | 74.2 | 2.9 | 39 |
| Upper Yakima wild | Male | 3 | 43.0 | 0 | 1 |
|  |  | 4 | 61.0 | 4.4 | 34 |
|  |  | 5 | 73.8 | 4.8 | 3 |
|  | Female | 3 |  |  | 0 |
|  |  | 4 | 60.8 | 3.9 | 104 |
|  |  | 5 | 70.2 | 3.0 | 18 |
| Upper Yakima Hatchery | Male | 3 | 40.5 | 4.7 | 8 |
|  |  | 4 | 59.1 | 4.2 | 26 |
|  |  | 5 |  |  | 0 |
|  | Female | 3 | 41.0 | 0 | 1 |
|  |  | 4 | 59.0 | 3.8 | 110 |
|  |  | 5 |  |  | 0 |

The POHP distributions of hatchery and wild age- 4 carcass recoveries (Table 8) were also examined for sexual dimorphism using a 2-way ANOVA (Sex, Origin [H/W], and Interaction effects). No significant Sex ( $\mathrm{p}=0.74$ ) or Sex*Origin interaction effects ( $\mathrm{p}=0.90$ ) were found in the upper Yakima age-4 carcass recovery data (Table 9). There were however, significant Origin effects ( $\mathrm{p}<0.01$ ) with both sexes of wild fish 2 cm larger than hatchery origin fish. In subsequent analyses of upper Yakima age-4 size-at-age below, we pooled over sexes.

Table 9. Two-way ANOVA results for POHP length estimating 2001 Sex (male and female) and Origin (upper Yakima wild and hatchery) effects from age-4 carcass recoveries.

| Source | Sums-of-Squares | df | Mean-Square | F-ratio | P |
| :--- | :---: | ---: | :---: | :---: | :---: |
| SEX | 1.7 | 1 | 1.7 | 0.1 | 0.744 |
| ORIGIN | 153.8 | 1 | 153.8 | 9.9 | 0.002 |
| SEX*ORIGIN | 0.3 | 1 | 0.3 | $<0.1$ | 0.898 |
| Error | 4199.4 | 270 | 15.6 |  |  |

Mean POHP length for Naches and American River populations are given in Table 8. We used a 2-way ANOVA (Sex, Age, and Interaction effects) to test for Sex effects (Table 10). There was no significant Sex or Age*Sex interaction effect in the upper Yakima ( $\mathrm{p} \geq 0.133$ ) and Naches ( $\mathrm{p} \geq 0.316$ ) POHP length carcass recovery datasets. In contrast, the American River population exhibited significant sexual dimorphism. Male POHP length was significantly greater ( $\mathrm{p}<0.001$ ) than female length in both age- 4 and -5 classes.

Table 10. Two-way ANOVA results comparing POHP length estimating Age (4- and 5year old), Sex (male and female) and Interaction effects for 2001 American River, Naches, and upper Yakima wild origin carcass recoveries.

| Population | Source | Sums-of- <br> Squares | df | Mean-Square | F-ratio | P |
| :---: | :--- | ---: | ---: | ---: | ---: | ---: |
| Upper <br> Yakima <br> Wild | AGE | 1158.3 | 1 | 1158.3 | 74.2 | $<0.001$ |
|  | SEX | 35.6 | 1 | 35.6 | 2.3 | 0.133 |
|  | AGE*SEX | 26.6 | 1 | 26.6 | 1.7 | 0.194 |
|  | Error | 2419.5 | 155 | 26.4 |  |  |
|  | AGE | 2061.8 | 1 | 2061.8 | 68.0 | $<0.001$ |
|  | SEX | 30.7 | 1 | 30.7 | 1.0 | 0.316 |
|  | AGE*SEX | 30.0 | 1 | 30.0 | 1.0 | 0.322 |
|  | American |  |  |  |  |  |
| River | Error | 5246.4 | 173 | 30.3 |  |  |
|  | AGEX | 5079.7 | 1 | 5079.7 | 192.4 | $<0.001$ |
|  | AGE*SEX | 692.0 | 1 | 692.0 | 26.2 | $<0.001$ |
|  | Error | 5094.9 | 193 | 26.5 | 3.4 | 0.067 |

## Development of Secondary Sex Characteristics

The difference (RAMF-CESRF) between paired lengths and weights from the same fish sampled at RAMF and then at CESRF were analyzed to determine the changes in traits over time (Table 11). All traits showed significant change in paired samples ( $\mathrm{p}<0.05$ )

Table 11. Mean differences between paired samples of fork length (FL), postorbital hypural plate length (POHP) and body weight (BW) measured on the same fish at RAMF and then subsequently at CESRF. Differences were calculated as RAMF value minus CESRF value. Differences were compared to a null hypothesis of 0 difference (paired sample t-test; $\alpha=0.05$; 2-tailed).

| Origin | Age | Sex | N | FL (cm) | POHP (cm) | BW (kg) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Wild | 3 | Male | 16 | -1.28** | 0.23 | 0.37** |
|  |  | Female | 0 |  |  |  |
|  | 4 | Male | 116 | -3.18** | 1.22** | 0.75** |
|  |  | Female | 195 | -1.83** | 1.04** | 0.62** |
|  | 5 | Male | 14 | -4.48** | 1.15* | 0.82** |
|  |  | Female | 19 | $-2.97 * *$ | 0.93* | 0.91** |
| Hatchery | 3 | Male | 0 |  |  |  |
|  |  | Female | 0 |  |  |  |
|  | 4 | Male | 4 | -4.03** | 1.73* | 0.56** |
|  |  | Female | 33 | -1.88** | 0.97* | 0.54** |

** indicates $\mathrm{p}<0.01$.

* indicates $\mathrm{p}<0.05$
except male age- 3 POHP ( $\mathfrak{p}=0.76$ ). The only negative change was in FL, due primarily to the elongation of the kype, a secondary sexual characteristic. Male FL increased by $4.4 \%$ and female FL increased by $2.6 \%$ on average during the $1-5$ months fish were held at CESRF. Both POHP length and body weight decreased. Age-4 male and female weight decreased by $16.3 \%$ and $13.4 \%$, respectively, and POHP length decreased by $2.0 \%$ and $1.7 \%$, respectively. As spring chinook complete the final stages of the maturation process, they convert calcium stores, lipids and muscle tissue into gametes and secondary sexual characteristics (e.g. large canine teeth and toughened epidermis), while depleting fat and lipid stores to sustain themselves (Hendry et al. 2000). This would explain the observed reduction in body weight. However, a small portion of the decrease in body weight we observed was also due to the "bleeding" process that occurs at CESRF during artificial spawning. Just after fish are sacrificed, but prior to being weighed, their gill arches are severed and the fish are bled. We do not have an estimate of what proportion of total body weight blood loss represents at this time. Therefore, our weight loss estimates are over-estimated to some degree.


## Size-at-age

## Hatchery and Wild Origin Returns

For hatchery origin fish, there was a lack of agreement between body size estimated from different data sets. When the age- 4 hatchery origin CESRF sample (mean $\mathrm{POHP}=60.9 \mathrm{~cm} ; \mathrm{n}=117$ ) was compared to the age- 4 hatchery origin spawning ground (mean $\mathrm{POHP}=58.8 \mathrm{~cm} ; \mathrm{n}=127$ ) and RAMF (mean $\mathrm{POHP}=59.3 \mathrm{~cm} ; \mathrm{n}=2,342$ ) samples, the CESRF sample was significantly larger than the other two ( $\mathrm{p}<0.001$; Tukey multiple comparisons test). Because the RAMF sample is the most representative comprehensive sample, it was used to estimate POHP length and body weight of naturally spawning
hatchery origin fish. In contrast to hatchery fish, the size of wild origin age-4 fish was not statistically different across the datasets. The POHP length distribution of the wild CESRF sample (mean $=61.4 \mathrm{~cm} ; \mathrm{n}=482$ ) was not significantly different ( $\mathrm{p}=0.251$; 1-way ANOVA) than the spawning ground POHP distribution (mean $=60.7 \mathrm{~cm} ; \mathrm{n}=142$ ).

When the RAMF age-4 hatchery fish sample (mean $=59.3 \mathrm{~cm}$ ) was compared to wild origin fish (mean $=61.4 \mathrm{~cm}$ ), there was a significant difference of $2.1 \mathrm{~cm}(\mathrm{p}<0.01$; 1 -way ANOVA). There was also a significant ( $\mathrm{p}<0.01$ ) difference between age- 3 hatchery fish (mean $=40.0 \mathrm{~cm} ; \mathrm{n}=473$ ) and wild fish (mean $=42.9 \mathrm{~cm} ; \mathrm{n}=32$ ). The same trend was found for body weight, with both 3- and 4-year old hatchery origin fish weighing approximately 0.3 kg less than wild fish of the same age ( $\mathrm{p}<0.05$ ). Thus, based on the 2001 RAMF and carcass recovery samples, both naturally spawning age- 3 and age- 4 hatchery fish were shorter and lighter than wild fish. As would be expected, hatchery and wild 4 -year olds were significantly larger than 3-year olds ( $\mathrm{p}<0.01$ ).

OCT vs SNT
There was no significant Treatment effect (OCT vs. SNT) found for POHP length $(\mathrm{p}=0.85)$ or body weight ( $\mathrm{p}=0.46$ ) in either age-3 or age 4's (Tables 12 through 14). There was less than 0.1 cm and 0.1 kg difference between the two treatment/control groups in 2001.

| Table 12. Summary statistics for body weight (kg) and POHP length (cm) of OCT <br> and SNT age 3 and 4 returns in 2001. Standard deviations are given in parentheses. <br> Age Treatment |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: |
| 3 | Body weight (sd) | POHP length (sd) | N |  |
|  | OCT | $1.42(0.40)$ | $40.0(3.6)$ | 184 |
| 4 | SNT | $1.42(0.36)$ | $40.0(3.6)$ | 189 |
|  | OCT | $4.30(0.81)$ | $59.3(3.8)$ | 792 |

Table 13. Two-way ANOVA results comparing POHP length estimating
Treatment (OCT/SNT) and Age (3 and 4) effects from RAMF recoveries.

| Source | Sums-of-Squares | df | Mean-Square | F-ratio | P |
| :--- | :---: | ---: | :---: | :---: | :---: |
| TREATMENT | 0.5 | 1 | 0.5 | $<0.1$ | 0.853 |
| AGE | 110403.2 | 1 | 110403.2 | 7080.8 | 0.000 |
| TRT*AGE | 1.1 | 1 | 1.1 | 0.1 | 0.793 |
| Error | 27270.1 | 1749 | 15.6 |  |  |

Table 14. Two-way ANOVA results comparing body weight estimating Treatment (OCT/SNT) and Age (3 and 4) effects from RAMF recoveries.

| Source | Sums-of-Squares | df | Mean-Square | F-ratio | P |
| :--- | :---: | :---: | :---: | :---: | :---: |
| TREATMENT | 0.3 | 1 | 0.3 | 0.6 | 0.459 |
| AGE | 2482.0 | 1 | 2482.0 | 4268.1 | 0.000 |
| TRT*AGE | 0.3 | 1 | 0.3 | 0.5 | 0.473 |
| Error | 1017.1 | 1749 | 0.6 |  |  |

American River and Naches mean POHP lengths by sex and age are given in Figure 4. Age-4 fish from the American River (male $=67.6 \mathrm{~cm}$; female $=64.3 \mathrm{~cm}$ ) were significantly larger ( $\mathrm{p}<0.05$ ) than Naches fish (male $=63.3 \mathrm{~cm}$; female $=63.3 \mathrm{~cm}$ ). American River fish (male $=83.3 \mathrm{~cm}$; female $=76.4 \mathrm{~cm}$ ) were also significantly larger at age- 5 than Naches fish (male $=74.2 \mathrm{~cm}$; female $=75.5 \mathrm{~cm}$ ). In general, both of these populations were larger-at-age than upper Yakima hatchery and wild origin fish. The only exception in 2001 was age- 5 upper Yakima males, which were larger than Naches age- 5 males.


Figure 4. Mean POHP length of age-4 (squares) and age-5 (triangles) American River, Naches system, and Yakima River wild populations by male (solid lines) and female (broken lines) estimated from carcass recoveries in 2001.

## Run/Spawn Timing

RAMF Passage Timing
Mean and median passage timing of hatchery $(\mathrm{n}=7,139)$ and wild $(\mathrm{n}=595)$ fish (all fish greater than 35 cm fork length, whether aged or not) at RAMF differed by 2 and 4 days, respectively, with wild fish passing earlier than hatchery fish (Fig. 5). The two group's passage timing distributions were significantly different in a K-S test ( $\mathrm{p}<0.01$ ). When the analysis was restricted to just age-4's ( $n=2,342$ hatchery and $n=482$ wild fish), representing the bulk of the both runs, there was no significant difference in temporal distributions ( $\mathrm{K}-\mathrm{S}$ test; $\mathrm{p}=0.064$ ), mean passage dates differed by less than 1 day, and medians were equal.


Figure 5. Cumulative passage timing at RAMF for hatchery and wild origin spring chinook in 2001.

## OCT vs SNT

We compared OCT and SNT treatment groups' passage timing at RAMF for age-$2,-3$, and -4 fish using K-S tests and found no significant treatment effect on passage timing ( $\mathrm{p}>0.232$ ) within any age class. OCT and SNT medians and means within age classes differed by no more than 2 days.

Relationship of CESRF Spawning Date to RAMF Passage Timing
Wild origin fish ( $\mathrm{n}=423$ ) exhibited a weak positive linear relationship between passage at RAMF and date of spawning at CESRF in 2001 (Fig. 6; $\mathrm{p}=0.01$ ). However, the relationship explained only $1.4 \%$ of the total variation in spawn timing. Hatchery origin fish ( $\mathrm{n}=79$ ) showed no significant correlation between date spawned and passage date at RAMF ( $\mathrm{p}=0.42$ ).

## Upper Yakima Spawn Timing

Mean spawn timing of both upper Yakima River hatchery ( $\mathrm{n}=150$ ) and wild ( $\mathrm{n}=175$ ) fish, based on in-river carcass recoveries, was Sept. 26 in 2001 and there was no significant difference in the two group's temporal distribution of carcass recoveries (K-S test; $\mathrm{p}=0.49$ ). The mean date of initial redd observation counts was Sept. 25 for the upper Yakima ( $\mathrm{n}=3,239$ total redds of both hatchery and wild origin).


Figure 6. Relationship between passage date at Roza Adult Monitoring Facility (RAMF) and date fish were spawned (SD) at CESRF.

## American River and Naches Spawn Timing

Mean and median spawn timing based on carcass recoveries was August 19 and 21, respectively, for the American River $(\mathrm{n}=209)$ and September 14 and 17, respectively, for the Naches ( $\mathrm{n}=182$ ) population.

## Carcass Recovery Bias

## Relationship between RAMF and Carcass recoveries

The proportions of hatchery and wild origin jacks passed upstream at RAMF were 13.8 and $6.3 \%$, respectively, in 2001. If jacks are recovered at the same rate as larger, older fish, then one would expect to find that jacks represent the same proportion in both the RAMF and carcass recoveries. However, hatchery and wild origin jacks made up only 5.9 and $0.6 \%$ of the carcasses recovered; a significantly smaller proportion than would be expected (Hatchery $X^{2}=6.83$ with Yates correction, $\mathrm{p}<0.01$; Wild $X^{2}=8.03$ with Yates correction, $\mathrm{p}<0.01$ ). There was no significant difference in the proportion of wild origin age-4 fish in the CESRF broodstock (84.9\%) and the carcass recovery (85.5\%) samples. However, wild origin age- 5 fish represented $9.2 \%$ of the CESRF broodstock and $13.9 \%$ of the carcass recovery sample ( $X^{2}=2.95$ with Yates correction; $p=0.09$ ). This difference is significant at the $\alpha=0.10$ level. The overall trend is that carcass recoveries were biased due to the youngest and oldest age classes being under- and overrepresented, respectively.

The F:M ratio of wild and hatchery origin fish (including jacks) was 1.66 in the CESRF sample ( $\mathrm{n}=706$ ) and 3.24 in the carcass recover sample ( $\mathrm{n}=305$ ). Females represented a significantly larger proportion of the carcass sample ( $X^{2}=17.97$ with Yates correction; $\mathrm{p}<0.001$ ), indicating that female carcasses are recovered at higher rates than
male carcasses. Since age-4 F:M ratios were nearly equal between CESRF (3.30) and carcass (3.27) samples, the difference in overall $\mathrm{F}: \mathrm{M}$ ratio was due to the effects of age-3 and age- 5 fish. The F:M ratio of the age- 5 CESRF sample was 0.86 and for carcass recoveries 6.67 ; almost 8 times greater. Also, age- 3 males were significantly underrepresented in the carcasses recoveries, further skewing the carcass F :M ratio toward females. The overall affect is that females are recovered at higher rates than males and larger, older fish at higher rates than smaller, younger fish.

## Discussion

## Sex Ratio

There was no significant difference between hatchery and wild sex ratios in 2001 between fish sampled at CESRF or as in-river carcass recoveries. However, significant differences were found between the sex ratios of the American, Naches and upper Yakima populations. This may be because fewer males residualize as nonanadromous precocials in the American River population and to a lesser degree in the Naches. Since precocials are virtually never recovered during spawning ground carcass surveys, they "drop out" and their contribution to the cohort is missed and very difficult to quantify.

We know a significant proportion of upper Yakima hatchery and wild males exhibit precocialism based on sampling pre-release hatchery juveniles (D. Larsen, NMFS, pers. comm.), snorkel survey observations (B. Ben James, Cascade Aquatics, pers. comm.) and underwater video (authors observations), yet virtually none are recovered as carcasses. Because they drop out, precocials skew anadromous adult sex ratios toward females. The greater the proportion of males exhibiting a precocial life history strategy, the larger the anadromous female:male ratio. In the American River, there are selection pressures that favor larger, older males relative to the upper Yakima River. The natural and sexual selection pressures in the American River may also select against the precocial life history strategy, making it less successful than in the upper Yakima River. In addition, lower water temperatures in the American River result in slower initial juvenile growth, which may reduce the likelihood of precocialism being expressed. An adult sex ratio of 1:1, as in the American population, will result in average adult male competition being greater than in a population with a F:M ratio of 3, such as the upper Yakima River. This is because there are more anadromous males per female in the American River, increasing the likelihood of competition between males for this more relatively scarce resource. It is interesting that both upper Yakima hatchery and wild populations had comparable F : M ratios, perhaps indicating that the total proportion of wild and hatchery males "dropping out" due to precocialism is very similar.

One final note, when the $\mathrm{F}: \mathrm{M}$ ratio is low the mean number of fish per redd will be higher. This is simply because low $\mathrm{F}: \mathrm{M}$ ratios indicate there are more males per female and, since theoretically each female constructs a redd, there will be more total fish per redd. Thus, there is some biological reason that the American River has historically
had a higher mean number of fish per redd than the upper Yakima River population (B. Bosch, YN, pers. comm.).

## Age Composition

Due to the lack of age-5 hatchery returns in 2001, no statistical comparison between hatchery and wild upper Yakima populations was made. However, there was little difference in age compositions in 2001 with age- 4 fish dominating in both populations. Age compositions were not different within samples either collected at RAMF or collected as in-river carcasses. However, as with sex ratios above, the RAMF and in-river carcass samples were significantly different. Relative to the RAMF collection, carcass recoveries overestimated the proportion of older, larger fish. This result is also in agreement with the results of Peterson (1954), Clutter and Whitesel (1956), Ward (1959), Boechler and Jacobs (1987), Knudsen (1992) and Zhou (2002). Larger fish are larger targets and thus easier to visually locate and recover. In addition, larger fish tend to tangle and catch in woody debris more easily, are more difficult for flow to displace downstream (particularly during low flows), and harder for predators to drag up out of the streambed. Age composition of spawning grounds samples should not be assumed to represent the spawning population without verification.

## Sexual Dimorphism and Development of Secondary Sex Characteristics

Naches and upper Yakima River hatchery and wild origin age-4 fish generally displayed no significant sexual dimorphism in body size (POHP length or body weight). We pooled the two sexes in subsequent analyses of upper Yakima River size-at-age. Knudsen (1991) also found that mean POHP lengths of age-4 upper Yakima River male and female carcass recoveries were not significantly different in 1991 returns. This is in contrast to the 2001 American River age-4's that exhibited significant sexual dimorphism.

## Size-at-Age

Hatchery fish were approximately 2 cm smaller than wild fish returning in 2001. This was true for both age- 3 and age- 4 fish based on both carcass recoveries and sampling live fish at RAMF. The difference in POHP length between first generation hatchery and wild origin fish is similar to results reported in two other hatchery supplementation projects: Tucannon River spring chinook and Cedar River sockeye. Tucannon River hatchery origin returns were significantly smaller-at-age during the initial years of operation and had significantly lower fecundity (Bumgarner et al. 1994). After 14 years of introgression between naturally spawning hatchery and wild fish, hatchery and wild size-at-age no longer differs (Gallinat et al. 2001). However, reduced average fecundity is still a problem in Tucannon hatchery female returns due primarily to the significantly larger eggs they produce (Gallinat et al. 2001). This suggests there may be a strong hatchery environmental component to this trait difference, rather than a genetic basis. Fresh et al. (in prep.) found that first generation Cedar River hatchery sockeye returns were as much as 5 cm smaller in POHP length than wild conspecifics and
in the majority of their comparisons hatchery fish were significantly smaller than wild returns. In addition, Unwin and Glova (1997) found that New Zealand hatchery reared male chinook returned 0.6 cm smaller than wild males.

The development of differences in traits between hatchery and wild origin fish derived from the same native stock may have a significant genetic component due to unintentional directional selection (domestication) or relaxation of natural selection pressures in the hatchery (Hard 1995; Lynch and O’Hely 2001). They may also be caused by phenotypic plasticity due to environmental variation (Stearns 1989) or be a result of a complex combination of both environmental and genetic factors (Riddle 1986; Taylor 1991). In both the Tucannon and New Zealand studies cited above, hatchery fish were much larger at release relative to their wild counterparts and this hatchery environmental factor, not selection on a heritable trait, was the cause of the observed differences in size at return. However, Cedar River sockeye fry are released into the river as unfed fry at the same size and time as wild fry and in CESRF spring chinook smolts are only slightly larger than wild smolts migrating contemporaneously. Thus, size-atrelease is not likely to be a cause of phenotypic plasticity in adult size in these two cases.

One environmentally driven difference between wild and hatchery upper Yakima River spring chinook that could potentially contribute to size-at-age differences is differential timing of the wild and hatchery smolt outmigrations and entrance into the Columbia River estuary. Because the size difference was observed in both age- 3 and -4 fish, the causal mechanism(s) must have occurred prior to age- 3 fish returning or between release and the first 18 months of post-release ocean rearing. We know that peak CESRF hatchery smolt passage at Chandler smolt passage facility in the lower Yakima River occurs later than wild origin peak smolt passage (see Fig. 26 in Sampson and Fast 2000) and hatchery origin fish exhibited slower mean in-river travel times from Roza Dam to McNary Dam on the Columbia River in 1999 (D. Neeley, IntStats, pers. comm.). Because of this, CESRF hatchery smolts would have also entered the Columbia River estuary later on average than wild fish. There may be a critical temporal window when growth of smolts entering the Columbia River estuary is maximized due to high food availability and/or lower competition. If this window is early in the season, wild fish will hit it, while later arriving hatchery fish miss it. It could also be simply that smolts entering the estuary first have the longest exposure to the more productive estuarine rearing area and/or transition into the ocean earlier, resulting in a growth advantage that later arriving smolts are not able to overcome before reaching maturity. Thus, the observed difference in age- 3 and -4 return body size could be simply an artifact of hatchery release timing, smolt outmigration speed, and estuary entrance timing.

Because hatchery age- 3 and -4 fish were 0.3 kg lighter than wild fish, the 8,634 hatchery fish that returned in 2001 would have produced 2.59 mt ( 5,700 pounds) more biomass had they been the same size as wild fish. However, it should be noted that hatchery fish from the 1997 brood produced an extremely high 30.3 adult spawners per recruit, compared to 4.8 adult spawners per recruit for wild fish. Thus, total returning biomass of hatchery fish was much greater than wild fish even with their reduced body weight.

Age-4 American River and Naches populations were significantly larger in POHP length than either upper Yakima River hatchery or wild populations. This was also true of age-5 fish, except upper Yakima males that were larger than Naches males. The selection pressures experienced by the American and, to a lesser degree, the Naches populations have caused local adaptations resulting in significantly larger size-at-age and historically older mean age compared to the wild upper Yakima River population. Total migration distance traveled within the Yakima River basin by each population to their respective upper spawning areas is not greatly different: upper Yakima fish travel up to 327 river kilometers (rkm) to Easton, American River up to 279 rkm, and Naches up to 259 rkm . Elevation of the respective spawning grounds is another significant selection pressure, and combined with distance traveled, determines the gradient or slope the fish must negotiate. American River fish spawn at the highest elevation ( $1,037 \mathrm{~m}$ ), followed by the Naches ( 801 m ) and upper Yakima fish ( 553 m ). Thus, the American River population must negotiate a much steeper gradient than in the upper Yakima. There are significant trade offs made between energy budgeted toward migration and other "bins" such as gametes (total mass, egg number, egg size), body size, secondary sexual characteristics, competition and nest guarding (Kinnison et al 2001). American River fish, and to a lesser degree Naches fish, must budget a much greater proportion of their total energy budget into migration related bins because of their steeper migration route. Life history theory suggests that within each population the allocation between all bins should coevolve so that lifetime reproductive success will be maximized (Pianka 1976; Roff 1988). Larger size and greater muscle mass should increase the likelihood of successfully completing a steeper gradient migration and this selection pressure is one reason American River fish are significantly larger at age and older at return. The length, elevation and gradient of the Naches population's migration lies intermediate between the American and upper Yakima rivers, and the size-at-age and mean age at return of Naches fish also falls intermediate between these two populations.

## Run/Spawn Timing

In 2001 there were no significant differences between hatchery and wild fish in terms of either run timing or spawn timing as represented by passage date at RAMF, redd observations over time, or in-river carcass recoveries. There were however, large differences between upper Yakima River, American River, and Naches populations in temporal distribution of redds and carcass recoveries. This has been noted by other researchers, as well (Major and Meghell 1969; Fast et al. 1991). The American River was the earliest spawning group, followed by the Naches and finally the upper Yakima River. Fry emergence is often synchronized across populations so that it occurs during the optimum spring period maximizing survival (Brannon 1987). American and upper Yakima River fry emergence timing does appear to be synchronized (Fast et al. 1991). The populations with the coldest water temperatures spawn first so that the eggs' total temperature unit accumulations, which determine fry emergence timing, will be equivalent across populations at emergence. Thus, temporal differences in spawning are driven by water temperatures during egg incubation, which are coldest in highest elevation American River, followed by the Naches, and warmest in the lower elevation
upper Yakima. In addition, since upper Yakima River fish spawn over a month later on average than American River fish, they must have the energy reserves to maintain themselves over an additional month of holding when mean water temperatures are warm and daily metabolic costs are high. Because of this we hypothesize that upper Yakima fish should invest more into somatic growth that can be quickly and efficiently metabolized (i.e. visceral fat stores) and devote relatively less growth into muscle mass, which is less efficiently converted back to energy, than American River fish. This would also tend to produce larger fish at age in the American River population.

## Carcass Recovery Bias

We found that in-river carcass samples differed significantly from our expectations based on RAMF samples with females and larger fish being recovered at higher rates than males and smaller fish. This result is consistent with the findings of Peterson (1954), Clutter and Whitesel (1956), Ward (1959), Eames and Hino (1981), Boechler and Jacobs (1987), Knudsen (1992) and Zhou (2002). The difference in recovery rates may be due to behavioral differences between males and females, the visibility and "catchability" of larger vs. smaller targets, the ease with which smaller carcasses are removed by terrestrial predators, and also displaced downstream by flow. The magnitude of the bias is likely affected strongly by each stream's hydrological characteristics. Also, for any river system in years with high flows and high turbidity, carcass recovery rates will likely be different than in low flow, high visibility years. Males spring chinook salmon tend to remain active on the spawning grounds over a longer period than females (Schroder et al. 2002). Spawning survey effort in the Yakima River typically drops off after new redd construction ceases. However, we may be able to increase the number of males carcass recoveries and reduce the sex bias by continuing surveys past the period of new redd construction. It may be possible to calculate a bias correction factor and apply it to the historical carcass recovery database. This can be done by comparing a representative sample collected at a weir or trap, such as RAMF, to the subsequent carcass recovery data or performing a mark-recapture study comparing the known trait distribution/age composition of the marked released fish to the estimates from recaptured carcasses. It may be possible to incorporate flow data, as well, (e.g. Zhou 2002).

## Conclusions

There were no significant differences between first generation hatchery and wild upper Yakima River origin fish for sex ratio and run timing based on temporal distribution of in-river carcass recoveries and redd observations. Returns from the first hatchery cohort ( 1997 brood) will not be completed until 2002 with the age- 5 component, but age compositions of hatchery and wild fish were very similar with both predominantly age-4's. There were however, significant differences in passage timing at RAMF and size-at-age. While statistically significant, the difference of 2-4 days in RAMF passage timing would appear borderline for biological significance since there was no relationship between RAMF passage date and spawning date and no difference found between spawn timing of hatchery and wild fish. We will continue to monitor this
trait to see if hatchery passage timing diverges further from the naturally produced population. Of more concern for project success is that hatchery fish were significantly smaller than wild fish by 2 cm POHP length and 0.3 kg body weight in age-4's and 2.9 cm and 0.3 kg body weight in age- 3 's. These hatchery-wild differences were similar in magnitude and direction across two independent hatchery cohorts, the 1997 (age-4's) and 1998 (age-3's) broodyears, arguing for a "domestication" effect that must occur prior to age-3's returning.

The observed differences in size-at-age after only a single generation of domestication in two successive cohorts is significant and, irrespective of cause, reduces the productivity and fitness of naturally spawning hatchery fish. For example, in upper Yakima River age- 4 spring chinook females fecundity is positively correlated with body size and a 2.1 cm reduction in POHP length translates to a $9 \%$ reduction in fecundity (see Chapter 2 of this report). If it is a genetic response to selection, it represents a potential selection response of 0.5 standard deviation generation ${ }^{-1}$ or 0.5 haldane (Haldane 1949). Size-at-age is a heritable trait shaped by both natural and sexual selection (Schroder 1981; Blair et al. 1993; Quinn and Foote 1994; Fleming and Petersson 2001; Hendry 2001), but subject to environmentally driven phenotypic plasticity, as well (Riddell 1986; Hard 1995). There is significant variation in traits between the three wild populations of spring chinook identified in the Yakima River basin, including size-at-age, age composition, sex ratios, sexual dimorphism, and spawn timing, which appear to reflect local adaptations to each population's unique set of selection pressures. Reducing average POHP length of age-4 upper Yakima spring chinook from the locally adapted mean by 2.1 cm will likely result in counter selection pressure against the smallest hatchery fish reducing their fitness and driving the size distribution back upward toward the locally adapted optimum. This will reduce the genetic impact on the progeny produced by the naturally reproducing hatchery population, but reduces the average fitness or productivity of hatchery fish in the wild. In addition, other unmonitored traits correlated with body size have also been shifted away from their optima and counter selection in the wild on these traits will also result in reduced natural productivity.

Identifying the mechanism(s) or cause(s) of the reduction in size-at-age is critical to understanding supplementation's impacts on natural productivity. A study to monitor and estimate the retarding affects of domestication in the YKFP is scheduled to begin in 2002 (Busack 2002). This effort will be crucial to helping us understand and identify the genetic component to any observed differences in traits such as size-at-age.

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## Chapter 2

# Monitoring Phenotypic and Demographic Traits of upper Yakima River Hatchery and Wild Spring Chinook: Gametic and Juvenile Traits 

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#### Abstract

As part of the Reproductive Ecology and Domestication Monitoring and Evaluation program in the Yakima/Klickitat Fishery Project (YKFP), we compared upper Yakima River hatchery and wild origin spring chinook returns in 2001 over an array of fitness related traits characterizing each group's gametes and progeny ("button up" stage fry).

\section*{Female Size/Fecundity Relationships}

Fecundity and female body size were positively correlated in both hatchery and wild origin age- 4 females. The fecundity/length and fecundity/weight slopes of age- 4 hatchery and wild origin females were not significantly different (common slopes $=863$ eggs $\bullet[\mathrm{kg} \text { body weight }]^{-1}$ and 165 eggs $\bullet[\mathrm{cm} \mathrm{POHP}]^{-1}$ ). Wild age- 5 females also had significant, but weaker, positive correlations between female body size and fecundity. However, compared to age- 4 females they exhibited significantly shallower linear relationships, producing less than half as many eggs per kg increase in body weight (389 eggs• $\bullet \mathrm{kg}$ body weight $]^{-1}$ ) and almost $1 / 3$ fewer eggs per cm increase in POHP length (113 eggs $\bullet[\mathrm{cm} \mathrm{POHP}]^{-1}$ ). Including body weight, mean egg weight and POHP in a multivariate fecundity regression equation significantly increased the amount of variation explained and improved the precision of fecundity estimates.


## Fecundity

Naturally spawning age-4 hatchery females (3,820 eggs) were $9 \%$ less fecund ( 340 eggs) than wild origin females ( 4,160 eggs) based on the observation that age- 4 hatchery females were on average 2.1 cm smaller in POHP length than age- 4 wild females (see Chapter 1 of this report). Age-5 wild origin females (5,101 eggs) were significantly more fecund on average than age- 4 wild females.

## Egg Weight

There was no significant difference between age-4 hatchery ( 0.195 g ) and wild $(0.192 \mathrm{~g})$ origin mean egg weights. Eggs of age- 5 wild origin females $(0.216 \mathrm{~g})$ were significantly heavier than age- 4 females. The variation in egg weights across females was over $300 \%$ greater than within-female variation ( $\mathrm{cv}=4 \%$ ). There were weak positive correlations between egg weight and female POHP and body weight. The relationship between egg size and fecundity was negative, weak and significant only in wild age-4's.

## Female Reproductive Effort

Female Reproductive Effort (RE), the ratio of the weight of gametes to total body weight, of hatchery females (mean=0.207) was greater than age-4 (mean=0.201) and age5 (mean $=0.193$ ) wild females in 2001. The difference between hatchery and age- 5 wild females was statistically significant.

## Egg-to-Fry Survival and Developmental Abnormalities

There was no significant difference in viability of eggs of hatchery (mean viability $=0.87$ ) and wild (mean viability $=0.89$ ) origin females. Both hatchery (mean=0.009) and wild (mean=0.004) origin females had very low proportions of abnormally developing fry, but the hatchery proportion was significantly greater than the wild proportion.

## Fry Size

There was no significant difference between wild and hatchery origin fry fork lengths or body weights at the "button up" stage. There were strong positive relationships between fry size and egg weight for both wild and hatchery origin females. The fry fork length/egg weight relationship explained 73 and $62 \%$ of the total variation and the fry body weight/egg weight relationship explained 93 to $82 \%$ of the total variation in wild and hatchery fish, respectively. There were weak positive correlations between fry size and adult female size, although the total variation explained ranged from only 5 to $10 \%$.

All findings in this report should be considered preliminary and subject to further revision unless previously published in a peer-reviewed technical journal.

## Introduction

A critical aspect of assessing success in the Yakima/Klickitat Fishery Project's (YKFP) spring chinook (Oncorhynchus tshawytscha) program is evaluating traits that determine natural production and to compare hatchery and wild origin fish across these traits. That is because project success is defined as increasing natural production and harvest opportunities, while keeping adverse ecological interactions and genetic impacts within acceptable bounds (Busack et al. 1997). Significant changes in locally adapted traits due to hatchery influences, whether of genetic or environmental origin, will likely be maladaptive, resulting in reduced population productivity and fitness (Taylor 1993; Hard 1995). Naturally spawning hatchery fish have been shown to be less reproductively successful then wild fish in some studies (Resenbichler and McIntyre 1977; Chilcote et al. 1986; Leider et al. 1990) particularly in populations that have experienced multiple years of domestication (see review in Schroder et al. 2002). Traits such as fecundity (Healey and Heard 1985; Fleming and Gross 1990; Beacham and Murray 1993), emergent fry size and fry energy reserves (Thorpe et al. 1984; Hendry et al. 2001), egg incubation rates, and emergence timing (Beacham and Murray 1993; Quinn et al. 1995) can have significant affects on the reproductive success and fitness of salmonids. These traits can also reflect local adaptations (Taylor 1991; Hendry et al. 1998; Quinn et al. 2001). Other traits such as the number of eggs produced per unit body size or the biomass of gametes per unit body size are indicators of how populations have responded to local selection forces and have optimized allocation of energy between somatic growth, gametes, migration, competition and mating (Heath et al. 1999; Kinnison et al. 1998; Kinnison et al. 2001).

In the following chapter of this report, we make comparisons between hatchery origin fish from the Cle Elum Supplementation Research Facility (CESRF) and upper Yakima River wild origin spring chinook returning in 2001over a suite of traits affecting fitness and reproductive success. These include fecundity, female body size/fecundity relationships, female reproductive effort, egg size, egg-to-fry viability, fry size, fry length/egg size relationships, female size/fry size relationships, and occurrences of developmental abnormalities in fry. Many of these traits have been measured on wild origin upper Yakima fish annually beginning with the first broodstock collection in 1997. However, in this report we restrict our analyses to the 2001 wild and hatchery origin returns. In future reports we will compare wild origin samples representing historical baseline years 1997-2000 to hatchery and wild origin samples from supplemented return years.

Two other facets of reproductive ecological monitoring in the YKFP cover phenotypic and demographic traits of adult upper Yakima River spring chinook (see Chapter 1 of this report) and the reproductive success of naturally spawning upper Yakima spring chinook in an experimental stream (Schroder et al. 2002). Tracking fitness related traits over time is also an important aspect of monitoring domestication effects to determine whether divergence in heritable traits is occurring between the supplemented naturally spawning population, a hatchery control line to be established in

2002, and a wild Naches basin control population (excluding the American River; see Busack et al. 2002). Thus, we will expand our comparisons to Naches females in future reports.


Figure 1. Yakima River basin showing the upper Yakima River, Roza Adult Monitoring Facility (RAMF), and the Cle Elum Supplementation Research Facility (CESRF).

## Methods and Materials

## Study Populations

The upper Yakima River is a tributary to the Yakima River, which discharges into the Columbia River (Fig. 1). Monitoring of the wild upper Yakima River population has occurred annually at Roza Adult Monitoring Facility (RAMF) since wild origin broodstock collection first began in 1997. The first hatchery reared cohort began returning in 2000 as anadromous age- 3 jacks and in 2001 as age- 4 adults. However, sexually mature non-anadromous age-2 hatchery origin males have been observed on redds within the same year of their release (B. Ben James, Cascade Aquatics, pers. comm.). Wild and hatchery origin precocial males have been observed spawning with adults (Knudsen and Schroder, unpubl. observations) and have demonstrated some
reproductive success in competition with adult wild males (Schroder et al. 2002). Thus, there is reason to believe that introgression of hatchery fish may have begun in 1999, the first year of CESRF hatchery releases.

Length, weight, and age data are collected from a subsample of returning spring chinook as they pass upstream through RAMF approximately 1 to 5 months prior to reaching full maturity. For a full description of the sampling, collection, and processing of hatchery and wild origin returns at RAMF see Chapter 1 of this report. The majority of fish sampled at RAMF in 2001 was of hatchery origin. Immediately after being sampled, these fish were released back into the river to continue their migration. A subsample of wild and hatchery origin fish are collected from throughout the run and taken to the CESRF. Data collected from wild origin fish selected for broodstock are used to represent the wild population's adult phenotypic and demographic traits, as well as, the following reproductive traits: total gamete mass weight (females), egg weight, female reproductive effort, fecundity, viability, incidence of abnormally developing fry, and fry size. In 2001, there were 596 wild origin fish collected for broodstock and reproductive success studies and 122 hatchery origin fish. Of these, 195 age- 4 and 18 age- 5 wild origin females and 33 age- 4 hatchery origin females were sampled for fecundity, reproductive effort, gamete mass, and egg weight.

## Traits

Total Gamete Mass, Egg Weight, Fecundity and Female Reproductive Effort
Gamete mass and mean egg weights were measured as females were artificially spawned at CESRF. A large portion of the ovarian fluid was drained off prior to a female's egg mass being weighed to the nearest 0.1 g . A subsample of approximately 30 50 eggs was then collected, weighed to the nearest 0.01 g , and the number of eggs in the subsample counted and used to calculate the mean "green" egg weight. A gravimetric estimate of fecundity was then calculated by dividing the total gamete mass weight by the mean green egg weight. Since it is not possible to drain off all ovarian fluid, gravimetric fecundity estimates are often biased, overestimating fecundity. In order to estimate whether this bias was occurring in our estimates, egg masses from 19 females were hand counted at the eyed-egg stage and compared to the gravimetric fecundity estimates so that a bias correction factor could be calculated. CESRF personnel also use a photocell-based automated egg counter to estimate fecundity. The hand counts of the same 19 females were compared to the machine counts to determine if any bias occurred in the machine counts.

The linear relationship between fecundity and female body weight, POHP length and egg size was estimated and comparisons of the slopes of the body size/fecundity regressions were made using ANCOVA. In addition, we looked at whether body weight, POHP length and egg size together as predictors of fecundity in a multivariate regression analysis explained more of the total variation in fecundity and increased precision.

We compared egg weight distributions of hatchery and wild origin females using ANOVA. The relatively large number of wild 5 -year old females ( $\mathrm{n}=18$ ) in 2001 allowed us to compare egg sizes of 4 - and 5 -year old females, as well. During the fecundity estimation process we collected five eggs from each female, held them in water for 1-3 days at approximately $3^{\circ} \mathrm{C}$ to water-harden, and then measured individual egg weights. These data was used to estimate within-female variation in egg weight, which we compare to between-female variation.

Reproductive effort (RE) was calculated for hatchery and wild origin females spawned at CESRF. This metric describes the proportion of a female's total biomass represented by gametes and is calculated by dividing the total egg mass weight (drained of ovarian fluid) by the total body weight including gametes and ovarian fluid.

A few females had significant proportions of unripe, overripe, injured, or abnormally developing eggs. We assumed these were primarily due to females being selected for spawning either too early or too late and/or from injuries incurred during handling, transfer and holding. Egg retention rates in wild naturally spawning Yakima River spring chinook females are generally very low (M. Johnston, YN, unpublished data; S. Young, WDFW, unpublished data). During holding of broodstock, particularly in the latter weeks of the spawning season, significant numbers of eggs are observed on the bottom of the adult holding raceway indicating that some females had prematurely released gametes. Females with RE values below 0.15 ( 10 of 256 total hatchery and wild origin females we examined) were considered to have a significant portion of either under- or over-developed, injured, or lost eggs prior to being sampled and consequently their fecundity and RE values were excluded from our analyses.

Table 1. Schematic of a six-by-six Hatchery/Wild factorial cross resulting in 36 single pair matings.

|  |  |  | Males types |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | Wild origin |  |  | Hatchery origin |  |  |
|  |  |  | W ${ }^{\text {® }} 1$ | W ${ }^{\text {® }} 2$ | W ${ }^{\text {® }} 3$ | $\mathrm{H}^{\text {® }} 1$ | $\mathrm{H}^{\text {® }} 2$ | $\mathrm{H}_{\bigcirc}^{\text {® }} 3$ |
| Females types | Wild origin | W ${ }^{\text {P }} 1$ |  |  |  |  |  |  |
|  |  | W Q 2 |  |  |  |  |  |  |
|  |  | W +3 |  |  |  |  |  |  |
|  | Hatchery origin | HQ1 |  |  |  |  |  |  |
|  |  | H¢2 |  |  |  |  |  |  |
|  |  | H 93 |  |  |  |  |  |  |

## Factorial Crosses: Egg-to-Fry Viability, Developmental Abnormalities and Fry Size

The standard protocol at CESRF for spawning broodstock is to spawn the fish in a series of factorial crosses (Busack et al. in prep). Each factorial cross typically is made up of 3 females and 3 males, creating 9 single pair matings. In order to make comparisons between hatchery and wild origin fish, we made four Hatchery/Wild factorial crosses in 2001. Our initial mating design was 3 wild and 3 hatchery origin females crossed with 3 wild and 3 hatchery origin males mated in a full $6 \times 6$ factorial
cross (Table 1). On average 183 eggs (range 71 to 217) per female were collected and placed into a dry $L L$ beaker with approximately $1 c c$ of milt from the respective male in the single-pair mating. The gametes were then activated by adding approximately 200 ml of well water. After a minimum of 2 minutes from the time the eggs and sperm were activated, the eggs from each single-pair mating were drained and placed into individual incubation containers or isolettes. Each isolette was labeled with the female and male's origin and individual identification numbers and placed into an Iodiphore bath for approximately 45 minutes. The isolettes from each female were then incubated in individual isobuckets to the eyed egg stage, shocked, and transferred to Heath trays for final incubation to the post-hatching yolk absorption or "button up" stage.

The isolettes were sampled twice: once, at the eyed egg stage just after shocking when all viable and nonviable eggs were counted and again, just after yolk absorption, when any additional mortalities were counted. Deformities and abnormalities (e.g. scoliosis, missing eyes, Siamese twining or inappropriate fin development) were also enumerated during the final sampling.

We analyzed the viability and deformity data in a two-way ANOVA estimating Origin (Hatchery/Wild), Factorial Cross, and Interaction effects. Unfortunately, in 2001 there were not enough hatchery origin adult males on station at CESRF to meet our factorial cross needs as well as supply the Spawning Channel Study which required 22 mature adult hatchery origin males. We gave precedence to the Spawning Channel Study and eliminated hatchery males from the last 3 of the 4 Hatchery/Wild factorial crosses (Table 2) making it impossible to estimate male Origin effects. Thus, the linear model for the ANOVA was:
where $i=$ Wild or Hatchery origin; $j=$ Factorial Cross 1 through $4 ; k=$ females 1 through 18 , and $\varepsilon_{i j k}$ is a random error term. We assumed that Female/Male interactions were not significant. In essence, we treated males like agricultural plots in which each female within a Factorial Cross was represented. We used the same basic linear model for analyzing the fry deformity data, replacing Viability ${ }_{i j k}$ with Proportion Deformed $_{i j k}$ in

Table 2. A six-by-three Hatchery x Wild factorial cross design in which only wild origin males are used. Egg viability, fry length and developmental abnormalities were estimated for each cell representing a subsample of eggs from the respective wild $\left(\mathrm{W}_{q}\right)$ or hatchery origin $\left(\mathrm{H}_{+}\right)$female fertilized by milt from a Wild (W ${ }_{\delta}^{\top}$ ) origin male.

equation (1). All proportions were arcsine square root transformed (Zar 1984) before analysis, but are reported as untransformed data in text, tables and figures.

Fork length and body weight were measured on five individual fry from one single-pair mating from each female within a factorial cross. Fry were anesthetized and blotted dry prior to being weighed. Because we did not collect fry size data from every female/male pairing, we could not estimate male effects. However, we were monitoring fry size at "button up" stage when maternal effects should overwhelm male effects (Iwamoto et al. 1984; Heath et al. 1999). Wild and Hatchery origin fry size was compared using ANCOVA to control for the effects of differences in egg weights.

## Results

## Fecundity and Fecundity/Female Size Relationship

Gravimetric estimates of fecundity were highly correlated with hand counts and were relatively precise. However, they were significantly biased ( $p<0.001$ ), overestimating fecundity by $3.8 \%$ on average in 2001 based on a paired-sample $t$-test of hand counts and gravimetric estimates (Fig. 2.A.). All gravimetric fecundity estimates were adjusted for bias by multiplying by the correction factor, 0.9618 , and the resulting corrected fecundity estimates are used in all analyses below. We also found that photocell based machine counts of eyed eggs significantly ( $p<0.001$ ) underestimated fecundity by $2.5 \%$ on average (Fig. 2.B.). The slopes illustrated in Figures 2.A. and B. were significantly different from a slope of $1.0(95 \% \mathrm{CI}=0.956$ to 0.968 and $0.969-0.980$, respectively). The machine counter would miss eggs as they passed by the optical sensors and the number of missed eggs was observed to increase as the rate eggs were introduced into the machine increased.


Figure 2. Relationship between $A$ ). hand counts (HC) and gravimetric estimates (GrvEst) of fecundity and B) hand counts (HC) and machine counts (MC) of eyed eggs. The regression (black lines) were forced through a 0 y -intercept and were all significantly different from 1.0 , indicated by the red line.


Figure 3. Linear relationship between CERSF POHP length and fecundity for hatchery age-4(*), wild age-4(■) and wild age-5 ( $\triangle$ ) origin upper Yakima River spring chinook in 2001.


Figure 4. Linear relationship between CERSF body weight (BW) and fecundity for hatchery age-4( $\uparrow$ ), wild age-4( $\bullet$ ) and wild age-5 ( $\quad$ ) origin upper Yakima River spring chinook in 2001.

Therefore, it is important to keep the rate eggs are poured into the machine constant so that the rate of under reporting (bias) is equal across all females.

There was a significant positive correlation between fecundity and POHP length (Fig. 3) and body weight at spawning (Fig. 4) in both hatchery and wild origin age-4 females (Table 3). In an ANCOVA there was no significant difference between the slopes of the two regressions ( $P O H P, p=0.314$ equivalent slopes; Body weight, $p=0.937$ equivalent slopes) or between the two age- 4 groups in either mean fecundity at a standardized body size ( $P O H$ : mean hatchery fecundity $=4,117$ and wild $=4,007$ at standardized POHP length of $60.5 \mathrm{~cm}, p=0.320$ of equal means; Body weight:

mean hatchery fecundity $=4,119$ and wild $=4,020$ at standardized body weight of 4.0 $\mathrm{kg}, p=0.356$ ). In comparison, age- 5 wild origin females had a weaker, shallower fecundity/body size relationship than age-4 females (Figs. 3 and 4). In the ANCOVA of age-4 and -5 year olds, fecundity/body weight slopes were significantly different ( $p<0.01$ ), but the fecundity/POHP slopes were not ( $p=0.08$ ). We estimated the age- 4 female (hatchery and wild combined) common fecundity/body size slopes as 863 eggs $\bullet(\mathrm{kg} \text { body weight })^{-1}$ and 165 eggs $\bullet(\mathrm{cm} \mathrm{POHP})^{-1}$, while age- 5 wild females had slopes of 389 eggs $\bullet(\mathrm{kg} \text { body weight })^{-1}$ and 114 eggs $\bullet(\mathrm{cm} \mathrm{POHP})^{-1}$.

Based on the common fecundity/POHP length relationships of age-4 hatchery and wild origin females observed in the regressions above (Fecundity $=$ [POHP*164.6]$5,937.8$ ) and the observation that naturally spawning age-4 hatchery female's POHP was 2.1 cm smaller than naturally spawning wild origin female's in 2001 (see Chapter 1 of this report), we estimated that age-4 wild origin females (mean fecundity=4,160) had $9 \%$ greater fecundity than naturally spawning age-4 hatchery females (adjusted mean fecundity $=3,820$ ). Thus, hatchery females are able to deposit fewer eggs on average than wild females, reducing their fitness. Wild origin age- 5 females (mean fecundity $=5,101$ ) had significantly greater fecundity than age-4 wild origin females $(p=0.03)$.

We also estimated fecundity of hatchery and wild origin females using three variables: female body weight at spawning, POHP length and mean egg size in a forward stepping multiple regression analysis. Mean egg size and body weight were significant variables in all three equations ( $p>0.001$; Table 4), while POHP did not explain a significant amount of variation in any ( $p \geq 0.25$ ). Including both body weight and egg weight in the regressions increased the total variation explain from $25-58 \%$ in the single variable regressions to $60-77 \%$ and reduced the SE of the regression lines from 524-670 eggs to 375-437 eggs.

Table 4. Forward stepping multiple regression results estimating fecundity using mean egg weight, POHP length and body weight. The significance of each variable's contribution to the equation is given under " $p$-value". Variables with $p$-values greater than 0.05 were excluded from the final regression equation.

| Origin | Effect | Coefficient | $p$-value | $\begin{gathered} \mathrm{SE} \\ \text { regression } \end{gathered}$ | Adjusted $\mathrm{R}^{2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| $\begin{gathered} \text { Wild } \\ \text { Age } 4 \\ (\mathrm{n}=192) \end{gathered}$ | Constant | 2890.2 | <0.001 | 375.4 | 0.753 |
|  | Egg Wt | -14830.1 | $<0.001$ |  |  |
|  | POHP |  | 0.608 |  |  |
|  | Body Wt | 1004.6 | <0.001 |  |  |
| Wild Age 5 ( $\mathrm{n}=18$ ) | Constant | 4029.8 | <0.001 | 437.0 | 0.596 |
|  | Egg Wt | -11667.8 | 0.001 |  |  |
|  | POHP |  | 0.739 |  |  |
|  | Body Wt | 610.9 | $<0.001$ |  |  |
| Hatchery$(\mathrm{n}=30)$ | Constant | 3359.6 | <0.001 | 416.8 | 0.774 |
|  | Egg Wt | -17557.5 | <0.001 |  |  |
|  | POHP |  | 0.251 |  |  |
|  | Body Wt | 1044.0 | <0.001 |  |  |



Figure 5. Mean green egg weight ( $\pm 1$ sd) for age-4 hatchery and wild and age- 5 wild females.

## Egg Weight

Based on data from 4-year old females spawned at CESRF, there was no significant difference (1-way ANOVA; $p=0.996$ ) in mean "green" egg weights of hatchery (mean=0.195 g; $\mathrm{n}=30$ ) and wild (mean= $0.192 \mathrm{~g} ; \mathrm{n}=192$ ) origin females in 2001 (Fig. 5). Age- 5 wild females (mean $=0.216 \mathrm{~g} ; \mathrm{n}=18$ ) had significantly larger eggs than both hatchery and wild origin age- 4 females ( $p=0.021$; Fig. 5). There were only very weak positive relationships between POHP length and egg weight in 2001 (Fig. 6). The relationship was not significant in hatchery females ( $p=0.13$ ), but was in wild females ( $p=0.02$ ). However, it explained just $2 \%$ of the total variation in wild egg weight. Only wild age- 4 females showed a significant positive relationship between body weight and egg weight ( $p=0.001$ ), but the total variation explained was just $5 \%$ (Fig. 7). Reanalyzing the egg weight data (see Fig. 7) as an ANCOVA and comparing hatchery and wild origin females, adjusting for differences in female body weight, also results in no significant difference in egg size ( $p=0.684$ ).


Figure 7. Linear relationship between female CERSF body weight (BW) and "green" individual egg weight (EggW) for age-4 hatchery ( $\uparrow$ ), age-4 wild (■) and age-5 wild ( $\triangle$ ) origin females in 2001.

The coefficient of variation (cv) in hatchery and wild origin age-4 females' egg weights was 14 and $13 \%$, respectively, and there was no significant difference in egg weight variation between the two groups (Levine's test; $p=0.412$ ). In comparison, the within-female cv of egg weights was only $3 \%$, based on 5 -egg samples from each female. Thus, egg size variation was over $300 \%$ greater between females than within individual females.

The relationship between egg weight and fecundity was negative in all groups of females (Fig.8). The relationship was statistically significant for both hatchery and wild


Figure 8. Linear relationship between fecundity and "green" individual egg weight for hatchery age-4 ( $\uparrow$ ), wild age-4 ( $\llcorner$ ), and wild age-5 ( - ) origin females in 2001.
age- 4 females ( $p<0.01$ ), but was weak, explaining between 1 and $4 \%$ of the total variation in egg weight.

## Reproductive Effort

Female Reproductive Effort (RE), the ratio of the weight of a female's gametes to total body weight, represents the proportion of total somatic growth allocated to producing gametes. The RE of age-4 hatchery females (mean $=0.207 ; \mathrm{n}=30$ ) was greatest followed by wild females (mean=0.201; $\mathrm{n}=192$ ) and wild age- 5 wild females (mean=0.193; $\mathrm{n}=18$ ) with the lowest mean RE. In a Tukey multiple-comparisons test hatchery and age- 5 wild female RE distributions were significantly different ( $p=0.048$ ).

## Relative Fecundity vs. Egg Weight and RE

Relative Fecundity (RF) standardizes fecundity to a unit body weight: eggs• $(\mathrm{kg}$ body weight $)^{-1}$ or for each kg of body mass how many eggs were produced. Regressing egg weight or reproductive effort against RF allows us to look at how females from each population and age class manage the tradeoffs between producing either many small eggs or fewer larger eggs. Figure 8 shows the linear relationship between RE and both RF (Fig. 9.A.) and egg weight (Fig. 9.B.) for hatchery and wild origin females. RE and RF are significantly positively correlated for age-4 hatchery ( $\mathrm{r}^{2}=0.25 ; p=0.01$ ) and wild $\left(\mathrm{r}^{2}=0.31 ; p<0.01\right)$ origin females. There was no significant difference between the slopes of age-4 hatchery and wild females ( $p>0.23$ ). In contrast, age- 5 wild females showed no trend between RE vs RF ( $\mathrm{r}^{2}=0.01 ; p=0.73$ ) over the range of RF values. Age- 5 females have larger eggs than age- 4 females (see Egg Weight results). There is an optimum egg size below and above which fitness decreases (see Hendry et al. 2001). It appears from Figure 8 that age- 4 female's eggs are closer to that lower size limit and as fecundity increases, RE (the proportion of total body weight gametes represent) increases (see Fig.
9.A.). In contrast, age- 5 eggs begin at the upper limit and more rapidly decrease in size as fecundity increases (Fig. 8), while RE shows no trend (Fig. 9.A.). Age-5 females will reduce egg size to increase fecundity, while age-4's will increase RE (the proportion of total body weight gametes represent) while reducing egg size at a much lower rate.


Figure 9. Relationship of A.) reproductive effort and B.) green egg weight to relative fecundity (eggs/kg body weight).

## Egg Viability and Developmental Abnormalities

There was no significant Origin effect ( $p=0.208$ ) in the ANOVA of egg viability of hatchery (mean viability $=0.865 ; \mathrm{n}=43$ ) and wild (mean viability $=0.889 ; \mathrm{n}=43$ ) origin females in 2001. Abnormally developing fry were rare in both hatchery and wild samples, occurring in less than $1 \%$ of the eggs we examined. However, such abnormalities occurred over twice as often in progeny of hatchery females. Mean proportions of fry with abnormalities in hatchery and wild fry were 0.009 and 0.004 , respectively, which was significant $(p=0.036)$.


Figure 10. Relationship between fry length (FL) and egg weight (EggW) for hatchery ( $\downarrow$; $n=44$ ) and wild $(\square ; n=63)$ origin spring chinook from the 2001 brood.

Fry Size
There was no significant difference between wild and hatchery origin fry length ( $p=0.530$ ) or weight ( $p=0.399$ ) after adjusting for differences in egg size using ANCOVA. It was necessary to adjust for egg size because of the strong positive correlation between egg weight and both fry length ( $\mathrm{r}^{2} \geq 0.62 ; p<0.01$; Fig. 10) and fry weight ( $\mathrm{r}^{2} \geq 0.82 ; p<0.01$; Fig. 11) and because by chance, for the particular subset of females selected for our experimental crosses, hatchery females' eggs (mean=0.197 g) were significantly ( $p<0.01$ ) heavier than wild eggs (mean=0.184 g). From Figure 12 it is clear that hatchery and wild fry also have similar fry weight/length relationships at the yolk-absorption stage.


Figure 11. Relationship between fry weight (FryW) and egg weight (EggW) for hatchery ( $\uparrow ; n=44$ ) and wild ( $\square ; n=63$ ) origin spring chinook from the 2001 brood.


Figure12. Comparison of hatchery ( $\downarrow$ ) and wild ( $\square$ ) origin fry weight (Weight) versus fry fork length (FL) for progeny of 2001 upper Yakima River spring chinook.

There were weak significant positive female body weight/fry size relationships (Table 5; Fig. 13 and 14), but the total variation explained was only 6 to $14 \%$. Wild females exhibited the only significant female POHP/Fry size relationship ( $p=0.04$ ), but it explained only $7 \%$ of the total variation in fry length. Thus, female body weight had
more influence on fry size than female POHP, but it only explained at most $14 \%$ of the total variation in fry size.


Figure 13. Linear relationship between female CERSF body weight (BW) and "green" individual egg weight (EggW) for age-4 hatchery ( $\uparrow$ ), age-4 wild (■) and age-5 wild ( $\triangle$ ) origin females in 2001.


Figure 14. Linear relationship between female body weight and fry fork length in hatchery ( $\downarrow$ ) and wild ( $\square$ ) origin spring chinook in 2001.

Table 5. Linear relationships between female body weight (FW) and POHP length (FL) and fry fork length (FLn) and fry body weight (FBW) by origin for upper Yakima River spring chinook.

| Relationship | Q Origin | $\mathrm{R}^{2}$ | $p$-value | n | Linear equation |
| :---: | :--- | :---: | :---: | :---: | :---: |
| FW by FLn | Hatchery | 0.089 | 0.104 | 44 | FLn $=\left(\mathrm{FW}^{*} 0.558\right)+32.40$ |
|  | Wild | 0.101 | 0.007 | 63 | FLn $=\left(\mathrm{FW}^{*} 0.403\right)+32.65$ |
| FW by FBW | Hatchery | 0.135 | 0.039 | 44 | FBW $=\left(\mathrm{FW}^{*} 0.025\right)+0.222$ |
|  | Wild | 0.058 | 0.058 | 63 | FBW $=\left(\mathrm{FW}^{*} 0.010\right)+0.269$ |
| FemL by FLn | Hatchery | 0.026 | 0.294 | 44 | FLn $=\left(\mathrm{FemL}^{*} 0.090\right)+29.25$ |
|  | Wild | 0.067 | 0.040 | 63 | FLn $=\left(\mathrm{FemL}^{*} 0.063\right)+30.40$ |
| FemL by FBW | Hatchery | 0.051 | 0.142 | 44 | FBW $=\left(\mathrm{FemL}^{*} 0.004\right)+0.055$ |
|  | Wild | 0.018 | 0.290 | 63 | FBW $=\left(\mathrm{FemL}^{*} 0.001\right)+0.240$ |



Figure 15. Linear relationship between female body weight and fry weight in hatchery ( $\uparrow$ ) and wild ( $\square$ ) origin spring chinook.

## Discussion

Any differences in heritable traits of CESRF hatchery and upper Yakima River wild origin fish, derived form the same native stock, would have to be due to a single generation of directional selection or relaxation of natural selection pressures in the hatchery. Trait differences can also have a non-genetic basis, caused by phenotypic plasticity due to environmental variation (Riddell 1986). An example is the larger size and later release of hatchery fish relative to wild conspecifics. This typically occurs because larger fish released later often have higher survival (Bilton et al. 1982). They are larger at release than naturally rearing juveniles because of the hatchery environment (rearing/feed regime) and outmigrate later due to human intervention (release timing), so this would occur even if the two groups shared identical parents. However, these environmentally induced differences can cause changes in adult returns such as reduced age at maturity (Beatty 1996) and size-at-return in hatchery chinook (Unwin and Glova 1997) and coho salmon (Bilton et al. 1982). In reality, there is likely to be a complex combination of both environmental and genetic factors affecting trait expression. The YKFP has begun to implement a domestication selection study (Busack et al. 2002) that will be crucial in helping us identify the magnitude of the genetic component in any observed trait differences.

Generally, we observed few significant differences between hatchery and wild origin spring chinook traits. The most significant difference was a $9 \%$ decrease in fecundity resulting in the reduced fitness of naturally spawning hatchery females. The fecundity reduction is a direct consequence of a reduction in size-at-age (see Chapter 1). In addition to fecundity, other traits correlated with body size have been shifted from their locally adapted optima, likely reducing fitness.

The allocation of energy between gamete production, somatic growth and behavior affects female fitness. There are significant trade offs made between energy budgeted toward gametes and other "bins" such as migration, body size, secondary sexual characteristics, competition and nest guarding (Kinnison et al 2001) and the allocation between all "bins" should coevolve under selection pressures so that lifetime reproductive success will be maximized (Pianka 1976; Roff 1988). For a naturally spawning fish, increasing gamete biomass above the optimum will either increase the number or mean size of eggs. However, this will divert energy from some other aspect of growth or behavior that has also been shaped by natural selection. The potential egg deposition or expected fry size may be greater because of this energy diversion however, if the female now lacks sufficient energy to build a quality redd or compete for quality redd sites, then the "potential" will not be realized and fitness can actually decrease. We can see how the trade offs between egg size, RE and RF differ between age- 4 and age- 5 females (Figs 9.A. and 9.B.) shaped by natural selection pressures. For artificially spawned fish, shifting energy into gamete production for example may have positive fitness consequences, since these fish experience a completely different set of selection pressures at spawning than naturally reproducing fish.

In the Tucannon supplementation project, hatchery females have experienced reduced fecundity relative to wild females because their eggs are significantly larger, but hatchery and wild female RE remains constant (Gallinat et al. 2001). However, this difference is likely environmentally driven perhaps due to the early rapid growth regime of hatchery females. Early growth has been shown to affect the number and size of eggs produced in female fish (Bagenal 1969; Thorpe et al. 1984; Jonsson et al. 1996). The growth trajectories of juvenile wild origin upper Yakima River spring chinook have been shown to differ significantly from their hatchery produced counter parts (Beckman et al. 2000), although we did not detect any significant differences in egg size or fecundity at a standardized size between hatchery and wild females.

All findings in this report should be considered preliminary and subject to further revision unless they have been published in a peer-reviewed technical journal.

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## Chapter 3

# Juvenile-to-Adult PIT Tag Retention In Yakima River Hatchery Spring Chinook: 2001 

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#### Abstract

To assess how well Passive Integrated Transponder (PIT) tags perform in hatchery spring chinook juvenile-to-adult survival studies we estimated tag loss from the time of release as yearling smolts to returns 1-3 years later. Annually from 1998 to 2001, approximately 40,0000 -age juveniles were tagged with a PIT tag, Coded-wire tag (CWT) injected into the snout, and an adipose fin clip primarily for the purpose of estimating downstream juvenile survival through the Yakima and Columbia rivers. Upon return fish fell into one of four categories based on tag retention: 1) PIT tagged/CWT/Ad clipped (all tags and marks retained), 2) CWT/Ad clipped (lost PIT tag), 3) PIT tagged/Ad clipped (lost CWT), or 4) Ad clipped only (lost both PIT and CWT). Returning age-2, -3 and -4 hatchery origin fish were monitored at Roza Adult Monitoring Facility (RAMF) in 2001 for the presence of each tag and mark, and retention rates were calculated. Separate adult male and female retention estimates stratified by time were also made.

PIT tag loss increased as return age increased. Age-2 ,-3 and -4 fish returning in 2001 were estimated to have lost 3,10 and $16 \%$ of their PIT tags, respectively. When the data for age-4 adults was stratified into temporal periods within 2001, we found that both females and males exhibited an increase in PIT tag loss over time. Adult females had PIT tag losses of 15,17 and $23 \%$ in May, June and July-September, respectively. Adult males had PIT tag losses of 11, 10 and $24 \%$ in May, June and July-September, respectively. While there was little difference between males and females, sexing errors likely confounded differences between the sexes. Spring chinook pass RAMF 1-5 months prior to spawning, while still not fully mature and if PIT tags continue to be shed over time, then our data underestimate actual loss at the time of spawning. This study utilized $400-\mathrm{kHz}$ PIT tags, which have a shorter detection distance than the $134-\mathrm{kHz}$ tags used since 1998. Some of our tag "loss" may actually be due to missed, undetected PIT tags causing us to generally overestimate loss. However, this would not explain the temporal trends we observed within a year. We will be repeating this analysis over the next 5 years to determine if these results are replicated with the newer generation PIT tags.


Juvenile-to-adult survival studies using PIT tags will underestimate actual survival rates when tag loss occurs. However, comparisons between similar groups of PIT tagged fish are valid relative survival comparisons. Care should be taken before extrapolating adult survival estimates from PIT tagged fish to untagged populations.

## Introduction

The Passive-Integrated-Transponder (PIT) tag (Prentice et al. 1990) has been an integral part of the Monitoring and Evaluation Program of the Yakima/Klickitat Fishery Project (Busack et al. 1997). Approximately 40,000 PIT tagged juvenile spring chinook have been released annually primarily to estimate juvenile in-river survival. In addition, PIT tags have been used to monitor smolt migration timing, movement of fish volitionally leaving Yakima/Klickitat Fishery Project (YKFP) acclimation sites, and to estimate a smolt entrainment/flow diversion relationship at Chandler Juvenile Fish Passage Facility (Sampsel and Fast 2001).


Figure 1. PIT tag loss over time in Skagit Hatchery adult male (■) and female ( $\uparrow$ ) coho salmon (adapted from Figure 15 in Prentice et al. (1994)). Weekly sample sizes of mature spawned fish are in parentheses.

Given the large number of juvenile PIT tags released by the YKFP, the question arose as to what could be learned from recoveries of adult PIT tagged fish. In addition, from a broader perspective there has been a significant effort by PTAGIS, NMFS and BPA to develop adult PIT tag monitoring sites throughout the Columbia River system (APTOC 2000). It is important to understand the limitations of each tagging technique in order to apply the most appropriate tag for each research purpose. Information regarding PIT tag loss in adult returns was first reported by Prentice et al. (1994) in the only published report to date on juvenile-to-adult PIT tag retention in free ranging adult Pacific salmon. They found that sometime prior to spawning, adult coho salmon PIT tagged as juveniles shed their tags at a high rate: overall $59 \%$ loss in females and $13 \%$ loss in males. Since PIT tag loss eight months after tagging was only $1 \%$ in their study (Prentice et al. 1993), essentially all of the tag loss had to occur sometime over the next 14 months prior to spawning. Prentice et al. (1994) collected five weekly samples as adult coho matured and were spawned. Their data demonstrate no temporal trend in PIT tag loss (Fig.1), indicating that the rate at which PIT tags were lost was not increasing
over the time period their samples were collected, although the sample sizes of the later collections are very small. Prentice et al. (1994) also reported PIT tag shedding had been observed in a sockeye salmon captive brood program. Since YKFP research objectives require tracking and recovering hatchery returns throughout their life history, including pre- and post-spawning, our goal in this study was to estimate whether significant PIT tag loss occurred in upper Yakima River hatchery spring chinook returns 1 to 3 years after tagging prior to spawning.

## Methods and Materials

The basic study design was to release yearling spring chinook each marked with a CWT, a PIT tag, and an adipose fin clip and examine returns 1-3 years later enumerating the number of fish retaining each type of tag. Beginning in 1998, approximately 40,000 age-0 hatchery origin spring chinook have been triple marked annually between October and December with 1) a PIT tag injected into the body cavity with the hand-held injector technique (Prentice et al. 1990), 2) a CWT injected into the snout (Jefferts et al. 1963), and 3) an adipose fin clip. Fish averaged between 103 and 110 mm fork length and 12 and 16 g weight at the time of tagging (Table X). In February, the fish were transferred by truck to three acclimation sites, held for approximately 1.5 months, and then allowed to volitionally emigrate as age-1 smolts between March 15 and May 30.

Table 1. Mean fork length (mm) and weight (g) of spring chinook PIT tagged in 1998 as 0 -age juveniles.

| Raceway | Length |  |  | Weight |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | ---: |
|  | Mean | SD | N | Mean | SD | N |
| 1 | 103.2 | 8.67 | 3998 | 12.0 | 3.53 | 129 |
| 2 | 102.9 | 9.80 | 3998 | 13.0 | 3.65 | 102 |
| 3 | 105.3 | 9.32 | 3996 | 14.1 | 3.95 | 159 |
| 4 | 107.2 | 8.55 | 3986 | 14.9 | 3.65 | 99 |
| 5 | 106.1 | 9.14 | 3995 | 14.3 | 4.06 | 100 |
| 6 | 108.9 | 7.87 | 1496 | 14.1 | 3.33 | 51 |
| 7 | 109.4 | 8.79 | 3998 | 15.0 | 2.95 | 100 |
| 8 | 108.5 | 8.19 | 3998 | 15.0 | 3.95 | 100 |
| 9 | 109.7 | 8.52 | 3998 | 15.9 | 3.30 | 100 |
| 10 | 106.0 | 8.09 | 3995 | 13.2 | 2.33 | 108 |

Quality control samples are collected after each raceway's tagging is completed and again between 2 to 4 months later in early February, prior to transfer to the acclimation sites. Beginning in 2000, functional PIT tag detectors were operating 24 hr per day at each acclimation site during the volitional release detecting an average of $98 \%$ of PIT tagged fish exiting the acclimation sites (Sampsel and Fast 2001).

Essentially all returning hatchery spring chinook are interrogated for PIT and CWT tags and fin clips at the Roza Adult Monitoring Facility (RAMF) as they pass


Figure 2. Map of the Yakima River Basin showing the Roza Adult Monitoring Facility (RAMF) and Cle Elum Research and Supplementation Facility (CERSF).
upstream to the upper Yakima River spawning areas (Fig. 2). Fish are interrogated for CWT's using an automatic CWT detector/hydraulic gate system that diverts fish from a passage flume into a holding tank. There, adipose fin clips visually identify study fish. All adipose fin clipped fish are checked for the presence of a PIT tag and snout CWT. Fish passage at RAMF begins in late April and continues through early September, approximately 1 to 5 months before spawning in September through October. The project's first 4 -year old adult returns of PIT tagged fish were in 2001. The PIT tags applied to those fish in 1998 were the older $400-\mathrm{kHz}$ models. From 1999 onward, we have used the $134-\mathrm{kHz}$ PIT tags.

Since each fish is tagged with a combination of CWT, PIT tag and adipose fin clip, each adult return would be expected to have all three tags ${ }^{1}$ assuming no tag loss. Returning fish will fall into one of four categories (Table 2): 1) PIT tagged/CWT/Ad clipped (all tags retained), 2) CWT/Ad clipped (lost PIT tag), 3) PIT tagged/Ad clipped (lost CWT), and 4) Ad clipped only (lost both PIT and CWT). Fish falling into categories 1) to 3) can be identified accurately and used to calculate the proportion of fish having lost each tag type. However, since all 810,000 juveniles released are adipose fin clipped, fish in category 4) that have lost both tags will be confused with other project fish that also lost their all of their tags, retaining the adipose fin clip. However, we can make an estimate of how large this loss of data may be.

[^0]| Table 2. The four possible combinations of PIT tag and CWT loss and retention. |  |
| :---: | :---: |
| CWT tag only, but no PIT tag (P1) | PIT tag only, but no CWT (P2) |
| Both a PIT tag and a CWT (P3) | No PIT tag or CWT (P4) |

Assuming tag loss within fish by tag type occurs independently, that is when a fish loses a PIT tag it does not affect whether it also losses or retains its CWT, we can calculate the following from the recoveries at RAMF:

P1 - Proportion with no PIT tag (Probability of a fish losing a PIT tag)

$$
\mathbf{P} \mathbf{1}=(\mathrm{CWT} \text { only }) /(\mathrm{CWT} \text { only }+ \text { PIT tagged only }+ \text { Both CWT+PIT })
$$

P2 - Proportion with no CWT (Probability of a fish losing a CWT tag)

$$
\mathbf{P 2}=(\text { PIT only }) /(\text { CWT only }+ \text { PIT tagged only }+ \text { Both CWT }+ \text { PIT })
$$

P3 - Proportion retaining all tags (Joint probability of a fish retaining both tags)

$$
\mathbf{P} 3=(1-\mathbf{P} 1)^{*}(1-\mathbf{P} 2)
$$

P4 - Proportion losing both PIT and CWT (Joint probability of fish losing both tags)

$$
\mathbf{P} 4=\mathbf{P} 1 * \mathbf{P} 2
$$

We can estimate how large $\mathbf{P 4}$ might be based on published data for $\mathbf{P 2}$. An average loss rate for CWTs ( $\mathbf{P} 2$ ) in chinook is $3 \%$ or less (Blankenship 1990). P1 is likely to be somewhere between 10 and $60 \%$. This results in a worst-case estimate for $\mathbf{P 4}$ of less than $2 \%$. Thus, not being able to factor in $\mathbf{P 4}$ will likely result in an error of less than $2 \%$ in estimated tag loss.

We stratified PIT tag loss estimates over time to see if there was any increasing temporal trend in tag loss. We also broke our data out by sex classification made at RAMF. However, these visual classifications on maturing fish are imprecise. For example, $66 \%$ of males and $96 \%$ of females were correctly identified to sex in 2001 (see Chapter 1 in this report). Thus, the analyses stratified by sex must be interpreted with some caution, because fish identified as "females" are actually a combination of true females and misclassified males.

## Results

From April through September 2001, there were 8,037 hatchery fish interrogated
for PIT tags, snout CWT's, and adipose fin clips at RAMF. A total of 615 PIT tagged fish were recovered and 695 with a snout CWT. Total adult (age-4), Jack (age-3), and Precocial (age-2) recoveries by tag combination are given in Table 3. We did not include recovery data from the initial sample collected in April ( $\mathrm{n}=6$ adult females). In April no PIT tags were detected in 5 of 6 fish that had snout CWTs, resulting in an estimated PIT tag loss of $83 \%$. This was larger than any other loss estimate and was likely due to a faulty PIT tag reader, since loss rates dropped off dramatically after the PIT tag detector was readjusted (M. Johnston, YN, pers. comm.).

## PIT tag loss over time

Female PIT tag loss averaged $16 \%$ over the entire sampling period, and increased over time from $15 \%$ in May to $23 \%$ in the July-to-September period (Table 4). CWT loss was $4 \%$ overall and did not demonstrate a trend over time. Male PIT tag loss was $13 \%$ over the total run and increased sharply to $24 \%$ in the July-September period from $10 \%$ in June, while CWT loss was 3\% (Table 5).

Table 3. Total recoveries of tag combinations in 2001 from adult, jack and precocial spring chinook originally triple marked with a PIT tag/snout CWT/adipose fin clip as juveniles. PIT tag loss (P1) estimates increase with older age-at-return, while CWT tag loss ( $\mathbf{P} 2$ ) is relatively stable over age classes.

|  |  | PIT tag loss <br> (P1) | CWT loss <br> $(\mathbf{P 2})$ | PIT and CWT <br> retained (P3) | Total <br> sample |
| :--- | ---: | :---: | :---: | :---: | :---: |
| Adults | N | 98 | 23 | 501 | 622 |
| (Age-4) | $\%$ | 15.8 | 3.7 | 80.5 |  |
| Jacks | N | 6 | 2 | 53 | 61 |
| (Age-3) | $\%$ | 9.8 | 3.3 | 86.9 |  |
| Precocials | N | 1 | 0 | 36 | 37 |
| (Age-2) | $\%$ | 2.7 | 0.0 | 97.3 |  |

Table 4. Monthly recoveries of tag combinations in 2001 over time for adult female spring chinook originally triple marked with a PIT tag/snout CWT/adipose fin clip as juveniles. PIT tag loss (P1) estimates increased over time, while CWT tag loss (P2) did not show a trend over time.

| Time <br> period |  | PIT tag loss <br> (P1) | CWT loss <br> $\mathbf{( P 2 )}$ | PIT and CWT <br> retained (P3) | Total sample |
| :--- | ---: | :---: | :---: | :---: | :---: |
| May | N | 43 | 12 | 240 | 295 |
|  | $\%$ | 14.6 | 4.1 | 81.4 |  |
| June | N | 18 | 2 | 86 | 106 |
|  | $\%$ | 17.0 | 1.9 | 81.1 |  |
| July- <br> September | N | 12 | 4 | 37 | 53 |
|  | $\%$ | N | 22.6 | 7.5 | 69.8 |

Table 5. Recoveries of tag combinations in 2001 over time for adult male spring chinook originally triple marked with a PIT tag/snout CWT/Adipose fin clip as juveniles. PIT tag loss ( $\mathbf{P} 1$ ) estimates increased in the last time period, while CWT tag loss ( $\mathbf{P} 2$ ) estimates showed no trend over time.

| Time <br> period |  | PIT tag loss <br> $\mathbf{( P 1 )}$ | CWT loss <br> $\mathbf{( P 2 )}$ | PIT and CWT <br> retained (P3) | Total sample |
| :--- | :---: | :---: | :---: | :---: | :---: |
| May | N | 9 | 2 | 68 | 79 |
|  | $\%$ | 11.4 | 2.5 | 86.1 |  |
| June | N | 6 | 3 | 49 | 58 |
|  | $\%$ | 10.3 | 5.2 | 84.5 |  |
|  | N | 6 | 0 | 19 | 25 |
| Over all <br> periods | $\%$ | 24.0 | 0.0 | 76.0 |  |
|  | $\%$ | 21 | 5 | 136 | 162 |



Figure 3. Estimated PIT tag loss over time for adult age-4 male (■) and females ( $*$ ) hatchery origin upper Yakima River spring chinook in 2001. Temporal sample sizes are in parentheses next to the plotted points.

Quality control samples
In the January or February quality control samples of approximately 200 fish per raceway are collected. PIT tagged fish ( 2,225 per raceway) represent about $5 \%$ of all fish within each raceway. The 200 fish are interrogated to identify PIT tags, CWTs, adipose fin clips, and, beginning in 1998, we began using colored elastomer injected into adipose eyelid tissue. Some fish are tagged in the cheek and nape with CWT's and these fish cannot be differentiated from fish with snout tags using a hand held CWT detector. Therefore, we could not identify with certainty fish that had lost their snout CWTs as juveniles. However, the 2001 data from adults, jacks and precocials confirm that snout CWT tag loss 1 to 3 years after release is on the order of $4 \%$ or less. A bigger problem is estimating PIT tag loss at the time of release. Within each raceway there are non-PIT tagged fish that have lost all of their tags except the adipose fin clip and either the cheek
or nape body tag. These fish cannot be distinguished as juveniles from PIT tagged fish that have lost their PIT tag, but still have their snout tag and adipose fin clip. We are still working through this issue.

## Discussion

There is clearly loss of PIT tags occurring between the time fish exit the YKFP acclimation sites and when they return 1 to 3 years after release. This is illustrated by loss rates increasing with increasing age of return. Age-4 adults had lost 16\% of their PIT tags compared to age- 3 and age- 2 loss rates of $10 \%$ and $3 \%$, respectively. When the data for age- 4 adults was stratified into temporal periods we found that both females and males exhibited an increase in PIT tag loss over time within the year. Adult females had PIT tag loss rates of 15,17 and $23 \%$ in May, June and July-September, respectively. Adult males had PIT tag loss rates of 11, 10 and 24\% in May, June and July-September, respectively.

Spring chinook pass RAMF 1-5 months prior to spawning, while still not fully mature. And if, PIT tag loss continues to occur over time, as suggested by the adult within-year temporal results (Fig. 3), then total loss could approach the levels observed by Prentice et al. (1994).

While there are still issues left to be worked out regarding accurate estimates of PIT tag loss at release, quality control samples of juveniles made just prior to release between 1998 and 2000, generally indicated that PIT tag loss prior to release and tagging quality was very similar across all years and does not explain the significant differences in retention across age classes.

Since we did not dissect fish that were determined to have lost their PIT tag, we cannot rule out that in some cases the PIT tag was actually present, but not functioning. However, Prentice et al. (1993) examined PIT tag failure rates in salmonids and found that over periods as long as 3 years failure rates were typically $0-1 \%$. They also found that nearly all failures were observed in the first sample collected within a few months after tagging and significant numbers of new failures were not detected after that. We have no reason to believe that PIT tag failure was higher than average in our releases and thus probably contributed $1 \%$ or less to the overall observed PIT tag loss. Project quality control samples taken a few months after tagging were collected when nearly all tag failures would be expected to have occurred. Thus, tag failures were factored into the pre-release tag "loss" estimate.

It is critical to understand the performance of any tag or mark in order to apply the most appropriate tag for the given situation and correctly interpret the results of tagging studies. When there is unaccounted for tag loss, survival will be underestimated and care should be taken before extrapolating the results to groups of untagged fish. However, when comparisons of juvenile-to-adult survival are made between similarly PIT tagged
groups, the results should be a valid relative survival comparison. PIT tag loss appears increase with increasing age of return and how close fish are to full maturity.

We had hoped to be able to make an estimate of PIT tag shedding from the time fish are collected at RAMF to the time they are spawned at Cle Elum Research and Supplementation Facility (CERSF). Any fish collected at RAMF with a juvenile PIT tag is identified and all fish collected at AMF are PIT tagged in the pelvic musculature with an 18 mm PIT tag (Johnston and McCutcheon in prep.) so each fish can be tracked from collection to spawning. Thus, it is theoretically possible to monitor whether fish shed their PIT tag after collection at RAMF prior to being spawned. However, in 2001 there were only 6 hatchery fish with juvenile PIT tags taken to CERSF and these few fish were not monitored through to spawning.

We will continue to monitor PIT tag loss rates to RAMF over at least the next 4 years. In addition, we will make a more concerted effort to track PIT tag retention in fish with juvenile PIT tags taken on station to CERSF.

All findings in this report should be considered preliminary and subject to further revision unless they have been published in a peer-reviewed technical journal.

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[^0]:    ${ }^{1}$ Technically, fin clips are marks, rather than tags. However, we will often refer to PIT tags, CWT's, and adipose fin clips generically as "tags" in the text.

