Reproductive Ecology of
Yakima River
Hatchery and Wild Spring Chinook
Yakima/Klickitat Fisheries Project Monitoring and Evaluation

Annual Report 2006

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Edited by:

Curtis Knudsen

Oncorh Consulting
2623 Galloway SE
Olympia, Washington 98501

Prepared for:

Yakama Nation
P.O. Box 151
Toppenish, WA 98948

Washington Department of Fish and Wildlife
600 Capitol Way North
Olympia, Washington 98501-1091

U.S. Department of Energy
Bonneville Power Administration
Division of Fish and Wildlife
P.O. Box 3621
Portland, Oregon 97283-3621

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This report covers four topics under the Yakima/Klickitat Fisheries Project's Monitoring and Evaluation Program (YKFPME) and was completed by Oncorh Consulting as a contract deliverable to the Yakama Nation and Washington Department of Fish and Wildlife. The YKFPME is funded under two BPA contracts, one for the Yakama Nation (Contract number 00027798) and the other for the Washington Department of Fish and Wildlife (Contract number 00027871, Project Number 1995-063-25). A comprehensive summary report for all of the monitoring and evaluation topics will be submitted after all of the topical reports are completed. This approach to reporting enhances the ability of people to get the information they want, enhances timely reporting of results, and provides a condensed synthesis of the whole YKFPME.

Executive Summary

This is the seventh in a series of annual reports that address reproductive ecological research and comparisons of hatchery and wild origin spring chinook in the Yakima River basin. This report is organized into four chapters with a general introduction preceding the first chapter. Summaries of each of the chapters in this report are included below. The first and second chapters are progress reports examining demographic and gametic data, respectively, collected in 2006 from hatchery and natural origin returns of spring chinook in the upper Yakima River. The third chapter compares hatchery and natural origin redds examined between 2002 and 2006. The fourth chapter presents an analysis of the long term loss of Passive Integrated Transponder tags and their effects on hatchery spring chinook survival, growth, and migration behavior.

Chapter 1. We compared age composition, passage timing, and size-at-age of supplementation hatchery (SH; first-generation) and hatchery control (HC; second-generation) and natural origin (NO) adult spring Chinook salmon returning to the upper Yakima River in 2006. In addition, spawn timing of HC and NO adults were compared.

In 2006, the majority of NO fish returned at age 4 (83%), age 5 adults made up 10%, and age 3 (jacks) comprised 7% of the returns. SH fish returned predominantly at age 4 (85%), while 5 adults made up 1% of annual returns. HC returns were made up of 6% age 3 and 94% age 4 adults. The first returns of age 5 HC adults will not occur until 2007. The proportions of age 3 SH and HC jacks were much lower in 2006 than in hatchery returns over the period 2001 to 2005. However, this is likely a harbinger of a poor return from all age classes in the BY2003 cohort rather than an actual reduction in the proportion of males adopting this life history strategy.

Age 4 mean SH and HC body length and weight distributions at RAMF were significantly smaller than NO adults by 1.0 to 1.3 cm and 0.2 to 0.3 kg, but did not differ significantly between each other. In contrast, HC, SH, and NO age 3's were not significantly different and HC adults were largest.

Over the period 2001 to 2006 there was a significant decline in body size in all age 4 Yakima River Basin wild/natural origin populations as well as the upper Yakima hatchery population. During the period 1990 to 2000 the wild populations exhibited no significant trend in body size of age 4 fish. Because the wild control population in the Naches experienced the same rate of decline as the upper Yakima natural and hatchery origin groups between 2001 and 2006, we concluded that the supplementation program was not the cause, and reduced size over time is more likely related to large scale phenomenon such as oceanic and/or main stem Columbia River environments shared by all the populations.

The body size of High and Low hatchery treatment groups differed significantly at the time of juvenile release, but after approximately 18 months of post-release ocean growth the body size of age 4 High and Low growth treatment groups were not significantly different. Thus, the differences in juvenile size-at-release did not translate into comparable size differences in adult returns.

For the first time in 9 years we observed sexual dimorphism in age 4 upper Yakima returns. Mean female POHP lengths were significantly greater than males (NO (male = 58.0, female = 59.6), HC (male = 56.8; female = 57.9), SH (male = 56.9; female

= 58.0)). Body weight dimorphism followed the same general trend, but was not statistically significant between the sexes (NO (male = 3.6; female = 3.7), HC (male = 3.4; female = 3.4), SH (male = 3.4; female = 3.5)).

Median passage timing of age 4 adults was significantly different (Kruskal-Wallis p=0.010), with HC and SH returns passing 6 and 2 days later than NO adults, respectively. Median passage timing of age 3 Types were not significantly different (KW p=0.468) and HC and SH returns were 2.5 days later and 2 days earlier than NO adults, respectively. As noted in previous years, jack (age 3) median passage was significantly later by 12-14 days than age 4 adults (all KW tests p<0.01).

Mean spawn timing or date of maturation of HC fish was significantly earlier than NO fish by 7.5 days, which was greater than the mean shift of 5 to 7 days earlier in SH spawn timing noted between 2001 and 2005.

These data should be considered preliminary until published in a peer-reviewed journal.

Chapter 2. Reproductive traits in second generation hatchery control (HC) and natural origin (NO) spring Chinook females from the upper Yakima River were compared to determine whether fitness related traits had diverged after two generations of artificial propagation. We also compared body size and survival of progeny from single-pair matings of HC-by-HC and NO-by-NO adults. Because fecundity (FEC), relative fecundity (RELFEC), egg mass (EM), and total gamete mass (TGM) are significantly correlated with female length, it was necessary to use length as a covariate in comparisons. In general, the fundamental body size vs gametic trait relationships of age 4 HC and NO females were not significantly different and means adjusted for body size did not differ. Due to significantly smaller HC mean body size of 2006 adults which is correlated with the reproductive traits, returning HC females would on average have lower reproductive trait values than NO females. The survival, body size and KD values of fry produced from HC-by-HC and NO-by-NO mating did not differ significantly.

These data should be considered preliminary until published in a peer reviewed journal.

Chapter 3. We compared the characteristics of redds constructed by naturally spawning upper Yakima River hatchery and natural origin female spring Chinook salmon (*Oncorhynchus tshawytscha*) between 2002 and 2006. We compared the redds in terms of size, water depth, velocity, substrate and habitat characteristics, date of initial redd construction, and distance to the nearest surrounding redd. Redds were sampled by snorkeling during the spawning period between September and early October. Female origin was identified by the presence (natural origin) or absence (hatchery origin) of an adipose fin. After females were no longer present, redd characteristics were measured (total sample size: hatchery n=152; natural n=201). After eliminating autocorrelated variables, a 2-way MANOVA testing for Origin (Hatchery and Natural) and Year main effects in the distribution of bowl depth, bowl velocity, percent sand within the bowl, and distance to the nearest redd demonstrated no significant Origin effect (p>0.86), despite the fact that hatchery female mean fork lengths (FL) were smaller by between 0.7 to 1.6 cm's. All distributions except bowl velocity showed significant Year effects (p<0.001). Only bowl length showed a significant, weak negative correlation with FL, explaining

just 5% of the total variation. Both hatchery and natural females preferred spawning habitat in the pool/riffle transition zone. Spawning density showed a weak negative correlation with apex height, distance to the nearest redd, and bowl length and explained between 6 and 9% of the total variation. Within years there were relatively small differences between hatchery and natural redds despite large interannual differences and no consistent trend in hatchery or natural origin means. However, our statistical tests did not have sufficient power to detect these relatively small differences due to low sample sizes.

Chapter 4. We tagged juvenile upper Yakima River hatchery spring chinook salmon (*Oncorhynchus tshawytscha)* with Passive Integrated Transponder (PIT) and Coded wire (CW) tags inserted into the snout in a double-tag study to test the assumptions often made that tags are not lost and do not effect survival, behavior, or growth. We estimated that PIT tags were lost on average in 17% of adults returning 8 months to 4 years after release. Tag losses were not significantly affected by the age of returns (ANCOVA p=0.40) indicating that after approximately 8 months of ocean rearing the majority of PIT and CW tag loss had already occurred. After correcting PIT tag recoveries for tag loss, recoveries were no longer significantly lower than expected (X^2-test p>0.05) indicating that there was no significant reduction in post-release survival due directly to the effects of PIT tags. The mean lengths and weights of PIT tagged adults were smaller than non-PIT tagged adults in all comparisons (age 4 mean POHP length difference = 1.1 cm; mean body weight difference = 0.1 kg). However, only age 4 PIT tagged adults were significantly smaller (ANOVA p<0.05). There was no significant difference in migration timing between PIT tagged and non-PIT tagged adults within the upper Yakima River (Mann-Whitney p>0.09). Observed PIT tag recoveries resulted in underestimating true survival by approximately 17% and underestimating body size, particularly in age 4 returns that make up over 80 percent of upper Yakima River hatchery origin returns.

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General Introduction

Raising fish in hatcheries can cause unintended behavioral, physiological, or morphological changes in Chinook salmon (Busack et al. 2007; Knudsen et al. 2007) due to either domestication selection or phenotypic plasticity. Domestication selection is defined as genetic changes within a captive population or between a captive population and its source population in the wild due to selection in an artificial environment (Busack and Currens 1995). The emphasis has often been on monitoring molecular traits, however Hard (1995a) points out that is at the quantitative trait level that polygenic traits, such as life history characters, are actually affected by selection resulting in those unintended changes in behavior and morphology that can lead to lowered survival and fitness in the natural environment.

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 quantitative traits due to hatchery influence would likely be maladaptive resulting in reduced population productivity and fitness (Taylor 1991; Hard 1995b). 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. 2006).

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 seventh in a series of annual reports that address reproductive ecological research and comparisons of hatchery and wild origin spring chinook in the Yakima River basin. This annual report summarizes data collected between April 1, 2006 and March 31, 2007 and includes analyses of some historical baseline data, 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. It is organized into four chapters with a general introduction preceding the first chapter. The first and second chapters are progress reports examining demographic and gametic data, respectively, collected in 2006 from hatchery and natural origin returns of spring chinook in the upper Yakima River. The third chapter compares hatchery and natural origin redds examined between 2002 and 2006 and will be submitted for publication. The fourth chapter presents an analysis of the long term loss of Passive Integrated Transponder tags and their effects on hatchery spring chinook survival, growth, and migration behavior. It was submitted to the North American Journal of Fisheries Management for publication

Additional field work 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. Data and findings should be considered preliminary until the results are published in a peer-reviewed journal.

Acknowledgments

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Chapter One

A Comparison of Life-History Traits of Natural origin and First- and Second-Generation Hatchery Origin Upper Yakima River Spring Chinook Salmon Returning in 2006

Prepared by:

Curtis M. Knudsen[1], Steve L. Schroder[2], Craig Busack[2], Todd N. Pearsons[2], Mark V. Johnston[3], and David E. Fast[3]

[1] Oncorh Consulting, 2623 Galloway SE, Olympia, WA 98501

[2] Washington Department of Fish and Wildlife, 600 Capitol Way North, Olympia, WA 98501-1091

[3] Yakama Nation, P.O. Box 151, Toppenish, WA 98948

Abstract

We compared age composition, passage timing, and size-at-age of supplementation hatchery (SH; first-generation) and hatchery control (HC; second-generation) and natural origin (NO) adult spring Chinook salmon returning to the upper Yakima River in 2006 were compared. Also, spawn timing of HC and NO adults were compared.

- In 2006, the majority of NO fish returned at age 4 (83%), age 5 adults made up 10%, and age 3 (jacks) comprised 7% of the returns. SH fish returned predominantly at age 4 (85%), while 5 adults made up 1% of annual returns. HC returns were made up of 6% age 3 and 94% age 4 adults. The first returns of age 5 HC adults will not occur until 2007.   The proportions of age 3 SH and HC jacks were much lower in 2006 then in hatchery returns over the period 2001 to 2005. However, this is likely a harbinger of a poor return from all age classes in the BY2003 cohort rather than an actual reduction in the proportion of males adopting this life history strategy.
- Age 4 mean SH and HC body length and weight distributions at RAMF were significantly smaller than NO adults by 1.0 to 1.3 cm and 0.2 to 0.3 kg, but did not differ significantly between each other. In contrast, HC, SH, and NO age 3's were not significantly different and HC adults were largest.
- Over the period 2001 to 2006 there was a significant decline in body size in all age 4 Yakima River Basin wild/natural origin populations as well as the upper Yakima hatchery population. During the period 1990 to 2000 the wild populations exhibited no trend in body size of age 4 fish. Because the wild control population in the Naches experienced the same rate of decline as the upper Yakima natural and hatchery origin groups between 2001 and 2006, we concluded that the supplementation program was not the cause, and reduced size over time is more likely related to large scale phenomenon such as oceanic and/or main stem Columbia River environments shared by all the populations.
- The body size of High and Low hatchery treatment groups differed significantly at the time of juvenile release, but after approximately18 months of post-release ocean growth the body size of age 4 High and Low growth treatment groups were not significantly different. Thus, the differences in size-at-release did not translate into comparable size differences in adult returns.
- For the first time in 9 years we observed sexual dimorphism in age 4 upper Yakima returns. In all Types, mean female POHP lengths were significantly greater than males (NO (male = 58.0, female = 59.6), HC (male = 56.8; female = 57.9), SH (male = 56.9; female = 58.0)). Body weight dimorphism followed the same general trend, but was not statistically significant (NO (male = 3.6; female = 3.7), HC (male = 3.4; female = 3.4), SH (male = 3.4; female = 3.5)).
- Median passage timing of age 4 adults was significantly different (Kruskal-Wallis p=0.010), with HC and SH returns passing 6 and 2 days later than NO adults, respectively. Median passage timing of age 3 Types were not significantly different (KW p=0.468) and HC and SH returns were 2.5 days later and 2 days earlier than NO adults, respectively. As noted in previous years, jack (age 3)

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median passage was significantly later by 12-14 days than age 4 adults (all KW tests p<0.01).

- Mean spawn timing of HC fish was significantly earlier than NO fish by 7.5 days, and was greater than the mean shift of 5 to 7 days earlier in SH spawn timing noted between 2001 and 2005.

These analyses focused primarily on comparisons within 2006 returns. Ultimately we intend to compare SH, HC, and NO upper Yakima River spring Chinook salmon returning between 2005 and 2008 in order to estimate whether the trends observed in first generation hatchery returns (2001-2004) continue as the project progresses into the second generation of returns and the Hatchery Control line implementation continues.

These data should be considered preliminary until published in a peer-reviewed journal.

Introduction

Life-history traits reflect local adaptations affecting population productivity and individual fitness (Stearns 1976; Roff 1992). Changes in demographic or life history traits, such as a reduction in age classes or skewed sex ratio, can reduce phenotypic variation, affect total annual egg production, and effective population size (Nunney 1991; Waples 2002). Moreover, changes in adult spawn timing may reduce fitness by shifting fry emergence timing outside locally adapted temporal windows (Brannon 1987; Smoker et al. 1998; Einum and Fleming 2000; Brannon et al 2004). In general, significant changes in locally adapted life-history traits will be maladaptive in the wild (Lynch and O'Hely 2001; Ford 2002; Goodman 2004, 2005), leading to reduced individual reproductive success (Taylor 1991; Fleming and Gross 1993; Fleming et al. 2000) and, particularly at low spawning densities, resulting in lower productivity of a naturally spawning population. Monitoring life-history traits of hatchery populations to determine if they are diverging from their native population's distributions is a necessary part of a hatchery monitoring plan (Hard 1995; Goodman 2005). Significant differences may indicate that the artificial rearing environment is causing genetic divergence to occur between the two groups. However, phenotypic changes alone are not sufficient to conclude that genotypic divergence has occurred. To do that, compared groups should be spawned, incubated, and reared in a common environment. Observed differences under these circumstances would represent genetic change.

This report is a continuation of work described by Knudsen et al (2006) who compared first generation hatchery and wild returns of upper Yakima hatchery spring Chinook returning from broodyears 1997 to 2000. The present analyses cover fish returning in 2006 and include age 3, 4, and 5 adults representing broodyears 2003, 2002, and 2001, respectively. Among the natural origin (NO) returns are the second cohort produced from a mixture of naturally spawning wild and hatchery origin fish (Table 1). The present analyses focus on comparisons within 2006 adult returns. Detailed analyses of broodyear specific comparisons and trends will be finished after the completion of the second generation of hatchery returns in 2008.

Methods

Study Population

The Yakima River is a tributary to the Columbia River and contains three genetically distinct, geographically separated wild spring Chinook populations (Busack and Marshall 1991; Knudsen et al 2005). The upper Yakima River population spawns primarily upstream of Roza Dam (rkm 206), an irrigation diversion dam through which all upstream migrating fish from this population must pass (Figure 1). The other two populations are located in the Naches system and consist of the American River (a tributary of the Naches River) and the Naches River and its tributaries, excluding the American River.
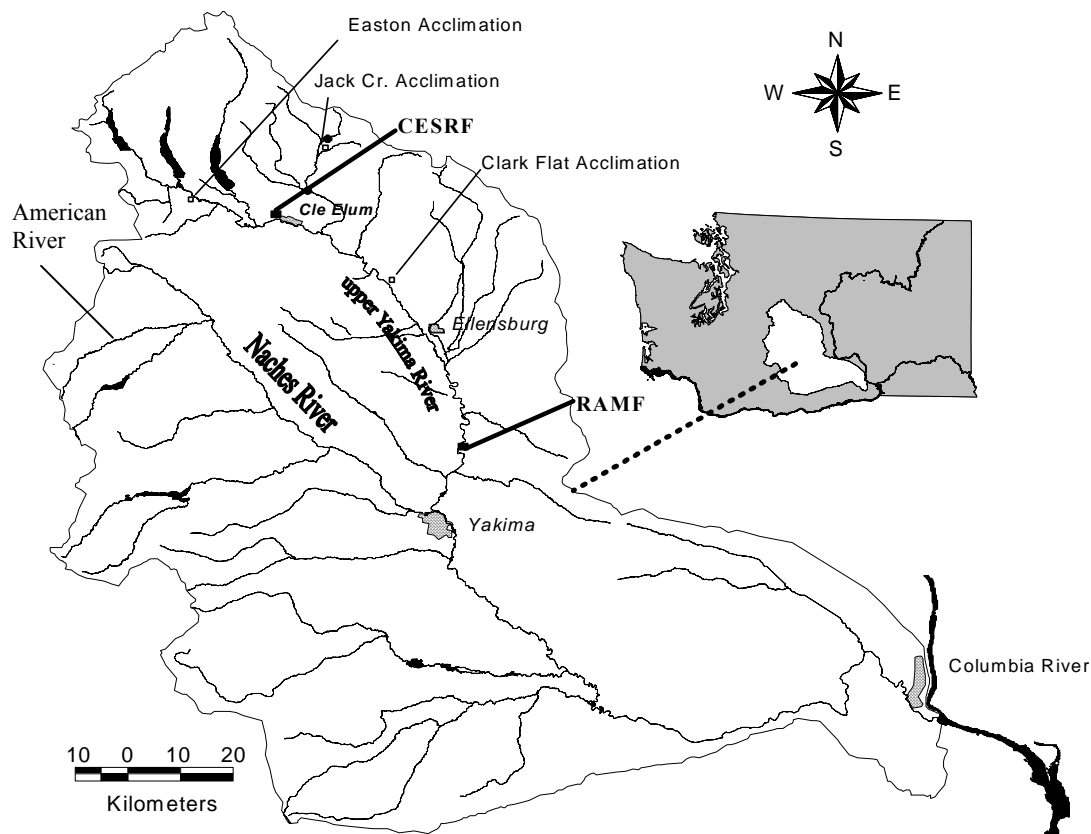
Figure 1. Yakima River basin showing the upper Yakima River, Roza Adult Monitoring Facility (RAMF), the Cle Elum Supplementation Research Facility (CESRF), acclimation sites, Naches River and American River.

The Yakima/Klickitat Fishery Project (YKFP) began operation of the CESRF spring Chinook hatchery near Cle Elum on the upper Yakima (rkm 290; Figure 1) in 1997. Broodstock are collected at RAMF, located adjacent to and upstream of Roza Dam, as spring Chinook pass upstream between April and September (Knudsen at al. 2006). Between 1997 and 2001, broodstock were exclusively of wild origin. Beginning in 2002, we established a Hatchery Control (HC) line founded exclusively by first generation hatchery origin returns in broodyears 2002, 2003, 2004, and 2005 (see Busack et al. (2004) for a detailed description). Beginning in 2006, with the return of our first adult HC age 4 adults, we began taking HC origin fish exclusively for HC broodstock. The 2006 age 4 and 5 natural origin adults were produced at least in part from the first generation of hatchery origin adults spawning in 2001 and 2002. The progeny produced from those naturally spawning hatchery and wild adults can no longer be considered

Table 1. Chronology of development of hatchery ancestry in natural-origin upper Yakima spring Chinook through first three generations of integrated hatchery operation. Calendar years of return for each brood year from 2000 to 2013 are shown. Entries denote age of returns.

		First generation				Second generation				Third generation			
		Initiation of hatchery operations and broodstock collection				Hatchery fish begin returning to spawn naturally				First returns of natural-origin fish produced by naturally spawning hatchery fish			
Broodyear→		1997	1998	1999	2000ᵃ	2001	2002	2003	2004	2005	2006	2007	2008
	2000	3											
Time	2001	4	3										
↓	2002	5	4	3									
	2003		5	4	3								
	2004			5	4	3							
	2005				5	4	3						
	2006					5	4	3					
	2007						5	4	3				
	2008							5	4	3			
	2009								5	4	3		
	2010									5	4	3	
	2011										5	4	3
	2012											5	4
	2013												5

ᵃ Some small contribution from age 3 hatchery adults spawning in 2000 is possible (see text).

purely wild in origin (Table 1), as it is highly likely that hatchery origin adults successfully spawned, and thus we are calling all natural production after broodyear 2000 natural Origin (NO) recruits.

HC and NO broodstock are transferred to CESRF and held together in one concrete raceway under the same water temperature, flow and rearing densities, until mature. Details of the broodstock collection process are given in Knudsen et al. (in press). Briefly, a fixed proportion of the total broodstock is collected each week over the entire run based on weekly mean historical passage proportions at RAMF. Broodstock collection is limited to no more than 50% of the NO population passing during any week and all returning HC adults are collected at RAMF and either used as broodstock, as experimental subjects, or contribute toward the YN tribal subsistence fishery. After reaching maturity, HC and NO fish were spawned separately in either 3x3 or 2x2 factorial matings in order to increase effective population size (Fiumera et al. 2004; Dupont-Nivet et al. 2006; Busack submitted) and maintain genetic diversity.

All returning fish passing through RAMF can be enumerated and sampled, if desired. All hatchery origin fish are sampled and NOR fish are sampled serially at a rate of between 1-in-4 to 1-in-10, depending on preseason run forecasts. To make possible collection and identification of broodstock origin, as well as other post-release monitoring, all hatchery releases are adipose fin clipped and tagged. A subset of 40,000

fish is PIT tagged and the remaining production is marked with a combination of colored elastomer in the adipose eyelid and a coded-wire tag in a specific body site. This allows identification of HC fish using a handheld CWT detector (to identify body tag location) and visual identification of the elastomer color.

Age Composition

Estimates of age composition for hatchery and natural origin fish returning in 2006 were made. The most appropriate way to calculate and compare age and sex composition is on a broodyear basis, rather than return year, in order to avoid the problem of unequal cohort strengths overwhelming within-cohort trends. However, in this annual report we do not make comprehensive comparisons across broodyears. Statistical analyses on a broodyear basis will occur in future reports.

Age composition of age 4 and 5 natural (NO n=485) and hatchery (HC n=134; SH n=447) origin adults was estimated from fish collected at RAMF. This includes all fish selected for broodstock and other experimental needs. All age 4 and 5 NO fish collected at RAMF were taken to CESRF. All HC fish (age 3, 4 and 5) were removed at RAMF and taken to CESRF. A subsample of SH fish were also taken to CESRF. Most SH origin adults sampled at RAMF were released upstream of RAMF and allowed to naturally spawn. Details of the sampling and broodstock selection methodology used at RAMF are given in Knudsen et al. (2006).

On a daily basis all hatchery fish passing RAMF were enumerated, anesthetized and examined for marks, classified as either an age 3 jack or an older adult (age 4 or 5) based on body size, and systematically scale sampled (~1-in-6 hatchery fish over the run). Also, 75 additional hatchery fish collected for broodstock and other experimental purposes were scale sampled. All scale sampled fish were measured for post-orbital hypural plate (POHP) length, fork length, body weight, and passage date recorded. Fish were held briefly to recover from the anesthetic and released back into the river to complete their spawning migration. Hatchery origin age composition of age 4 and 5 adults was estimated from the RAMF systematic sample of scales. Two scale analysts independently aged all scales and resolved disagreements. Ages were designated as the number of years from the year of conception (broodyear) to return year. Thus, a fish produced from parents spawning in the fall of 2000 and returning in 2005 was age 5. Under this convention, precocious males (nonanadromous males maturing in their first [natural only] or second [natural and hatchery] year) are designated age 1 and age 2, respectively (see Larsen et al. (2004) and Pearsons et al. (2004) for a full description of natural and hatchery precocious male production in the upper Yakima River). Returning spring Chinook in the Yakima River are greater than 99% yearling outmigrants based on adult return scales (J. Sneva, WDFW, personal communication).

Natural and hatchery origin age 3 jack returns are identified visually based on the significant body size differences between age 3 and age 4 fish and the presence or absence of an adipose fin as fish pass RAMF. The daily passage numbers of age 3 jacks and age 4 and 5 adults combined at RAMF were used to represent run timing.

Size-at-Age

The NO, SH and HC length and weight samples were measured at RAMF. Sex specific data were collected from fish that were measured at CESRF as broodstock and

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for other experimental purposes were sex could be confirmed by visually examining gametes. Length and weight data collected at RAMF, prior to fish reaching full maturity, were used to compare hatchery and natural size-at-age distributions using a one-way ANOVA (Type effect). RAMF body weights are significantly heavier than body weights of the same fish at full maturity 1 to 5 months later (Knudsen et al. 2004), and this should be kept in mind when making comparisons between RAMF and other data from mature spawners. When there was a significant Type effect, a Tukey Multiple Comparisons Test (MCT) was used to estimate which Types differed.

Beginning with BY 2002 and continuing through BY 2005, a precocious male minimization study was implemented at CESRF. This study manipulated juvenile growth trajectories in order to reduce the rate of precocious male production. Half of the raceways were placed on a High growth treatment and half on a Low growth treatment resulting in different juvenile size-at-release (Figure 2). Age 4 (High growth n= 286; Low growth n= 207) from broodyear 2002 were recovered in 2006. We compared the lengths and weights of fish from the High and Low treatment groups by Acclimation site to determine whether the size differences at release persisted after 18 months of post-release growth using a 2-way ANOVA estimating Aclimation (Easton, Clark Flats, Jack Creek), Treatment (High, Low), and interaction effects. The sample sizes for age 3 cells were as low as 1 fish, and no age 5 High or Low treatments from BY2001 were released. Thus it was not possible to estimate Acclimation and Treatment effects for these ages.

Size Over Time

We examined body size over time (return years) to determine whether there were any trends in size over time for upper Yakima NO, hatchery origin (HC and SH combined based on results above), and the Naches wild population using ANCOVA. Significant declines in the body size of NO adults over time could be an indication that the supplementation project is negatively effecting the naturally spawning population. The Naches wild population serves as a wild control to the upper Yakima River and is a useful benchmark that is independent of the supplementation project. We used age 4 adults because they were most abundant across the populations compared and restricted the analysis to means based on 10 or more samples.
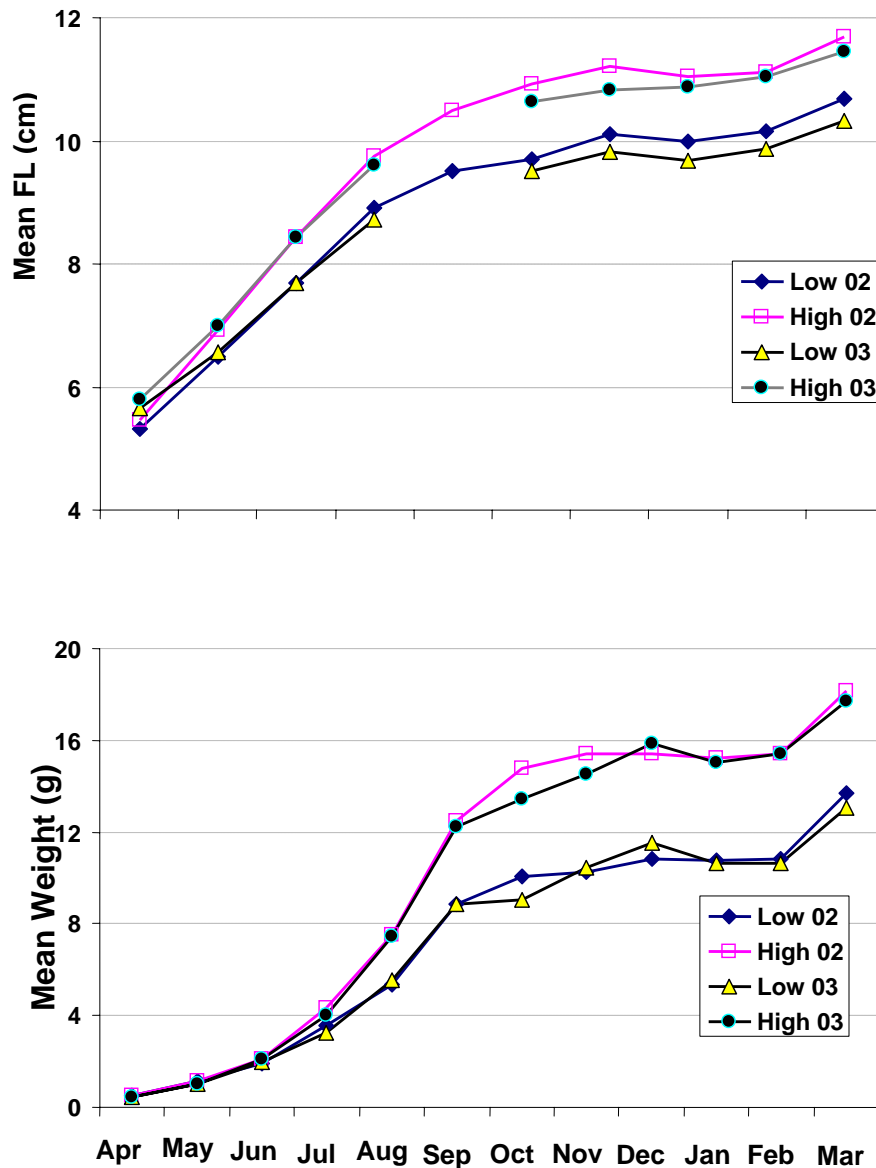
Figure 2. Growth in fork length (FL) and body weight (Weight)
during rearing of 2002 and 2003 broodyear juveniles from the High
and Low growth treatment groups.

Passage and Spawn Timing

Passage timing distributions of hatchery and natural origin fish at RAMF were
compared using a Kruskal-Wallis non-parametric ANOVA (KW test; Zar 1999) because
of the highly skewed temporal distributions. Because of significant differences in
passage timing of jacks (age 3) and adults (age 4 and 5) (Knudsen et al. 2006), we made
passage timing comparisons between origins within adults and jacks.

Artificial spawning occurs at CESRF over a five-to-six week period from early
September through early October. HC (n=70) and NO (n=364) spawn timing

distributions were compared with a t-test testing for an Origin (HC vs NO) effect. Spawn timing distributions are not skewed like RAMF passage timing distributions (Knudsen et al. 2006) and therefore could be analyzed with a parametric test. All passage and spawning dates were converted to ordinal dates (day-of-year) prior to analysis.

Results

Age Composition

The over 80% of HC, SH, and NO fish returned at age 4 (Table 2). Age 5's were represented by 1 and 10% of SH and NO adults, respectively. The increasing proportion of hatchery origin age 3 returns compared to the relatively stable proportion of NO age 3 adults noted by Knudsen et al. (2006) was not detected in 2006, although Knudsen et al.'s analyses were based on broodyears, rather than return year.

Table 2. Age composition of 2006 upper Yakima River natural and hatchery origin spring Chinook based on body size at RAMF (Age 3 vs Ages 4 and 5) and scales ages (Age 4 vs Age 5). Scale age sample sizes are in parentheses.

Origin	Age 3[a]	Age 4	Age 5
HC	5.6	94.4 (134)	NA[b]
SH	14.2	85.2 (444)	0.6 (3)
NO	7.2	82.7 (432)	10.1 (53)

[a] Jack percentages are based on visual counts as fish pass RAMF. Other age class percentages are then adjusted to account for the jack component.
[b] The first year of age 5 HC returns is 2007.

Table 3. Mean Postorbital-Hypural Plate (POHP) lengths (cm) and Body Weight (BW; kg), and sample sizes (n) of Hatchery Control, Supplementation (SH) and Natural Origin (NO) returns in 2006. Measurements were collected at RAMF and males and females are combined. Standard deviations are in parentheses.

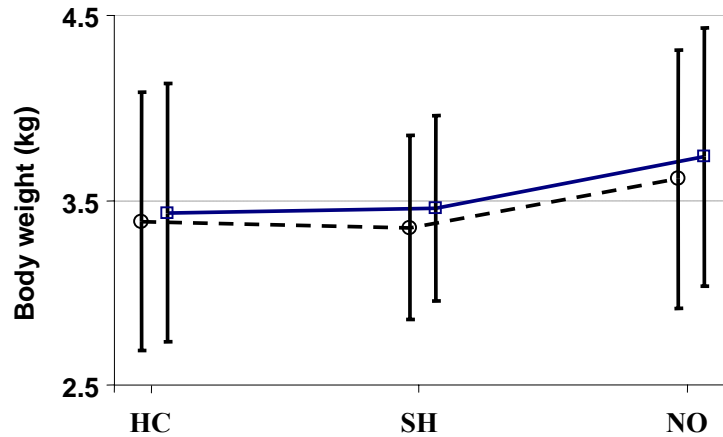| Age	Origin	POHP (sd)	BW (sd)	n
	HC	42.6 (3.9)	1.5 (0.4)	8
3	SH	40.4 (3.7)	1.3 (0.3)	27
	NO	41.1 (2.8)	1.4 (0.3)	32
	HC	57.7 (4.1)	3.5 (0.8)	134
4	SH	57.4 (4.2)	3.4 (0.7)	444
	NO	58.7 (4.2)	3.7 (0.7)	430
	HC			NA
5	SH	71.0 (4.6)	6.8 (1.3)	3
	NO	71.1 (4.0)	6.4 (1.4)	57

A)
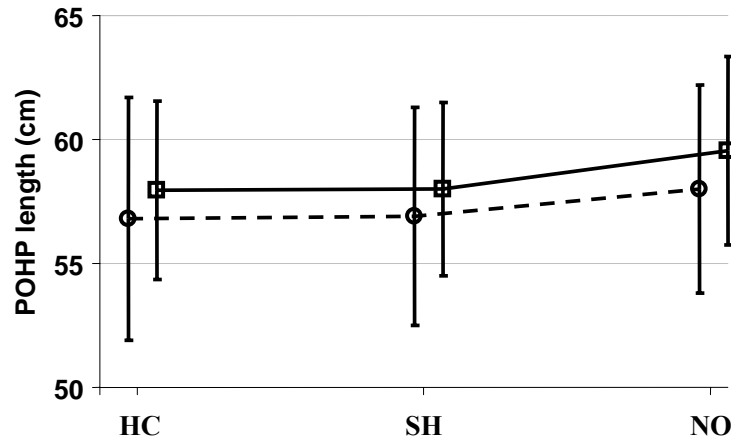
B)

Figure 3. Age 4 female (□; solid line) and male (○; dashed line) mean A) POHP length and B) Body weight ($\pm$ 1 sd) in 2006 for Hatchery Control (HC), Supplementation Hatchery (SH), and Natural Origin (NO) upper Yakima River spring chinook collected at RAMF.

Size-at-age

Mean POHP lengths and body weights of HC, SH and NO returns by age are given in Table 3 along with sample sizes and standard deviations. On average, SH age 3 fish were 0.7 cm and 0.1 kg smaller than NO age 3 fish. However, HC age 3 fish were larger than either SH or NO fish and there was no significant difference between the 3 Types (all Tukey MCT p>0.20). Only 8 fish were collected from the HC population unfortunately, and this small sample may not represent it well and also results in low statistical power. NO age 4 and age 5 adult body sizes were greater on average than either HC or SH body sizes (1.0 to 1.3 cm POHP length and 0.2 to 0.3 kg body weight).

One-way ANOVA (Type effects) of POHP and body weight distributions showed that age 4 HC, SH, and NO differed significantly (Type $p \leq 0.001$). NO fish were significantly

Table 4. Age 4 male and female body size (POHP length and body weight) means, standard deviations, and samples sizes by Type.

Type	Sex	POHP lengths (cm)		Body weight (kg)		n
		Mean	sd	mean	sd	
HC	Female	57.9	(3.6)	3.4	(0.7)	45
	Male	56.8	(4.9)	3.4	(0.9)	41
SH	Female	58.0	(3.5)	3.5	(0.5)	52
	Male	56.9	(4.4)	3.4	(0.7)	33
NO	Female	59.6	(3.8)	3.7	(0.7)	241
	Male	58.0	(4.2)	3.6	(0.8)	141

larger than both HC and SH fish (Tukey MCT $p<0.05$), while HC and SH means did not differ significantly (Tukey MCT p>0.72).

Between 2001 and 2005 we found no evidence of sexual dimorphism in age 4 body size. In marked contrast, in 2006 we found significant sexual dimorphism in age 4 adults. Age 4 females of NO, SH and HC origin were significantly larger (Sex effect p=0.003) than males by 1.1 to 1.6 cm (Table 4; Figure 3). Females of NO and SH origin were also heavier, but were not significantly larger (Sex effect p=0.236). We were not able to make comparable tests for either age 3 or 5 returns because of small sample sizes for some age/sex cells.

We found that by the time age 4 adults returns from BY 2002 arrived at RAMF the Low and High growth treatment differences in juvenile length- and weight-at-release shown in Figure 2 no longer exist (Table 5). In no case was there a significant Treatment or Acclimation site effect, nor were there significant interactions. Knudsen et al. (2006) found that age 3 returns from BY2002 also demonstrated no significant Treatment effect, but did find significant Treatment effects in age 2 returns from both BY2002 and 2003. Age 2 precocious males were sampled approximately 6 to 8 months after release

Size Over Time

When we examined the trend in POHP length of age 4 fish returning in 2001, the first year of SH adult returns, to 2006 (Figure 4A) we found there was a significant decline in hatchery origin adults (-1.04 cm•year^{-1}, r^2=0.397, p=0.014,). We also found a decline in upper Yakima NO (-0.56 cm•year^{-1}, r^2=0.535, p=0.014), Naches wild control (-0.53 cm•year^{-1}, r^2=0.243, p=0.014), and American wild (-0.86 cm•year^{-1}, r^2=0.460, p=0.014) adults over the same time period. ANCOVA demonstrated that the populations all had statistically equivalent slopes (p=0.554). In contrast, over the period 1990 to 2000 there was no significant trend in body size of age 4 wild Naches (wild control), American River, or upper Yakima populations (regression p=0.204, Figure 4B).

Table 5. Two-way ANOVA results of POHP and Body weight distributions of age 4 High and Low treatment groups.

Source	SSq	df	Mean-Sq	F-ratio	P
POHP length					
Treatment	6.863	1	6.863	0.390	0.533
Acclimation site	37.178	2	18.589	1.056	0.349
Treatment x Acclimation	42.371	2	21.186	1.204	0.301
Error	8571.006	487	17.600		
Body weight					
Treatment	0.011	1	0.011	0.022	0.883
Acclimation site	0.347	2	0.174	0.337	0.714
Treatment x Acclimation	0.515	2	0.257	0.500	0.607
Error	250.984	487	0.515		

Passage and Spawn Timing

Age 3 jack passage at RAMF differed significantly from adult passage timing (KW test $p<0.0001$) with HC, SH and NO jack median dates being 14, 12, and 14 days later than age 4 and 5 adults, respectively (Table 6). For this reason, we compared passage timing by origin for adults and jacks separately. NO adults median passage date was significantly earlier than HC and SH adults by 6 and 2 days, respectively (KW test $p=0.010$). There was no significant difference between passage of HC, SH and NO jacks (KW test $p=0.468$).

A)   B)

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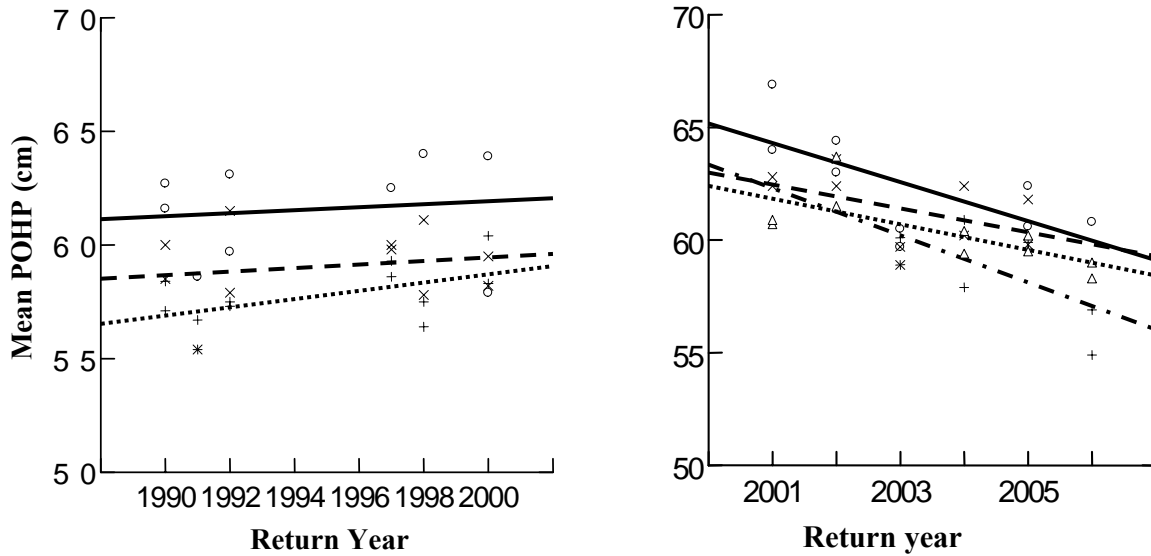
Figure 4. Mean annual POHP lengths of American (o, solid line), Naches wild control (x, dashed line), upper Yakima natural (Δ, dotted line), and upper Yakima hatchery (+, dash-dot line) origin from A) 1990 to 2000 and B) 2001 to 2006.

Table 6. Median 2006 passage timing at RAMF by Life history Type: Jack (age 3) or Adult (ages 4 and 5 combined). Sample sizes (n) are total Adult and Jack run sizes passing RAMF.

Origin	Type	Median	n
Natural Origin	Jack	179.0	31
	Adult	165.0	446
Hatchery Control	Jack	185.0	7
	Adult	171.0	122
Supplementation	Jack	179.0	20
	Adult	167.0	433

In 2006, HC fish spawned 7 days earlier (Sept. 14; Table 7) and were significantly earlier (t-test, p<0.0001) from NO fish (Sept. 21). Since 2001, hatchery origin fish have consistently spawned earlier than natural origin fish by 5 to 7 days.

Table 7. Mean 2006 spawning date at CESRF. Sample sizes (n) are the total number of fish spawned.

Origin	Ordinal date	sd	n
Natural Origin	265 (Sept. 21)	7.8	364
Supplementation	258 (Sept. 14)	6.9	70

Discussion

Hatchery origin returns in 2006 demonstrated many of the same trends relative to natural origin returns noted by Knudsen et al. (2006) between 2001 and 2004. Knudsen et al. (2006) found that most upper Yakima hatchery and natural origin fish reached maturity at age 4 ($\geq$76%) followed in magnitude by ages 3 and 5. However, hatchery mean age-at-maturation was declining over time due to an increasing proportion of age 3 returns, while wild origin mean age-at-maturation was stable. The significance of the relatively low proportion of age 3 males observed in 2006 (based on return year numbers) is not clear at this time. We will not know whether this is due to an actual reduction in the proportion of males adopting this life history strategy within the BY 2003 cohort or simply a harbinger of a poor return from all age classes in that cohort, until the data are analyzed on a broodyear basis. This requires analyzing age 4 and 5 returns in 2007 and 2008, respectively. However, the upper Yakima River run forecast is that the 2007 return will be lower than expected (B. Bosch, personnel communication).

Mean lengths and weights of age 4 HC and SH fish were significantly lower than those of NO fish and represented a difference in body size of approximately 0.5 SD. This is similar to body size differences observed by Knudsen et al. (2006) in SH returns between 2001 and 2004.

The two most striking observations we noted were that upper Yakima River age 4 males and females demonstrated sexual dimorphism for the first time in our analyses, and that there was a significant decline in body size across all populations beginning in 2001.

Median arrival timing of hatchery (SH and HC combined) and NO fish at RAMF showed no consistent difference. However, median arrival date of age 3 fish, regardless of Type, was 19-20 days later than for ages 4 and 5.

Mean spawn timing of hatchery fish was significantly earlier than wild fish by an average of 5.1 days in a "common garden" experiment.

These analyses examined traits of the hatchery and natural origin adult spring Chinook salmon returning to the upper Yakima River in 2006. This information will be used in a more comprehensive future report comparing hatchery and natural origin spring Chinook salmon in order to estimate whether the trends observed in first generation hatchery returns continue throughout the second generation. Detailed analyses of broodyear specific comparisons and trends will be finished after the completion of the second generation of hatchery returns in 2008.

Acknowledgements

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Chapter Two

Comparison of Gametic Traits of Second Generation

Hatchery- and Natural-Origin Upper

Yakima River Spring Chinook Salmon

Curtis M. Knudsen[1], Steve L. Schroder[2], Mark V. Johnston[3],

Todd N. Pearsons[2], and Charles R. Strom[4]

[1]Oncorh Consulting, 2623 Galloway SE, Olympia, WA 98501

[2] Washington Department of Fish and Wildlife, 600 Capitol Way North, Olympia, WA 98501-1091

[3] Yakama Nation, P.O. Box 151, Toppenish, WA 98948

[4] Yakama Nation, Cle Elum Supplementation and Research Facility, 800 Spring Chinook Way, Cle Elum, WA 98922

Abstract

Reproductive traits in second generation hatchery control (HC) and natural origin (NO) spring Chinook females from the upper Yakima River were compared to determine whether fitness related traits had diverged after two generations of artificial propagation. We also compared body size and survival of progeny from single-pair matings of HC-by-HC and NO-by-NO adults. Because fecundity (FEC), relative fecundity (RELFEC), egg mass (EM), and total gamete mass (TGM) are significantly correlated with female length, it was necessary to use length as a covariate in comparisons. In general, the fundamental body size vs gametic trait relationships of age 4 HC and NO females were not significantly different and means adjusted for body size did not differ. Due to significantly smaller HC mean body size of 2006 adults which is correlated with the reproductive traits, returning HC females would on average have lower reproductive trait values than NO females. Fry characteristics produced from HCxHC and NOxNO matings did not differ significantly in survival, body size or KD values.

Introduction

   Washington state has practiced artificial propagation of salmon and steelhead for over a century and during this period significant advances have been made in fish culture technology resulting in hatchery spawner/recruit rates that can considerably exceed replacement rates. The importance of hatchery operations has increased because of continuing losses of natural production from over-harvest, habitat degradation, and disappearance of spawning habitat due to hydroelectric development, irrigation, logging and transportation (Lichatowich 1999). However, artificial production's affects on native populations is not well understood (Goodman 2005). Use of integrated hatchery programs in the Columbia River basin has recently increased (Goodman 2004) making the issue of deliberate interbreeding of hatchery and natural origin fish even more significant (Goodman 2005; Mobrand et al. 2005). The demographic risks of integrated programs have been recognized (Hard 1995; Goodman 2004; Mobrand et al. 2005) and aspects of the genetic risks of integrated programs have been modeled (Lynch and O'Hely 2001; Ford 2002; Goodman 2005). In a recent review (Berejikian and Ford 2004) compared the fitness of natural and hatchery origin fish. Seventeen of the 18 studies reviewed examined the effects of intentional selection, multiple generation effects, use of non-local broodstock, or combinations of these factors. However, empirical assessments of integrated programs are just beginning (Busack et al. in press; Knudsen et al. 2006).

   Life-history traits, particularly those directly associated with reproduction, reflect local adaptations affecting fitness (Stearns 1976; Roff 1992). Relaxation of natural selection for larger eggs combined with domestication selection for greater fecundity was suggested as the reason egg size declined in a Chinook salmon captive breeding program (Heath et al. 2003; however see Beacham 2003 and Fleming et al. 2003). Such traits as egg size, reproductive effort (biomass of gametes relative to total body biomass) and fecundity are maternal traits. However, they also have direct consequences for progeny affecting yolk reserves and fry body size (Einum et al. 2004). Other maternal traits also have direct consequences for progeny. Where a females chooses to construct her redd will determine the quality of the incubation substrate as well as early fry rearing habitat. When a female spawns will significantly affect emergence timing and thus the state of her progeny's early rearing environment. If a female spawns too early, food will be scarce, although density and competition will likely be low. Spawning too late, when productivity is higher, also results in greater competition with earlier emerging, larger fry. Life history theory suggests that natural selection will maximize female fitness. Assuming there is some maximum to the resources a female can devote to gametes (either space or biomass), there must be a trade-off between egg size and egg number. An increase (decrease) in egg size results in a decrease (increase) in egg number. In general, significant changes in locally adapted traits will be maladaptive in the wild (Lynch and O'Hely 2001; Ford 2002), and can result in reduced individual fitness (Taylor 1991; Fleming and Gross 1993; Fleming et al. 2000). Monitoring hatchery populations to determine if they are diverging from their native population's life-history trait distributions is a necessary part of a hatchery monitoring plan (Hard 1995; Goodman 2005). Significant differences may indicate that the artificial rearing environment is causing genetic divergence to occur between the two groups. However, trait differences may be due to phenotypic plasticity and are not sufficient to conclude that genotypic

divergence has occurred. On the other hand, a lack of differences may be heavily influenced by the proportion of hatchery fish naturally spawning and consequently shifting the trait distributions naturally produced fish toward the hatchery origin distributions. To determine clearly whether there is a genetic effect, fish from both groups should be spawned, incubated, and reared in a common environment. Under these conditions observed trait differences represent genetic change.

To evaluate the risks and benefits posed by integrated programs, appropriate demographic and genetic data need to be collected (Hard 1995), preferably from the beginning of a program. Assuming native broodstock were used, this permits documenting whether first generation hatchery fish diverge from their founder population prior to hatchery introgression. After progeny of first generation hatchery fish begin naturally spawning with wild origin fish, their progeny may possess characteristics that are intermediate between those of progeny of pure wild and hatchery origin individuals. Accordingly, when naturally produced individuals of mixed hatchery and wild ancestry are compared to second generation hatchery fish, their genetically based trait distributions will differ less than between pure wild and first generation hatchery individuals.

In 1997 an integrated hatchery program was begun to supplement the Upper Yakima spring Chinook population (Fast and Craig 1997). The program uses only natural origin (NO) fish as broodstock and the proportion of hatchery origin fish on the spawning grounds is not controlled. Beginning in 2002 a hatchery control (HC) population was initiated at the Cle Elum Supplementation Research Facility (CESRF) and currently represents 11% of total CESRF production (2 of the 18 raceways). Initial HC broodstock were taken from first-generation hatchery returns beginning in broodyear 2002. For the next 3 years, first-generation hatchery origin adults were used to found the subsequent HC brood lines. The first HC age 4 returns occurred in 2006 and from that year forward HC broodstock are taken from only HC adult returns. In addition, no HC returns are allowed to spawn naturally. Thus, the HC line is completely separate from natural production and subjected to continuous hatchery propagation resulting over time in multiple generations of domesticating effects. Isolation and removal of HC returns from the naturally spawning population is accomplished by marking them uniquely as juveniles and collecting them as maturing adult returns at an upstream adult trapping weir adjacent to Roza Adult Monitoring Facility (RAMF) which monitors 100% of upstream passage.

In this report we analyze data collected from 2006 adults and compare NO and second-generation HC female's mean egg mass, total gamete mass, reproductive effort, fecundity, and relative fecundity distributions. Then, extending the comparisons into the next generation, we compare the survival and body size of fry produced from single-pair *inter se* (HC-by-HC or NO-by-NO) matings

Methods

Upper Yakima River spring Chinook

The Yakima River is a major tributary to the Columbia River located in south central Washington state (Figure 1). The upper Yakima River supports a genetically distinct (Busack and Marshall 1991), naturally sustaining population of 'stream type' spring Chinook (Healey 1991). After rearing for 1 to 3 years in the North Pacific Ocean,

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adults migrate upstream into the Yakima River basin in the spring and spawn in the early fall, and juveniles spend a full year in freshwater before migrating to the ocean. Some males mature precociously in freshwater during their first or second year (Larsen et al. 2004; Pearsons et al. 2004).
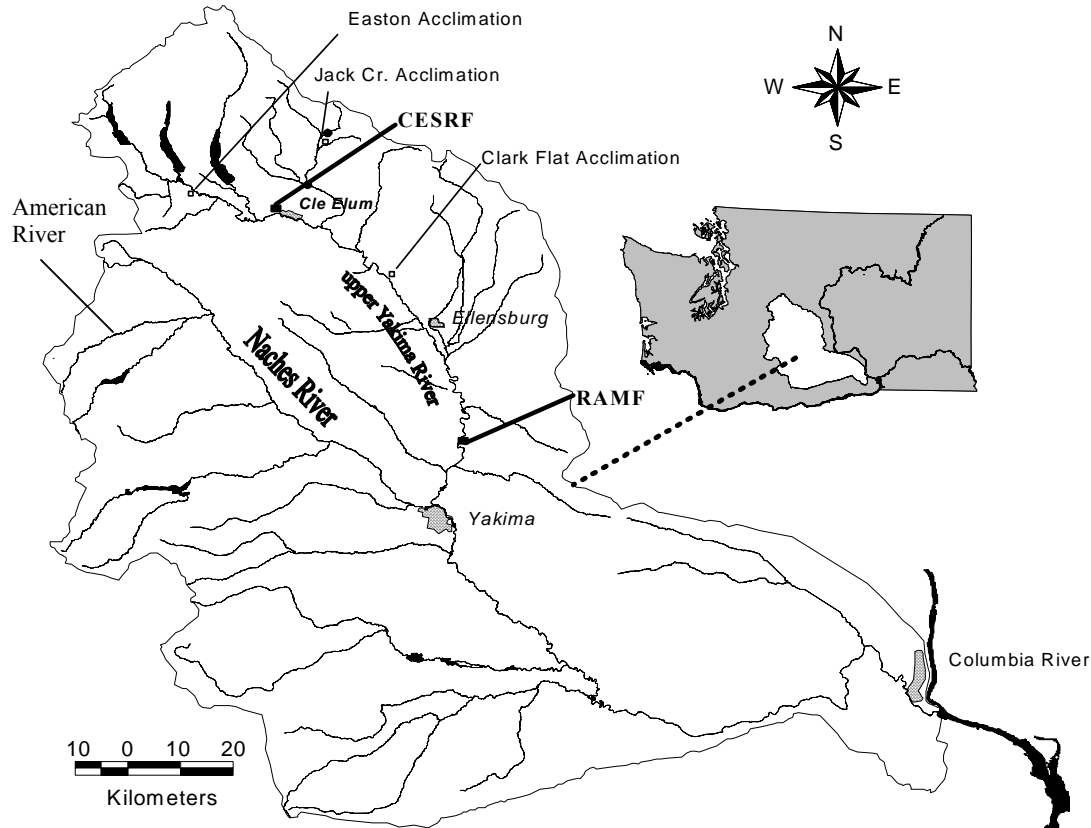

Figure 2. Yakima River basin showing the upper Yakima River, Roza Adult Monitoring Facility (RAMF), the Cle Elum Supplementation Research Facility (CESRF), acclimation sites, Naches River and American River.

As integrated programs proceed beyond the first generation, it is inappropriate to call fish resulting from natural spawning "wild", because they may be the progeny of naturally spawning hatchery fish. Thus, these fish are more appropriately called "natural origin" fish because they were produced from broodyears 2001 through 2003 when between 59 and 75% of natural spawners were of hatchery ancestry (Table 1).

Hatchery spring Chinook in this study were progeny of adults collected as they passed upstream through Roza Adult Monitoring facility (RAMF). The adults were transported via tanker truck to CESRF where they were held for 1 to 5 months until fully mature. For a full description of the collection of adults at RAMF see Knudsen et al. (2006). We were able to use a "common garden" experiment to test for differences between hatchery and wild origin gametic traits because all adults were held together in a single concrete raceway at CESRF. Beginning in early September and continuing into early October adults were checked for ripeness and spawned weekly. Ripe females were

Table 1. Chronology of development of hatchery ancestry in natural-origin upper Yakima spring Chinook through first three generations of integrated hatchery operation. Calendar years of return for each brood year from 2000 to 2013 are shown. Entries denote age of returns.

		First generation				Second generation				Third generation			
		Initiation of hatchery operations and broodstock collection				Hatchery fish begin returning to spawn naturally				First returns of natural-origin fish produced by naturally spawning hatchery fish			
	Broodyear→	1997	1998	1999	2000ᵃ	2001	2002	2003	2004	2005	2006	2007	2008
	2000	3											
Time	2001	4	3										
↓	2002	5	4	3									
	2003		5	4	3								
	2004			5	4	3							
	2005				5	4	3						
	2006					5	4	3					
	2007						5	4	3				
	2008							5	4	3			
	2009								5	4	3		
	2010									5	4	3	
	2011										5	4	3
	2012											5	4
	2013												5

ᵃ Some small contribution from age 3 hatchery adults spawning in 2000 is possible (see text).

identified when eggs were extruded with gentle manual pressure or by the firmness of the ventral surface. A soft ventral surface that sagged slightly when the head was pointed head down indicated a female was ripe.

After reaching maturity, HC and NO female broodstock were sampled for the following gametic traits as they were spawned: total gamete mass (TGM), mean egg mass (EM), reproductive effort (RE), fecundity (FEC), and relative fecundity (RELFEC). In addition, the following traits were monitored post-fertilization: survival to the eyed-egg stage and fry body length and weight. Descriptions of the data collection processes are given below.

Only age 4 females were analyzed because they were the first adult female age class to return from the initial HC juveniles produced from adults spawned in 2002.

Total gamete mass (TGM), Egg mass (EM), and Fecundity (FEC)

The weight of the TGM and average EM were measured as females were artificially spawned at CESRF. Details of the methods are described in Knudsen et al. (2006b). The gravimetric estimates of fecundity were biased by small amounts of residual ovarian fluid remaining within the egg mass. We corrected the bias using a

factor based on comparisons of hand counts and gravimetric estimates of fecundity (n= 110 females). The correction factor, 0.9447, was applied to all gravimetric fecundity estimates used in the analyses below.

Reproductive effort (RE)

Reproductive Effort (RE), also called gonadal-somatic index, was calculated by dividing the TGW by the fish's total body weight (including gametes) and represents the proportion of body mass allocated to gamete production.   Because RE is a dimensionless ratio of two weight measures, an arc sin square root transformation (Zar 1999) was used to normalize its distribution during analyses, but we report the values as untransformed ratios in the text.

A few females had a significant proportion of unripe, overripe, or injured eggs. We assumed these occurred because either females were selected for spawning too early or too late or the eggs were injured during handling, transfer and holding. In addition, during the latter weeks of the spawning season a few eggs were observed on the bottom of the adult holding raceway indicating that some females had released gametes before being selected for spawning. For these reasons, we excluded TGM, FEC and RE values of females with RE values below 0.12 (20 out of 847 wild and 5 out of 189 hatchery females). An RE value of 0.12 was 2-3 standard deviations from the mean RE value in each broodyear.

Relative fecundity (RELFEC)

Relative fecundity (RELFEC) was calculated by dividing a female's total fecundity (FEC) by either her POHP length or body weight and represents the number of eggs produced per unit change in body size (eggs·cm^{-1} or eggs·kg^{-1}).

Factorial mating protocols and egg incubation

The fish used in the matings for survival and post-fertilization analyses were spawned in a series of factorial crosses typically made up of 3 females and 3 males, resulting in 9 single pair matings. However, there were cases where other combinations such as 2x2 or 2x3 crosses were used. In general, three aliquots of between 150 and 250 eggs per female were collected and placed into a dry 1 *L* beaker with approximately 0.2 *cc* of milt (3 drops from a 5 cc syringe) from the respective male in the single-pair mating. The gametes were then activated by adding approximately 200 *ml* of ambient well water, initiating fertilization. After a minimum of 2 minutes from activation, the eggs from each single-pair mating were drained, placed into individual incubation containers called isolettes, and held in an Iodiphore bath for 45 minutes. Each isolette was labeled with the female and male's origin and individual identification numbers. The eggs from each female were then incubated to the eyed egg stage, shocked, and mortalities enumerated and removed. The remaining eggs were incubated to the post-hatching yolk absorption or "button up" stage. Any additional mortality was then noted.

Fork length and body weight were measured on five fry from one single-pair mating from each female. Fry were anesthetized and blotted dry prior to being weighed. Because we collected fry size data from only one *inter se* single-pair mating per female, we could not estimate male effects on fry body size. However, we were monitoring fry size at the "button up" stage, when maternal effects, particularly those due to egg size,

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should overwhelm paternal effects (Iwamoto et al. 1984; Heath et al. 1999). Wild and Hatchery origin fry body size distributions were compared by ANCOVA using egg mass as a covariate.

The amount of yolk reserves a juvenile possess at emergence can affect its survival in two opposing ways. First, yolk material can serve as an important food reserve as an individual transitions from an endogenously feeding fish to one that must rely on external prey. Second, yolk materials may also make an individual conspicuous, reduce its swimming speed, and therefore increase the risk that a predator will consume it (Fresh and Schroder 1987). Therefore, the amount of yolk material a fish has at emergence is likely a compromise between these two competing selection pressures. Under hatchery conditions these pressures will be relaxed and it is uncertain how KD will respond. If it changes in either direction negative survival consequences could occur when fish incubate and emerge under natural conditions.

Knudsen et al. (2006) showed that first generation CESRF hatchery spring Chinook salmon adults were significantly smaller than wild adults. Chinook salmon body size has been shown to be positively correlated with fecundity and egg size (Beacham and Murray. 1993; Healey and Heard 1984; Knudsen et al. 2006b), and total egg mass (Kinnison et al. 2001; Knudsen et al. 2006b) and negatively correlated with relative fecundity (Heath et al. 2003; Knudsen et al. 2006b).

Our general procedure for comparing traits began by comparing slopes of the POHP vs Trait regression. If the slopes were equivalent, we compared HC and NO types to determine whether they had equal adjusted means. That is, did they have the same fundamental relationships between body size and trait distributions or had exposure to hatchery culture altered this relationship somehow. If the equal-slope assumption was not accepted we used a t-test to compare the traits. However, in the past broodstock have differed significantly from the general population in some traits due to sampling and random error.

Statistical tests were considered significant when p-values were less than or equal to 0.05. All analyses were executed using SYSTAT version 11 software (SYSTAT 2004).

Results

In all traits but TGM, the POHP vs gametic trait slopes were equal (all $p \geq 0.22$) confirming the necessary assumption for ANCOVA. In the ANCOVA the adjusted means of HC and NO females were not significantly different for fecundity, egg mass, reproductive effort, and relative fecundity ($p \geq 0.39$). Thus, for fecundity, egg mass, reproductive effort, and relative fecundity the regression lines of HC and NO females are equivalent.
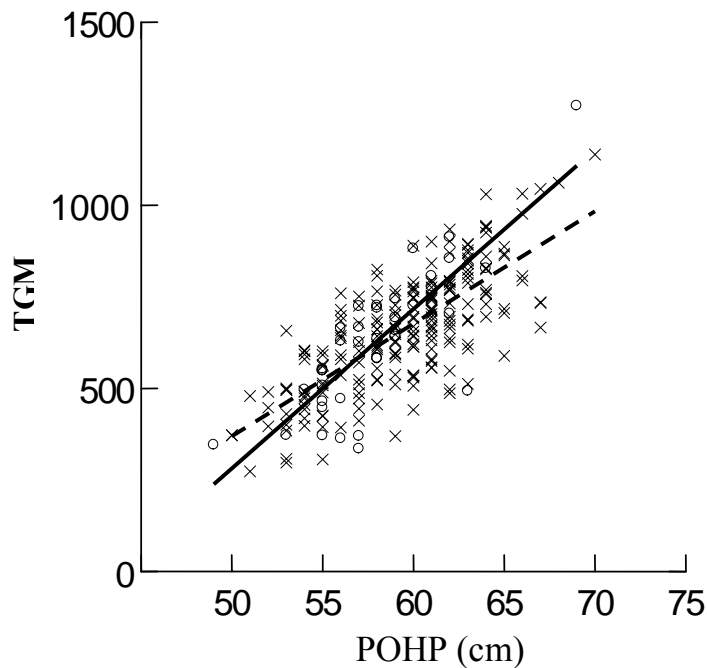
Figure 2. Total gamete mass (TGM) vs POHP length for HC (○; dashed line) and NO (x; solid line) females.

Total gamete mass (TGM)

For TGM, HC females had a significantly steeper POHP vs TGM slope than natural origin females (Figure 2; slopes are equal p=0.018). Because of the failure to accept the assumption of equal slopes, we used a t-test to compare the TGM distributions. Average TGM of HC females (625.1 g; n=39) was less than that of NO (659.6 g; n=208) age 4 females, although this was not significant (Table 2). As in previous years, TGM was positively correlated with POHP length (p<0.0001; r^2 =0.557; Figure 2) and body weight (p<0.0001; r^2 =0.782).

Table 2. Results of a t-test comparing Total gametic mass testing for Origin (HC vs NO) effects.

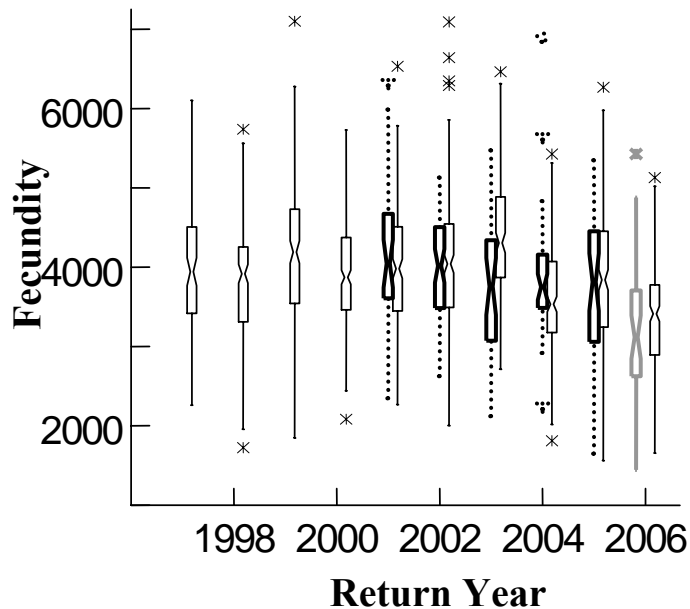| Trait	Source	Sum-of-Squares	df	Mean-Square	F-ratio	P-value
Total gamete	Origin	38194.5	1	38194.5	1.404	0.237
mass	Error	6637508.2	244	27202.9		

Figure 3. Mean fecundity (notch) and distribution of age 4 female fecundities from 1997 to 2006. Wild/Natural origin females are represented by thin solid black lines, Hatchery females by thick box dotted lines, and Hatchery Control females (only 2006) by thick solid gray lines and box.

Egg mass (EM)

Adjusted average egg mass of HC (187 mg; n=38) and NO (186 mg; n=208) age 4 females were essentially equal and there was no significant difference between EM distributions (p=0.369). EM was positively correlated with both POHP length (p<0.001; r^2 =0.399) and body weight (p<0.001; r^2 =0.501).

Fecundity (FEC)

After adjusting for POHP, mean FEC of HC (3,319.7 eggs; n=38) and natural (3,328.8 eggs; n=208) origin age 4 females were not significantly different (p=0.923). As in previous years, FEC was highly correlated with POHP length (p<0.0001; r^2 =0.430) and body weight (p<0.0001; r^2 =0.516). Mean fecundity in 2006 was the lowest observed since 1997 (Figure 3), reflecting the fact that age 4 body size in 2006 was also the smallest since 1990.

Female Reproductive effort (RE)

Adjusted mean RE of HC (0.198; n=38) and natural (0.197; n=208) origin age 4 females were statistically equal (p=0.950). In 2006, POHP length and body weight were significantly correlated with RE (p=0.020 and p<0.001, respectively); but only explained 2% and 6% of the total variation, respectively.

Relative fecundity (RELFEC)

   Mean RELFEC of HC (995.4 eggs*kg^{-1}; n=38) and NO (1,019.3 eggs*kg^{-1}; n=208) age 4 females were not significant different (p=0.390) after adjusting for POHP length. As in previous years, POHP length was negatively correlated with RELFEC (p=0.001), but explained very little of the total variation (r^2 =0.046).

   Results for body weight were similar to POHP. Mean RELFEC of HC (57.1 eggs*cm^{-1}; n=38) and NO (57.5 eggs*cm^{-1}; n=208) age 4 females were not significant different (p=0.435) after adjusting for POHP length. As in previous years, body weight was negatively correlated with RELFEC (p<0.0001), explaining 21% of the total variation.

Fry Survival

   We analyzed egg-to-fry survival distributions for Origin effects using a t-test. We had samples of from each of the 3 factorial crosses a female was used and there were a total of 27 HC and NO females. In 2006, NO fry (mean survival= 0.677) survived at approximately the same rate as HC fry (mean survival= 0.681, n=xx) (t-test p=0.849). This was the poorest in-hatchery survival since we began monitoring in 1997.

Fry Body Size

   We tested for Origin effects in fry body weight distributions by ANCOVA using egg mass as a covariate. HC fry (mean body weight = 0.300 g) were slightly smaller than NO fry (mean body weight 0.304 g) (Figure 4), but there was no significant Origin effect (p=0.209) indicating that, given eggs of the same weight, hatchery fry will have equivalent body mass as wild fry in 2006.

KD-value Comparisons

   Mean KD of HC (0.772; n=38) and NO (0.767; n=208) fry from were not significant different (p=0.665) after adjusting for EM differences.

Discussion

Body size, both POHP length and body weight, are significantly correlated with female reproductive traits, except RE (Knudsen et al. 2006b). Because the body size of 2006 Yakima River basin were the smallest observed in the previous 6 years (Knudsen et al. 2007), reproductive traits values were also relatively low (see Figure 3). This trend in body size was observed in both hatchery and natural origin populations from throughout the Yakima River Basin and indicates that the CESRF supplementation project is not the root cause of the temporal decline in body size and female reproductive traits. However, this is ared flag warning and if the decline in size and per capita productivity (fecundity) continues, these populations will be increasingly at higher demographic risk.
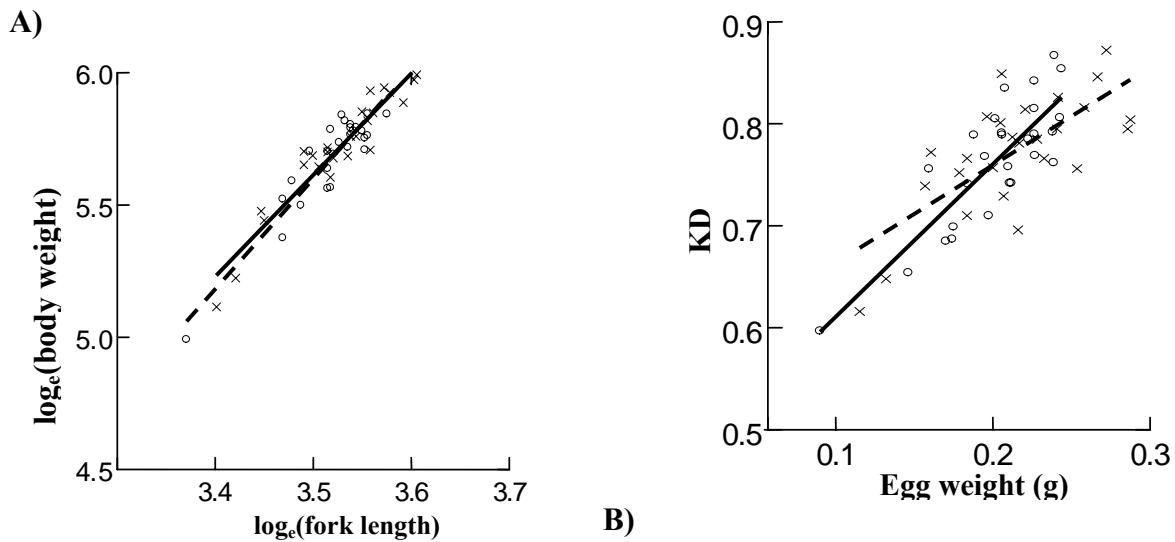
Figure 4. A) Log$_e$(fork length) vs log$_e$(body weight) and B) KD vs mean egg mass (g) of progeny from single-pair matings of either HC (○; dashed line) and NO (x; solid line) parents in 2006.

Acknowledgements

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Chapter Three

Spawner and Redd Characteristics of Hatchery and

Natural Origin

Upper Yakima River Spring Chinook

Curtis M. Knudsen[1], Todd N. Pearsons[2], Brenda L. Ben–James[3],

and

Steven L. Schroder[2]

[1] Oncorh Consulting, 2623 Galloway SE, Olympia, WA 98501

[2] Washington Department of Fish and Wildlife, 600 Capitol Way North, Olympia, WA 98501

[3] Cascade Aquatics, 7620 Manastash Road, Ellensburg, WA 98926

Abstract

We compared the characteristics of redds constructed by naturally spawning upper Yakima River hatchery and natural origin female spring Chinook salmon (*Oncorhynchus tshawytscha*) between 2002 and 2006. We compared the redds in terms of size, water depth, velocity, substrate and habitat characteristics, date of initial redd construction, and distance to the nearest surrounding redd. Redds were sampled by snorkeling during the spawning period between September and early October. Female origin was identified by the presence (natural origin) or absence (hatchery origin) of an adipose fin. After females were no longer present, redd characteristics were measured (total sample size: hatchery n=152; natural n=201). After eliminating autocorrelated variables, a 2-way MANOVA testing for Origin (Hatchery and Natural) and Year main effects in the distribution of bowl depth, bowl velocity, percent sand within the bowl, and distance to the nearest redd demonstrated no significant Origin effect (p>0.86), despite the fact that hatchery female mean fork lengths (FL) were smaller by between 0.7 to 1.6 cm's. All distributions except bowl velocity showed significant Year effects (p<0.001). Only bowl length showed a significant, weak negative correlation with FL, explaining just 5% of the total variation. Both hatchery and natural females preferred spawning habitat in the pool/riffle transition zone. Spawning density showed a weak negative correlation with apex height, distance to the nearest redd, and bowl length and explained between 6 and 9% of the total variation. Within years there were relatively small differences between hatchery and natural redds despite large interannual differences and no consistent trend in hatchery or natural origin means. However, our statistical tests did not have sufficient power to detect these relatively small differences due to low sample sizes.

Introduction

In order to successfully spawn, females Chinook salmon (*Oncorhynchus tshawytscha*) must accomplish four main tasks: select and defend a suitable spawning site, excavate a redd, attract a mate and deposit and cover fertilized eggs, and defend her redd from superimposition by other females; and all of this must occur during the appropriate time of year. The survival and growth of a female's progeny, and thus her fitness, are intimately tied to her attributes and choices that affect her ability to perform these tasks.

Salmon that are raised in hatcheries and expected to contribute to natural production must be able to select appropriate spawning areas and construct redds that have the same features as natural origin fish. Otherwise natural production of hatchery fish may be compromised. The choice of spawning site will determine the quality of incubation substrate and influence the survival of incubating eggs and pre-emergent fry (Einum and Fleming 2000a; Hendry et al. 2001). Because fry remain relative close to their redd during initial post-emergence rearing, the female's choice of spawning site. Poorly placed and constructed redds are more susceptible to scour from flooding (Montgomery et al. 1996), entombment by sediments (Chapman 1988), and desiccation by decreasing water flows (Crisp and Carling 1989; Groot and Margolis 1991). Spawn timing has been shown to be a heritable trait with fitness consequences (Smoker et al. 1998) and can be affected in Chinook salmon by domestication (Quinn et al. 2002). If redds are constructed too early, then they are at higher risk to superimposition by other females. Too early or late and eggs and fry will experience suboptimal water temperatures during incubation and emerge at less favorable times (Brannon et al. 2004).

Fish that are exposed to a hatchery environment might locate and construct redds inappropriately because selection pressures on their artificially spawned parents were relaxed or because hatchery fish may differ in physical or other characteristics from natural origin fish. The parents of hatchery fish are typically trapped, held on site, and then spawned artificially, removing natural selection pressures on spawning site choice and redd construction. This relaxation of selection may be detrimental to production of offspring in natural environments. Body size and spawn timing (Knudsen et al. 2006), and morphology (Busack et al. in press) of spring Chinook have been demonstrated to be affected by hatchery programs after only one generation, although the authors were not able to determine whether the observed changes were due primarily to selection pressure or phenotypic plasticity. These authors also estimated that the observed changes could reduce fitness by up to 5 percent.

Despite the potential importance of redd placement and construction by hatchery fish, there are no published studies we are aware of that have compared these traits for hatchery and natural origin Chinook salmon. However, some studies have shown that the reproductive success of hatchery fish in natural environments is lower than natural origin fish (Chilcote et al. 1986; Leider et al. 1990; Fleming and Gross 1993; McGinnity et al. 1997; Fleming et al. 2000; Araki et al. 2006). One of the factors that may contribute to the reduction in reproductive success is the placement and construction of redds.

We assumed that the redd characteristics of natural origin females were a complex product of many vectors of natural selection affecting the survival of a female's progeny

during incubation; a period of high nonrandom mortality (Montgomery et al. 1996; Einum and Fleming. 2000a; Einum and Fleming. 2000b), and post-emergence. Significant changes in locally adapted traits due to hatchery influences will likely be maladaptive and result in reduced fitness (Taylor 1991; Hard 1995). Thus, if hatchery redds differ significantly from natural origin redds, then hatchery females will likely suffer some reduction in fitness due to increased egg and/or fry mortality directly related to the female's performance and choices made while spawning.

In this study we compared the redds of naturally spawning hatchery and natural origin spring Chinook females in terms of redd width, length, water depth and velocity, initiation of redd construction, substrate composition, and spawning habitat type over the years 2002 to 2006. We also estimated whether female fork length and spawning density were correlated with redd characteristics and compared the timing of initiation of redd construction.

Methods

Our study site was located on the upper Yakima River in south-central Washington State, which flows into the Columbia River (Figure 1). Upper Yakima River spring Chinook are ''stream-type'' Chinook salmon (Healey 1991); adults migrate into the basin in the spring and spawn in the early fall, and juveniles spend a full year in freshwater before migrating to the ocean. Our redd surveys began immediately downstream of Easton Dam (river kilometer [rkm] 326 measuring from the confluence with the Columbia River) and extended downstream to the Eatson Acclimation Site covering approximately 2 rkm's (Figure 1). The spring Chinook hatchery population are part of the Yakima–Klickitat Fishery Project, located at the Cle Elum Supplementation Research Facility (CESRF) near Cle Elum (rkm 297; Figure 1) targeting the upper Yakima River spring Chinook population.

Redds were sampled by snorkeling 3 to 7 days per week throughout the spawning period during September and October each year (Table 1). All spring Chinook released from the CESRF are adipose fin clipped and thus female origin was identified based in the presence (natural) or absence (hatchery) of an adipose fin. During surveys, a female's fork length (FL) was estimated visually and the status of her redd and behavioral stage was noted. Behavioral stages were classified as: New – some substrate had been moved due to either a test dig prior to committing to that site; Constructing – the redd is in progress with one or more males attending or the female is digging and still has eggs remaining; and Guarding – there is no attending males, the female has no observable eggs left within her body and she is guarding the redd; and Empty - fish absent. When estimated FLs for a female differed between surveys we averaged across the estimates.

The Yakama Nation made weekly float surveys in our study area and identified and individually flagged each new redd constructed during the previous week. We used these weekly redd counts as a measure of Weekly Spawning Density (WSD) in the survey area and these values were assigned to a female's redd based on the week she was first observed in the Constructing stage.
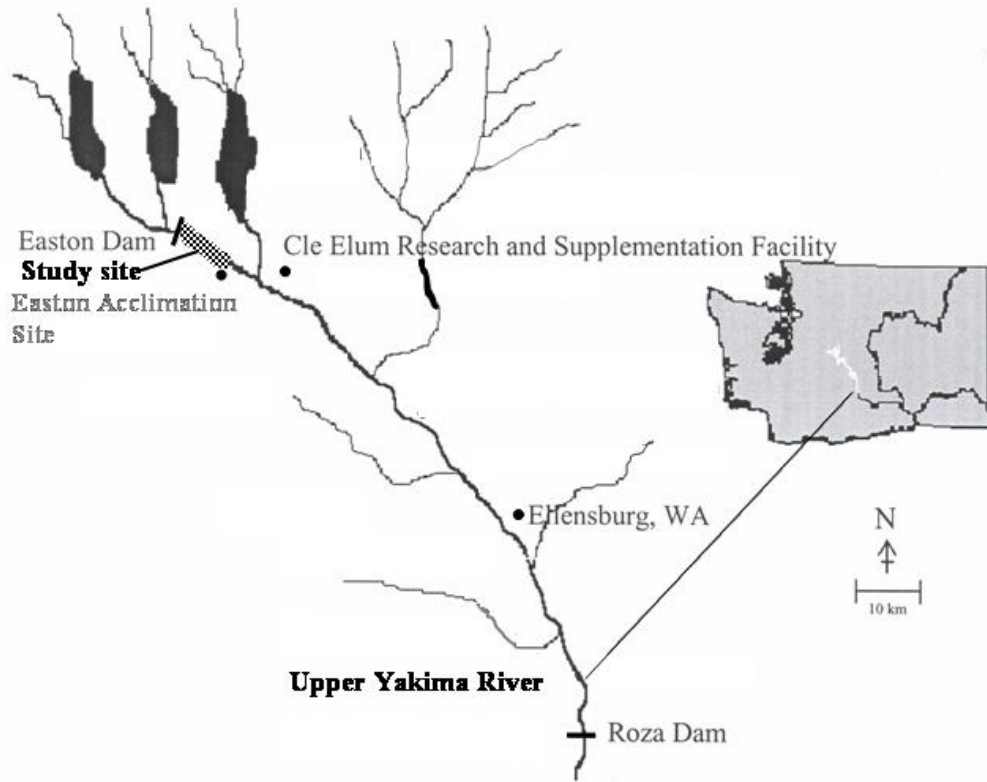
Figure 1. The study site was located between Easton Dam and the Easton Acclimation Site covering approximately 2 rkm.

Table 1. Beginning, ending, and mean dates, sample sizes, and standard deviations of A) female observations and B) redd measurements each year.

	2002	2003	2004	2005	2006
A)					
N of observations	659	72	635	735	280
Beginning date	9/9	9/18	9/12	9/13	9/18
Ending date	10/2	10/6	10/11	10/18	10/13
B)					
N of redds	131	23	140	103	27
Beginning date	9/9	10/1	9/20	10/6	10/10
Ending date	10/2	10/14	10/21	10/19	10/16

After spawning and redd construction were completed, a suite of metrics were collected from each known origin redd. Redd physical dimensions, water depth and velocity (at each point length measurements were collected) were measured, as well as visual estimates of substrate characteristics: percent sand, gravel, cobble and boulder in the bowl and the tail areas (Figure 2; Table 2). Redd habitat types were given an categorical score: riffle=1, riffle/pool transition=2, pool=3, pool/glide=4, and glide=5. In some cases
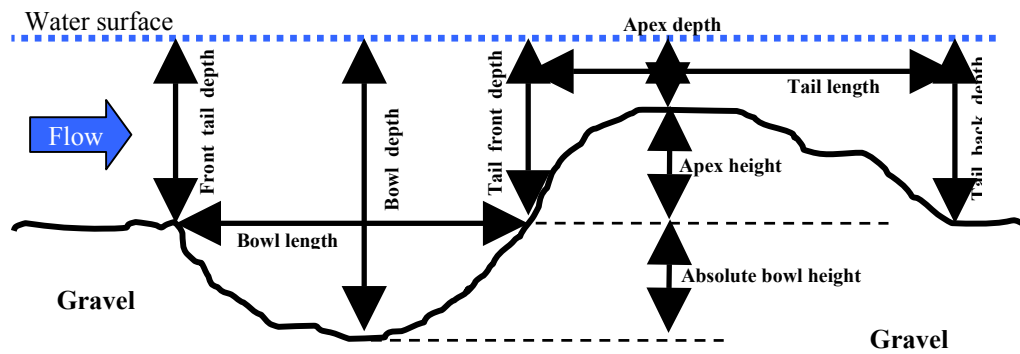
Redd Side View
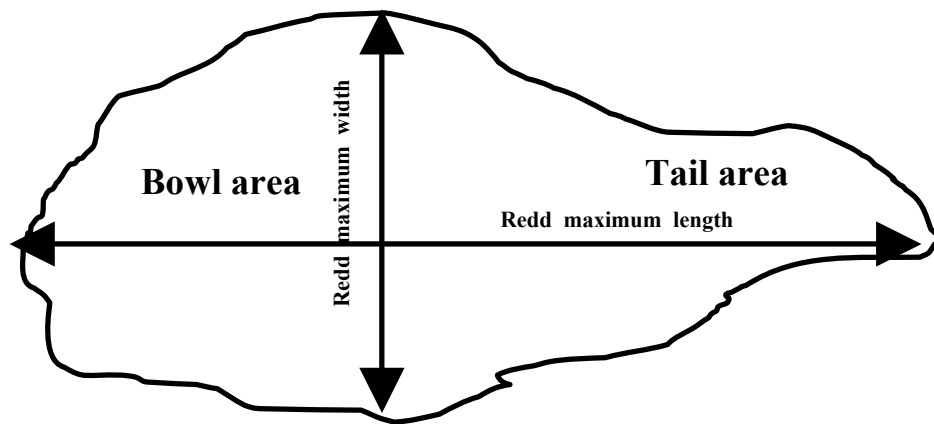
Redd Top View

Figure 2. Side and top views of a redd showing the dimensions measured. Water velocities were measured at each point a depth and width measurement were collected.

Table 2. Velocity, Depth, Length-Width, and Substrate measurements collected from redds.

Velocity (m·sec^{-1})	Depth (m)	Width-Length (m)	Substrate %
Bowl front	Bowl front	Tail length	Bowl % sand
Tail front	Maximum bowl	Bowl length	Bowl % gravel
Tail back	Tail front	Redd maximum width	Bowl % cobble
Redd left	Tail back	Redd maximum length	Bowl % boulder
Redd right	Tail apex		Tail % sand
	Redd left		Tail % gravel
	Redd right		Tail % cobble
			Tail % boulder

habitats were given an intermediate value (e.g. 3.5) when the habitat type fell between the two types. All water velocity measurements (m·sec^{-1}) were taken at 0.6 depth.

As an indicator of spawning density in the immediate vicinity of an individual redd, we measured the distance from each redd to the nearest adjacent redd. As spawning density increases one would expect this distance to decrease. Also, in order to remove any variability in depth variables due to flow fluctuations between the time females began constructing redds and when the redds were actually measured, we calculated Absolute Bowl Depth (ABD) by subtracting the Bowl depth from the average of the depth to the left and right of the redd at the maximum width points. We also calculated the Apex Height (AH), the height of the mound in the tail area downstream of the bowl, as the difference between the average of depths at the left and right of the bowl minus the Apex Depth (see Figure 2). In a few cases, due primarily to redd superimposition, ABD and AH values were negative.

We examined correlations of variables within four groups of variables: Velocity, Depth, Length/Width, and Substrate. We eliminated from our analyses highly correlated variables to reduce colinearity (Zar 1999). A Bonferroni method was used to adjust test statistics for multiple pairwise comparisons (Sokal and Rohlf 1995). The remaining variables were entered into a 2-way MANOVA to test for Origin (Hatchery vs Natural origin) and Year (2002, 2004 to 2006) effects, as well as Origin*Year interactions. The Year 2003 was not included in this analysis due to the small sample size for natural origin redds (n=4). Pillai Trace was used as the MANOVA main and interaction effects test statistic (Zar 1999). The SYSTAT 11.0 software was used to perform all statistical analyses (SYSTAT 2004).

Results

The great majority of our redd measurements were highly correlated within types Depth, Velocity, Substrate, and Length, and therefore present a problem of colinearity in multivariate analyses such as MANOVA. The seven velocity measurements were all significantly correlated (Bonferroni adjusted $p<0.001$) with Pearson correlation coefficients of 0.37 to 0.82. We eliminated all but one of these measurements retaining Bowl Velocity (BV). We did the same analyses with the other three groups and found that within groups 80% or more of the variables were significantly correlated (Bonferroni adjusted $p<0.001$) with correlation coefficients of 0.12 to 0.89. From the Depth, Length/Width, and Substrate groups we retained Absolute Bowl Depth (ABD), Bowl length (BL), and Percent Sand within the Bowl (PSB), respectively. Since PSB is a ratio, we used the arc sin square root transformation (Zar 1999) to normalize the distribution. We also used the Distance-to-the-nearest-redd (DNR) and Weekly Spawning Density (WSD) variables in our analyses. Because the variance in WSD increased as the mean increased, we $\log_e$ transformed WSD to normalize the variance. Correlations between these remaining variables are given in Table 3 while means, standard deviations and sample sizes by Origin and Year are given in Table 4.

We estimated Origin effects by comparing Hatchery and Natural origin redd variables using a 2-way MANOVA (Origin and Year effects) and found no significant Origin effect (*df*= 5, 298; Pillai Trace p=0.477). The test for Year effects was significant (*df*= 15, 900; Pillai Trace p<0.001), while Origin*Year interaction effects were not significant (*df*= 15, 900; Pillai Trace p=0.932). Of the variables included in the MANOVA, only BV varied little interannually exhibiting no significant Year effect (p=0.721) in a univariate 2-way ANOVA (Year and Origin effects).

Table 3. Pearson correlation coefficients (r) between pair-wise comparisons of variables (pooled across years and hatchery and natural origin females n=284). Bolded values were significant at the p<0.05 level. *P*-values were Bonferroni adjusted to account for multiple pair-wise comparisons. Log transformed Weekly Spawning Density ($\log_e$(WSD)), Absolute Bowl Depth (ABD), Bowl Velocity (BV), Apex Height (AH), Bowl Length (BL), Distance to nearest Redd (DNR), and arc sin Bowl Substrate$^{0.5}$ (ASBS).

	$\log_e$(WSD)	ABD	BV	AH	BL	DNR	ASBS
$\log_e$(WSD)	1.000						
ABD	-0.028	1.000					
BV	0.066	-0.052	1.000				
AH	**-0.265**	-0.140	0.065	1.000			
BL	**-0.304**	0.098	0.025	-0.067	1.000		
DNR	**-0.248**	0.076	-0.086	**0.180**	0.118	1.000	
ASBS	0.002	0.080	-0.121	0.110	-0.081	0.006	1.000

Table 4. Means, standard deviations, and sample sizes for hatchery and natural origin redd variables: $\log_e$Weekly Spawning Density ($\log_e$WSPD), Riffle-glide (RIF-G), Absolute Bowl Depth (ABD), Bowl Velocity (BV), Apex Height (AH), Bowl Length (BL), Distance to nearest Redd (DNR), and arc sin Bowl Substrate$^{0.5}$ (ASBS).

	Hatchery														
	2002			2003			2004			2005			2006		
Variable	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
$\log_e$ (WSPD)	4.80	0.59	76	3.53	0.76	14	5.50	1.38	38	3.72	0.49	16	2.98	0.31	8
RIF-G	4.34	0.85	73	2.69	0.48	13	2.42	0.47	38	2.75	0.45	16	2.50	0.53	8
ABD	0.10	0.08	63	0.09	0.08	14	0.35	0.18	38	0.08	0.06	16	0.10	0.06	8
BV	0.68	0.23	76	0.77	0.22	14	0.74	0.20	36	0.63	0.25	16	0.67	0.26	8
AH	0.10	0.12	62	0.46	0.12	14	0.02	0.07	38	0.14	0.06	16	0.11	0.06	8
BL	2.01	0.73	72	1.44	0.46	14	1.53	0.53	37	2.18	0.89	16	2.68	0.72	8
DNR	3.77	4.51	67	15.86	12.20	14	3.03	2.95	38	5.92	3.34	16	15.79	10.54	8
ASBS	0.08	0.03	63	0.09	0.02	14	0.07	0.02	36	0.07	0.02	16	0.06	0.04	8

	Natural														
	2002			2003			2004			2005			2006		
$\log_e$ (WSPD)	4.86	0.58	43	3.78	0.27	4	5.52	1.05	72	3.93	0.43	68	2.98	0.32	14
RIF-G	4.25	0.93	40	2.00	0.00	4	2.51	0.47	72	2.66	0.54	68	2.50	0.52	14
ABD	0.13	0.05	42	0.15	0.04	4	0.32	0.20	71	0.09	0.06	68	0.09	0.05	14
BV	0.68	0.23	43	0.74	0.12	4	0.68	0.24	71	0.62	0.19	68	0.70	0.17	14
AH	0.09	0.07	42	0.42	0.10	4	0.01	0.05	71	0.14	0.08	68	0.12	0.05	14
BL	2.10	1.01	41	1.35	0.62	4	1.46	0.72	67	2.16	0.82	68	2.57	0.46	14
DNR	5.45	9.02	39	3.88	4.63	4	4.50	5.19	72	5.42	5.14	68	14.18	12.98	14
ASBS	0.08	0.03	40	0.10	0.02	4	0.07	0.02	70	0.07	0.02	68	0.06	0.02	14

We found that Riffle-Glide demonstrated no significant difference between hatchery and natural origin redds in Kruskal-Wallis tests made for each year (all p≥0.34). Year 2003 was not tested due to low sample size.

In the 2002 and 2004 to 2006, natural origin females were larger than hatchery females by between 0.7 to 1.6 cm's (Table 5). Natural origin females were represented by only 4 individuals in 2003 and therefore this year was eliminated from the 2-way ANOVA of FL. There was no significant difference in FL distributions due to Origin effects (p=0.127). However, there was a significant Year effect (p<0.001) and between year differences in means were as large as 18 cm. The Origin-Year Interaction effect was not significant (p=0.989).

Table 5. Mean female fork length, standard deviation (sd), and sample sizes (n).

Year	Origin	Mean	sd	n
2002	Hatchery	69.6	3.6	76
	Natural	71.3	2.5	43
2003	Hatchery	74.8	8.5	14
	Natural	68.3	7.7	4
2004	Hatchery	71.9	6.6	37
	Natural	73.4	8.4	72
2005	Hatchery	60.4	4.6	13
	Natural	61.6	4.2	61
2006	Hatchery	55.4	2.7	7
	Natural	56.1	4.3	14

Table 6. Mean date hatchery and natural origin female spawning was initiated based on in-river visual observations during snorkel surveys.

Year	Origin	Mean date	sd	n
2002	Hatchery	Sept 25	6.1	76
	Natural	Sept 26	4.6	43
2003	Hatchery	Sept. 23	3.5	14
	Natural	Sept. 26	1.5	4
2004	Hatchery	Sept. 20	3.8	38
	Natural	Sept. 22	3.9	72
2005	Hatchery	Sept. 26	4.5	16
	Natural	Sept. 23	4.2	68
2006	Hatchery	Sept. 28	5.6	8
	Natural	Sept. 27	3.8	14

Within years, the mean dates spawning was initiated (SpDate) for hatchery and natural origin females differed by no more than 3 days and were variable over years (Table 6). There was a significant Origin*Year interaction for SpDate (p=0.019) in a 2-way ANOVA (Origin and Year effects) making any conclusions about Origin effects questionable. To determine whether there were hatchery-natural differences within years we reanalyzed the data on a year-by-year basis using a t-test. For the years 2002, 2003

and 2006 there was no significant Origin differences (all t-test *p*-values ≥0.116). In both 2004 and 2005, there were significantly different SpDates (both *p*-values<0.02). However, in 2004 Hatchery females began spawning earlier on average, while in 2005 the reverse was true. Thus, there was no consistent difference in SpDate due to Origin.

Mean BV and BL values are shown in Figure 3 and illustrate the relatively small differences between hatchery and natural origin redds within years. In addition, the large between year and large difference across years we observed across all measurements.

A)

B)
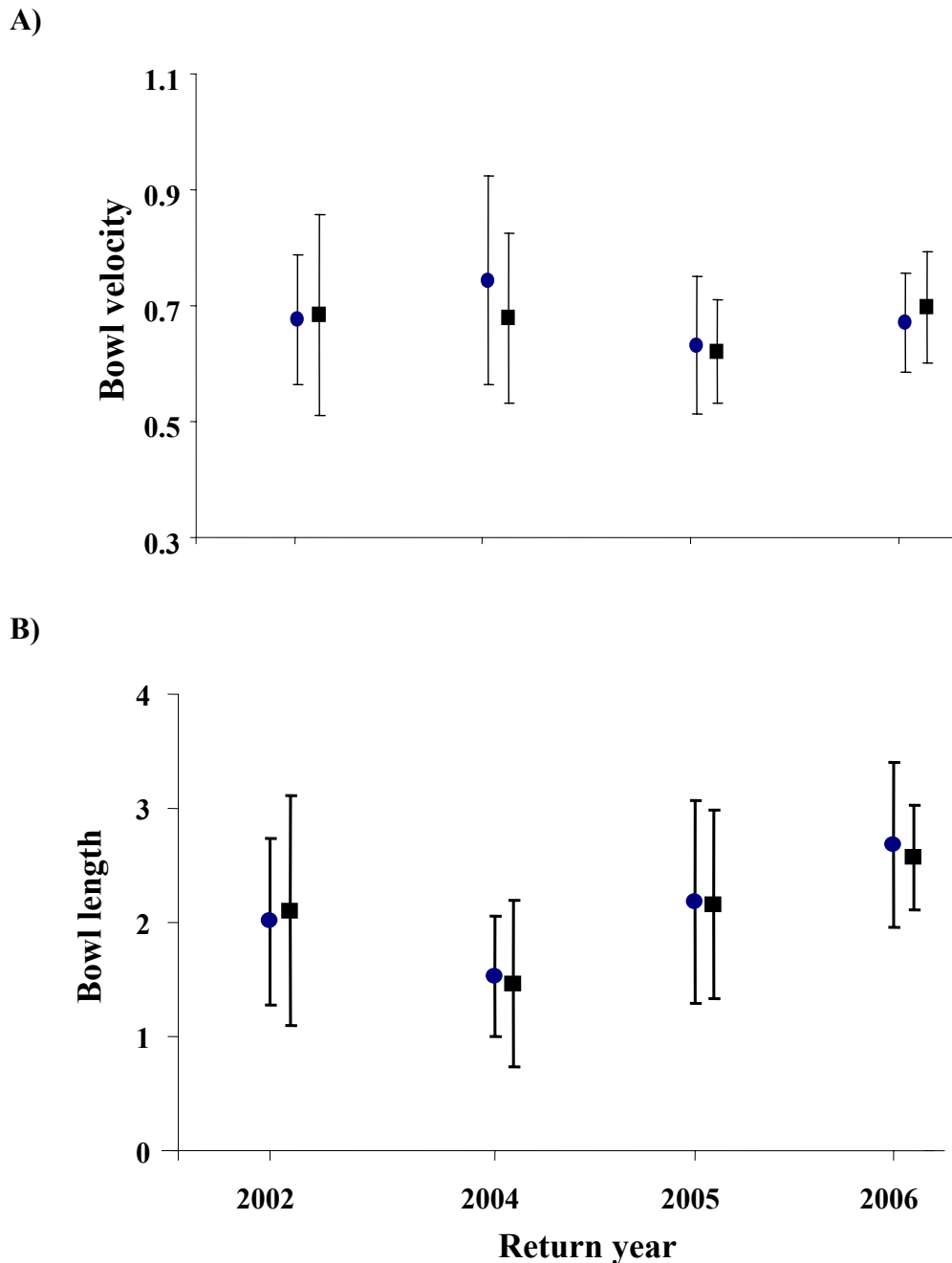
Figure 3.   Mean (+1 sd) Hatchery (●) and natural (■) origin A) Bowl velocity (m·sec^{-1}) and B) Bowl length (m).

We investigated whether FL or $\log_e$(WSD) might contribute to redd parameter variation by examining the relationship between either FL or $\log_e$(WSD) with redd characteristics (Table 7). In only 1 of 7 pair-wise comparisons to FL was there a significant correlation, and in that case BL was negatively correlated and explained only 5.2% of the total variation (Figure 4). AH, BL, and DNR were significantly negatively correlated with $\log_e$(WSD), and only explained between 5.3 to 8.8% of the total variation again. Thus, both FL and $\log_e$(WSD) indicated some density dependent effects (negative correlations) with either AH, BL, or DNR. However, the correlations were weak and explained 9% or less of the total variation in any variable.

Table 7. Correlations between female fork length or $\log_e$(WSD) and redd variables. The *p*-values were Bonferroni adjusted for multiple pair-wise comparisons.

Redd variable	Fork length		$\log_e$(WSD)	
	Pearson correlation	Bonferroni *p*-values	Pearson correlation	Bonferroni *p*-values
RIF-G	0.106	1.000	0.028	1.000
ABD	0.056	1.000	-0.016	1.000
BV	0.126	1.000	0.023	1.000
AH	0.079	1.000	-0.297	0.000
BL	-0.228	0.002	-0.271	0.000
DNR	-0.114	1.000	-0.231	0.002
ASBS	0.095	1.000	0.051	1.000
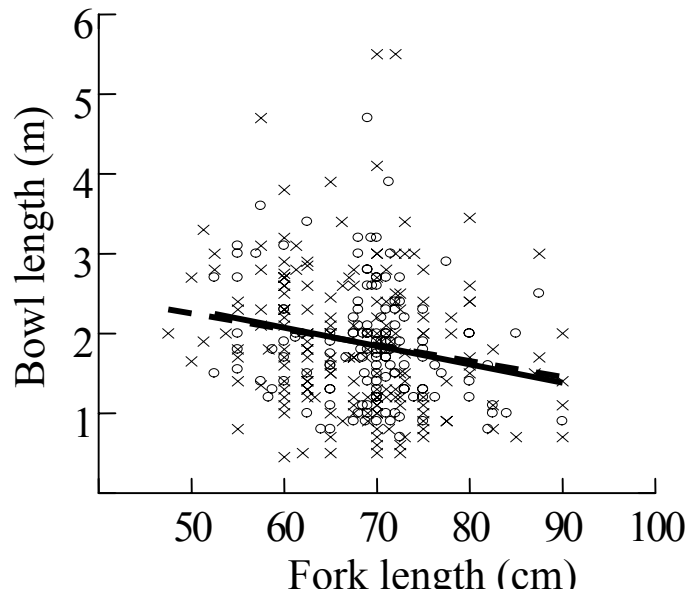
Figure 4. The relationship between Fork length and Bowl length for Hatchery (o, solid line), Natural (x, dashed line) origin females.

Discussion

We did not detect a significant difference between the redds of hatchery and natural origin female spring Chinook salmon. However, in another study of upper Yakima spring Chinook salmon, Schroder et al. (submitted) reported that, after controlling for body size-fecundity differences, first generation hatchery origin Chinook females had 7% lower reproductive success than natural origin females in an artificial stream channel. The fish used in Schroder et al. (submitted) were collected between 2001 and 2005 from the same population as the fish in our study. The main difference between the studies was that we evaluated fish in an uncontrolled natural environment and Schroder et al (submitted) evaluated fish in an artificial stream channel. Based on our results, it doesn't appear that the 7% difference in reproductive success in the artificial channel was due to the capability of females to construct appropriate redds.

Differences in hatchery and wild fish body size and morphology might plausibly influence redd characteristics. In our study area, we estimated differences in the mean FL of hatchery and natural origin females of between 1 and 2 cm, which are comparable to the length differences observed by Knudsen et al. (2006) between age 4 hatchery and wild upper Yakima River spring Chinook returning between 2001 to 2004. We found no strong correlations between female FL and redd characteristics, indicating that over the range of lengths we observed (FL ranged from 48 to 90 cm) FL did not strongly contribute to the variation in redd parameters and should not strongly affect redd characteristics. However, smaller female size does result in lower fecundity and 1 to 2 cm reduction in fork length will result in approximately 111 to 222 fewer eggs; an approximately 3 to 7% reduction (CMK unpublished upper Yakima R spring Chinook data: n=274; r^2=0.538, p<0.001; FL vs Fecundity regression slope =111 eggs*cm^{-1} FL; mean fecundity 3,347). In addition, Busack et al. (in press) found that upper Yakima River hatchery females had proportionately larger heads, narrower bodies, longer maxillaries, wider anal fins, and shorter caudal peduncles than wild females of the same stock. Apparently, these difference in morphology combined with smaller body size were not sufficiently large to produce detectable differences in redd characteristics.

One reason we were unable to detect a statistically significant difference between hatchery and wild redd characteristics was that our statistical tests had insufficient statistical power: the probability to reject the null hypothesis when it is false and the alternative hypothesis is true (Sokal and Rohlf 1995). For example, given the variation we observed in BV, the observed between year variation, and assuming α=0.05 and β=0.10; we can model statistical power in a 2-way ANOVA for a given effect size (SYSTAT 2004). We want to have at least 90% power to reject our main effect null hypothesis, given that it is false. We estimated we had 12% power to detect an approximately 2% Origin main effect difference in BV (Figure 5), given a balanced model design with 34 samples per Origin-Year cell (4 Years by 2 Origins) or a total of 272 samples. Our actual BV sample size was 341, but was not balanced and ranged from 8 to 76 per cell. Thus, we were unable to detect an effect size in BV of 2% with 90% power. We would actually need an effect size of approximately 11% in order to have 90% power given our sample sizes (Figure 5). However, supporting the argument that there is no large Origin effect in our redd measurements are 1) the average differences we observed between hatchery and natural origin redd measurements within years were relatively small over multiple years despite the fact that there were large significant

between-year differences, 2) the Origin effect *p*-value in the MANOVA was relatively high at 0,477, and 3) hatchery and natural origin fish demonstrated no consistent trend of having greater or larger values. Therefore, while we cannot say with statistical certainty that there are no differences between hatchery and natural origin redds, there are qualitative reasons to believe that if there are significant differences, they are relatively small; particularly relative to between year-differences.
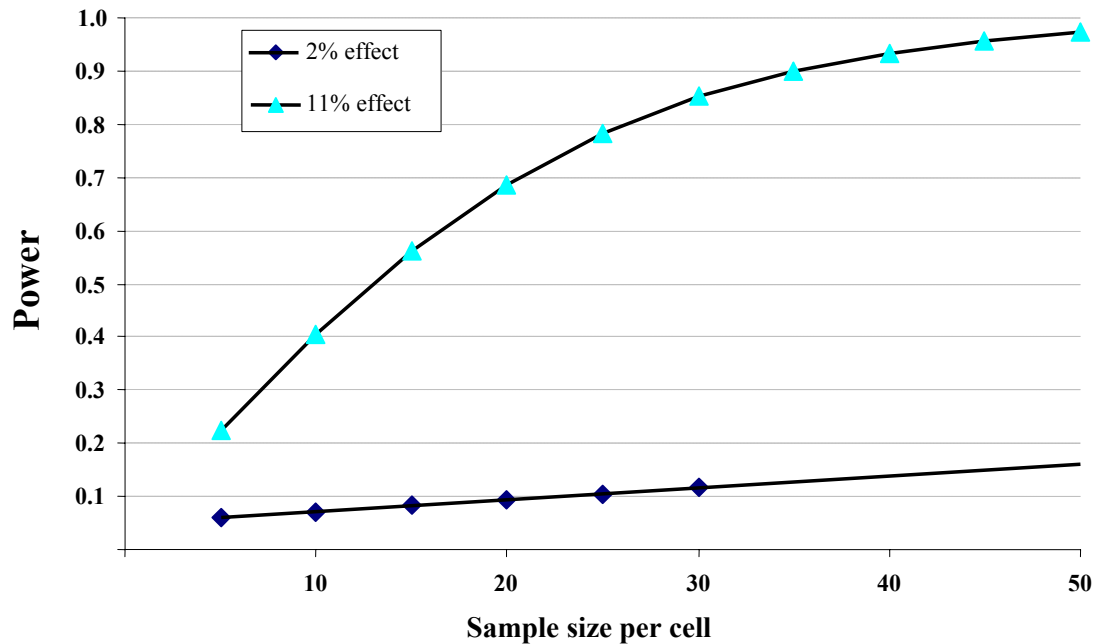

Figure 5. Bowl velocity power analysis. Given a balanced sample size of approximately 35 per cell or 280 total, it would be possible to detect a differences in hatchery and natural origin means of 11% with approximately 0.90 power assuming α=0.05. Our actual total sample size was 332 but was not balanced, ranging from 8 to 76 per cell. The actual effect we observed was approximately 2% on average.

Bowl Length was negatively correlated with FL. This result is somewhat counter intuitive in that it implies larger females tend to have smaller BL, which does not seem likely, given all other things are held equal. However, this result likely occurred because FL and $\log_e$(WSD) are themselves more highly correlated (r=0.433, p<0.001) than FL and BL. In years of higher than average freshwater and ocean productivity, ocean survival is higher than average because of a favorable environment which promotes good growth and larger size-at-age (Cox and Hinch 1997; Beamish et al. 2004; Wells et al. 2006). In years when freshwater productivity is high, but ocean productivity is low, survival and growth will be low due to density dependence and size at age will be smaller than average.

In the case of AH, BL, and DNR, which were significantly negatively correlated with $\log_e$(WSD), higher spawning densities were associated with lower AH, smaller BL, and shorter DNR. That is, as the number of females spawning increases, the distance between redds diminishes. This leads to more interactions between females, reducing the

size of defendable territories leading to smaller redds and, in our case, smaller BL. Because BL is smaller, the apex, created downstream of the bowl from excavated gravel, is also smaller. These results are in agreement with what Schroder (1981) observed in chum salmon (*O. keta*).

Bowl velocity was relatively constant across years and was the only variable exhibiting no significant Year effect. BV is the only variable directly under the control of the female, rather than influenced by such factors as river discharge, water temperature, substrate, and spawning density. That is, a female can pick a spawning site under a wide range of habitats and conditions and, by properly excavating her redd, create a preferred BV, and by extension inter-gravel flow that directly affects progeny survival and fitness. The preferred inter-gravel flow should be relatively site independent and stable across years, as was demonstrated in our study.

Knudsen et al. (2006) found that in a "common garden" experiment at the Cle Elum Supplementation and Research Facility the maturation timing of hatchery fish averaged 5 days earlier than wild origin upper Yakima River spring Chinook between 2001 and 2004. In addition, this same trend in maturation timing was repeated at CESRF between 2002 and 2006 (CMK unpublished data). However, we did not find a similar consistent trend in the initiation of redd construction in this study. The onset of maturation at CESRF, as indicated by a soft abdomen and loose gametes, was compared rather than initiation of redd construction. There may be differences between hatchery and natural origin females in the onset of maturation and gamete development that are not as tightly correlated with redd construction.

It is possible that other hatchery programs that have less stringent protocols than were used in the YKFP could produce different results than we did. Our fish were descendants of endemic brood, experienced only one generation of fish culture, and were cultured in ways thought to minimize adverse genetic effects. Fish that are reared for multiple generations in a non-integrated hatchery program, that are not endemic to the watershed, or that experience stronger selection regimes in the hatchery; could produce redd characteristics that are different than wild fish. Such differences in Chinook salmon spawn timing have been demonstrated (Quinn et al. 2002). In short, if hatchery programs affect redd characteristics, then we would expect that our hatchery fish to produce relatively small differences compared to natural fish at this point in its development.

Acknowledgements

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Chapter Four

Long Term Loss of Passive Integrated Transponder Tags
and Effects On Survival, Growth, and Behavior of
Yakima River Hatchery Spring Chinook Salmon

Submitted by

Curtis M. Knudsen[1], Mark V. Johnston[2], Steven L. Schroder[3],

William J. Bosch[2], David E. Fast[2], and Charles R. Strom[4]

[1]Oncorh Consulting, 2623 Galloway SE, Olympia, WA 98501

[2]Yakama Nation, P.O. Box 151, Toppenish, WA 98948

[3]Washington Department of Fish and Wildlife, 600 Capitol Way North, Olympia, WA 98501

[4]Yakama Nation, Cle Elum Supplementation Research Facility, Cle Elum, WA 98922

Abstract

We tagged juvenile upper Yakima River hatchery spring chinook salmon (*Oncorhynchus tshawytscha*) with Passive Integrated Transponder (PIT) and Coded wire (CW) tags inserted into the snout in a double-tag study to test the assumptions often made that tags are not lost and do not effect survival, behavior, or growth. We estimated that PIT tags were lost on average in 17% of adults returning 8 months to 4 years after release. Tag losses were not significantly affected by the age of returns (ANCOVA p=0.40) indicating that after approximately 8 months of ocean rearing the majority of PIT and CW tag loss had already occurred. After correcting PIT tag recoveries for tag loss, recoveries were no longer significantly lower than expected (X^2-test p>0.05) indicating that there was no significant reduction in post-release survival due directly to the effects of PIT tags. The mean lengths and weights of PIT tagged adults were smaller than non-PIT tagged adults in all comparisons (age 4 mean POHP length difference = 1.1 cm; mean body weight difference = 0.1 kg). However, only age 4 PIT tagged adults were significantly smaller (ANOVA p<0.05). There was no significant difference in migration timing between PIT tagged and non-PIT tagged adults within the upper Yakima River (Mann-Whitney p>0.09). Observed PIT tag recoveries resulted in underestimating true survival by approximately 17 and underestimating body size, particularly in age 4 returns that make up over 80 percent of upper Yakima River hatchery origin returns.

Introduction

The use of tags and marks in fish studies has a long history dating from at least the 1800's (see review in McFarlane et al. 1990). Estimation of fish population size, emigration and migration rates, exploitation rates, gear selectivity, natural and fishing mortality, growth, age, reproduction, and physiology have been developed using marked and tagged fish (Ricker 1975; Seber 1982; Burnham et al. 1987). Two critical assumptions typically made are that tag loss does not occur and tags do not cause mortality. In addition, tags are assumed to have no significant affect on growth or behavior. Significant violations of these assumptions can bias study results (Robson and Regier 1966; Arnason and Mills 1981; Seber 1982; MacDonald et al. 2003; Rotella and Hines 2005) and make extrapolation of results to the untagged population invalid. Essentially all tags and marks violate some study assumption to a degree, so it is important to understand the strengths and limitations of any tagging technique in order to select the tag violating the fewest or least important assumptions in the proposed research (Seber 1982; Krebs 1998).

With the development of the Passive Integrated Transponder (PIT) tag in the late 1980's (Prentice et al. 1990), it became possible to mark individuals uniquely in large quantities and recover tag codes remotely. More sophisticated individual-based models for data analysis were also developed (Burnham et al. 1987) and applied using PIT tags in the Columbia River basin to monitor juvenile salmonid survival and migration timing (Skalski et al. 1998; Muir et al. 2001; Budy et al. 2002).

Estimating post-release tag loss and tag effects occurring one or more years after release is a difficult and often expensive problem. In order to estimate long term tag loss, rather than holding fish in a protected vessel buffered from the rigors of a free ranging migratory life, it is important that marked fish experience the types of stresses and challenges all fish within the population of interest experience. For this purpose, Beverton and Holt (1957) and Seber (1982) suggest using a double-tagging design and releasing fish under actual study conditions allowing estimation of tag loss for each tag type after release.

Prentice et al. (1994) used a double-tag study design to estimate PIT tag loss in the only published report we are aware of on juvenile-to-adult PIT tag retention in free ranging adult Pacific salmon. They found that mature adult coho salmon (*Oncorhynchus kisutch*) PIT tagged as juveniles lost their tags at high rates at some point in their life prior to spawning, estimating an overall 59% loss rate in females and 13% in males.

In this study we 1) estimate PIT and CW tag loss in upper Yakima River hatchery spring chinook returns approximately 6 months to 4 years after tagging and 2) estimate whether PIT tags had an impact on post-release survival, size-at-age, and in-river migration timing.

Methods and Materials

PIT and CW Tag Loss

We used a double-tag study design (Seber 1982) in which two marks or tags are applied to each fish in the study population. In our study these were a PIT tag injected into the body cavity with a hand-held injector (Prentice et al. 1990) and a Coded Wire (CW) tag injected into the snout (Jefferts et al. 1963). It was then possible to estimate tag

loss rates for PIT and CW tags, as well as the number of fish losing both tags. Beginning in 1998, approximately 40,000 age-1 hatchery origin spring chinook (fish in their first year post-hatching) were double marked annually at the Cle Elum Supplementation and Research Facility (CESRF) between October and December (Table 1). An additional mark, an adipose fin clip, was applied to distinguish all CESRF fish from natural origin fish. Each year an equal proportion of each raceway, varying between approximately 5 and 10% over the study (Table 1), was PIT and CW tagged and reared in common with the fish not PIT tagged. In February, fish within a raceway were transferred by truck to a raceway at one of three acclimation sites (Clark Flats, Easton, and Jack Creek; Figure 1), held for approximately 1.5 additional months, and then allowed to volitionally emigrate as age-2 smolts (~18 months after fertilization) between March 15 and May 30.   Thus, fish were held at acclimation sites for between 70 to 125 days after tagging before volitional releases began. The fish not PIT tagged were marked with a combination of a colored elastomer material inserted in the adipose eyelid (Bonneau et al. 1995) , a CW tag imbedded in the musculature at one of five specific body sites (not including the snout), and an adipose fin clip. Thus, all hatchery fish were marked with an adipose fin clip allowing quick visual identification and enumeration of all hatchery returns. All tagged hatchery fish were marked during October to December each year under similar environmental conditions. Body placement of CW tags (snout vs other body sites) and/or the presence of PIT tags allowed separation and identification of PIT tagged fish post-release.

Table 1. The number of juvenile hatchery spring chinook PIT and snout CW tagged, the number not PIT or snout CW tagged, the total release (PIT tagged plus not PIT tagged), and the percentages of tagged and untagged fish released by broodyear. All fish released were adipose fin clipped.

| | Broodyear | | | | |
Juvenile Releases	1997	1998	1999	2000	2001
PIT/CW tagged	39,892	37,385	38,791	37,580	30,087
Not PIT tagged	346,156	552,298	719,998	796,705	301,043
Total released	386,048	589,683	758,789	834,285	331,130
Percent PIT tagged	10.33%	6.34%	5.11%	4.50%	9.09%

For the first brood (1997), the number of fish released from each acclimation raceway was calculated as the number of fish PIT-tagged adjusted for observed pre-release mortalities. Beginning with the 1998 brood, improved tag technology and detection equipment at the acclimation sites allowed the number of PIT tag releases to be based on tag detections at raceway outlets. There were a small proportion of tags (less than 1%) missed by acclimation site detectors which were detected at downstream monitoring sites and these tags were not included in the analysis. The total number of PIT tagged and number of marked hatchery juveniles released by broodyear are given in Table 1.

Returning fish were examined 6 months to 4 years after release. During the post-release period fish migrated downstream below the Roza Adult Monitoring Facility (RAMF; Figure 1). Fish age 3 and older migrated through the Yakima and Columbia rivers to the Pacific Ocean where they reared for 1 to 3 years, eventually returning to the
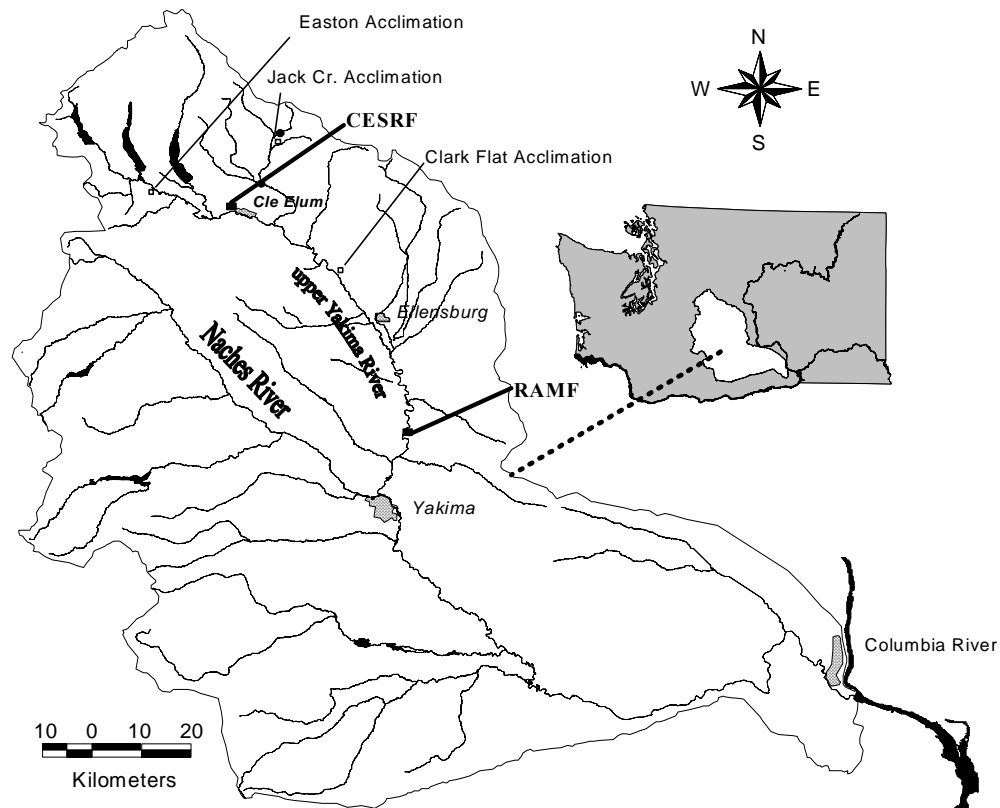
Figure 1. Map of the Yakima River Basin showing the Roza Adult Monitoring Facility (RAMF) and Cle Elum Research and Supplementation Facility (CERSF).

upper Yakima River as maturing fish to spawn between September and October. Age 2 fish, called precocious males, return approximately 6 months after release and likely do not migrate beyond the Columbia River basin before returning to spawn (see Pearsons et al. (2004) and Larsen et al. (2004) for a description of precocious males in the Yakima River and Beckman et al. (2005) for a discussion of Columbia River spring chinook precocious male directed movements). On a daily basis at RAMF, all returning hatchery fish (adipose fin clipped) were diverted to a short term holding tank containing the anesthetic MS222 (Bell 1964), examined for marks and tags including PIT and CW tags in the snout, and the number of fish retaining each tag type was recorded. Post-orbital hypural plate (POHP) body length and body weights were collected, as well. Fish without PIT tags were scale sampled to age fish and determine broodyear. Fish passage and trapping at RAMF began in late April and continued through early September.

PIT tagged adult recaptures fell into one of four categories: 1) PIT tagged/snout CW tag/Ad clipped (all tags retained), 2) snout CW tag/Ad clipped (lost PIT tag), 3) PIT tagged/Ad clipped (lost snout CW tag), or 4) Ad clipped only (lost both PIT and snout CW tag). Captured wild origin chinook were identified by an intact adipose fin.

Recaptured PIT tagged fish falling into categories 1) through 3) could be identified unambiguously. However, PIT tagged recaptures which have lost all their marks in category 4 are confused with other hatchery juveniles released which were also adipose fin clipped. Identifying category 4 fish in double-mark studies is a common problem and a method was developed by Seber (1982) to account for them.

Assuming tags are lost within fish independently, that is losing a PIT tag does not influence whether a fish losses or retains its snout CW tag, we can calculate the following from tagged fish recovered at RAMF:

R_{cwt} = the total number of fish retaining only a snout CW tag.

R_{pit} = the total number of fish retaining only a PIT tag.

$R_{pit,cwt}$ = the total number of fish retaining both a PIT and snout CW tag.

R is the total number of tagged fish recovered and is

$$R = R_{cwt} + R_{pit} + R_{pit,cwt} + \{\text{Number of fish losing both tags}\}$$

R' is the total number of tagged fish retaining one or more tags and is

$$R' = R_{cwt} + R_{pit} + R_{pit,cw}.$$

Using the methodology of Seber (1982), we can then estimate the probability of PIT and snout CW tag loss:

$$\text{Pr}_{PIT} = [\text{Probability of losing a PIT tag}] = \frac{R_{cwt}}{(R_{cwt} + R_{pit,cwt})}$$

$$\text{Pr}_{CWT} = [\text{Probability of losing a snout CW tag}] = \frac{R_{pit}}{(R_{pit} + R_{pit,cwt})}$$

Since fish that have lost both tags (category 4) cannot be separated out from other non-PIT tagged, adipose fin clipped fish that have lost both of their tags, they cannot be directly enumerated to calculate R and it is necessary to correct for them in order to estimate the total number of captures, $\hat{R}$. Following Seber (1982) this involves defining k, the joint probability of losing each tag, assuming independence of each tag, then

$$k = \frac{R_{cwt} * R_{pit}}{(R_{cwt} + R_{pit,cwt})(R_{pit} + R_{pit,cwt})}$$

and

$$c = \frac{1}{1-k}.$$

Then an estimate of the total number of PIT recaptures, including those losing both tags, is

$$\hat{R} = c(R_{cwt} + R_{pit} + R_{pit,cwt}).$$

We estimated the loss rates of both PIT and snout CW tags by broodyear. We also examined trends in loss rates by age. If tag loss is a continuous process over time, then one would expect that older fish would have higher rates of tag loss. We used an ANCOVA to compare trends over ages (2, 3, 4, and 5) by tag type (PIT and CW tag).

Survival, Migration Timing, and Body Size Comparisons

For these analyses we used recovered adult returns that were PIT tagged as juveniles and compared them to recoveries from the same cohort that were not PIT tagged. We did not use age 2 returns as they were sampled in a less rigorous manner over the course of the run in the initial years of the project. After being PIT tagged, juvenile fish were returned to their respective raceways and reared together with non-PIT tagged fish which made up 90-95% of each raceway population. Raceways were volitionally released and therefore both PIT tagged and non-PIT tagged fish had the opportunity to experience the same outmigration and post-release rearing conditions. Thus, body size, adult migration timing distributions, and survival rates of PIT tagged and non-PIT tagged fish can be compared and differences should reflect the effects of tagging.

The proportion of adult fish retaining PIT tags was compared to the proportion of fish PIT tagged and released as juveniles for each broodyear using a X^2-test with Yates correction (Zar 1999). If both PIT tagged and non-PIT tagged releases survive at equal rates, then the two proportions should be equal. However, if PIT tags are lost, then survival of PIT tagged adults will be underestimated (missed tags) resulting in apparent higher mortality in PIT tagged fish. Therefore, in addition to using the actual adult PIT tag recoveries ($R_{pit} + R_{pit,cwt}$) in our comparisons, we also used $\hat{R}$ to calculate the proportion of PIT tagged adult recoveries corrected for tag loss.

Because all returning fish passing RAMF were monitored on a daily basis, all retuning hatchery fish were identified to date of passage. These data were transformed to ordinal numbers representing the day of the year, i.e. January 1 was represented by 1 and December 31 by 365. The distributions of ordinal passage dates of PIT and non-PIT tagged recoveries were compared by age within each broodyear using a Mann-Whitney test (Table 2; Zar 1999). Ages and broodyears were treated separately because it has been shown that both significantly affect the timing of adult spring chinook upstream passage at RAMF (Knudsen et al. 2006).

Body weight and length distributions collected at RAMF were compared using 2-way ANOVA examining Tag type (PIT tagged vs. not PIT tagged) and Broodyear effects with interactions (Table 2). We analyzed data representing age 3, 4 and 5 returns and did not include any age class/tag type represented by less than 10 recoveries within a year. The age 4 group represents greater than 80 percent of hatchery returns each year (Knudsen et al. 2006) and were the only age class represented in all five broodyears (1997-2001). Age 3 recoveries were represented by broodyears 1998, 2000 and 2001, while age 5 recoveries were represented by broodyears 1997 and 1998.

Table 2. Sample sizes by broodyear and age for PIT tagged and non-PIT tagged recoveries used in comparing migration timing, POHP length and body weight distributions.

Broodyear	Age	PIT tagged recoveries	Non-PIT tagged recoveries
	3	0	0
1997	4	190	1795
	5	18	18
	3	16	289
1998	4	432	1196
	5	41	179
	3	0	0
1999	4	25	231
	5	0	0
	3	38	357
2000	4	148	334
	5	0	0
	3	27	25
2001	4	69	155
	5	0	0

Results

PIT and CW tag loss

From April through September, as maturing hatchery fish passed upstream past RAMF and were interrogated for PIT tags, snout CW tags, and adipose fin clips, a total of 252 recovered fish had lost their PIT tag, 63 had lost their snout CW tag, and 1,492 had retained both their PIT and CW tag (Table 3). Over broodyears, estimates of PIT tag loss ranged from 8 to 20% and from 2 to 7% for CW tags, respectively, and averaged 17% and 5%, respectively. Estimates of the number of fish losing both tags ($\hat{R} - R'$) within broodyears ranged from 1 to 8 fish.

We estimated tag retention by age (Figure 2) and in an ANCOVA found no significant trend as fish aged for either CW or PIT tags (Figure 2; slopes differ from 0.0; p=0.40) indicating that tag loss does not change significantly over ages. However, there was a significant difference between overall tag loss rates, and PIT tags were lost at approximately 3 times the rates of CW tags (equality of means in ANCOVA p<0.001).

Survival Impacts of PIT tags

In Figure 3 we present by broodyear the percentage of recovered adult fish actually retaining their PIT tags ($R_{pit}+R_{pit,cwt}$), the expected percentage based on release numbers of PIT tagged and untagged hatchery juveniles, and the percentage of PIT tagged fish after correcting for PIT tag loss including the estimated number of fish losing both tags. In each broodyear, the percentage of PIT tagged fish actually recovered is significantly less than the expected percentage (each broodyear X^2-test with Yates
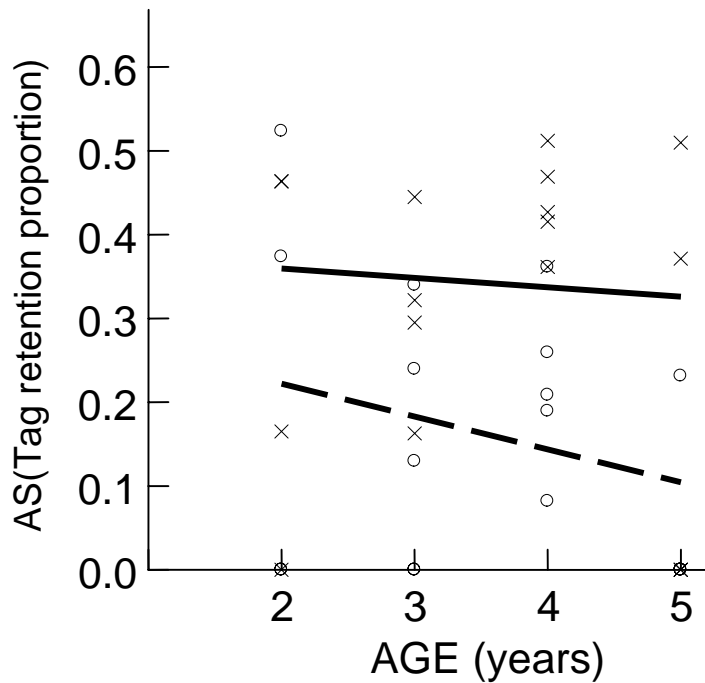
Figure 2. Linear regression of arc sin square root (AS) transformed PIT tag (solid line; x) and snout CW tag (dashed line; ○) retention proportions by adult age for broodyears 1997 to 2001.

correction $p<0.01$). After correcting for tag loss, the percentage of PIT tag recoveries increased in every broodyear although estimates were less than the expected in 2 of 5 broodyears. However, in no broodyear was there a significant difference between expected and corrected recoveries (X^2-test with Yates correction $p>0.05$). The difference between the expected recovery rate and the corrected recovery percentage can be interpreted as an estimate of PIT tag induced mortality. It is not a generic "tagging/handling mortality" because all released fish were marked with more than one tag, reared in common raceways, and experienced common conditions throughout rearing, release and migration. Our estimates of PIT tag induced mortality indicate that in no broodyear was a corrected estimate significantly different than the expected proportion at release.

Migration Rate Comparison

We found no difference between passage timing for PIT and non-PIT tagged fish at RAMF in age 3, 4 and 5 fish over the broodyears 1997 to 2001 (Figure 4). Results of Mann-Whitney tests showed that in the 10 age/broodyear comparison all the p-values were greater than 0.09.
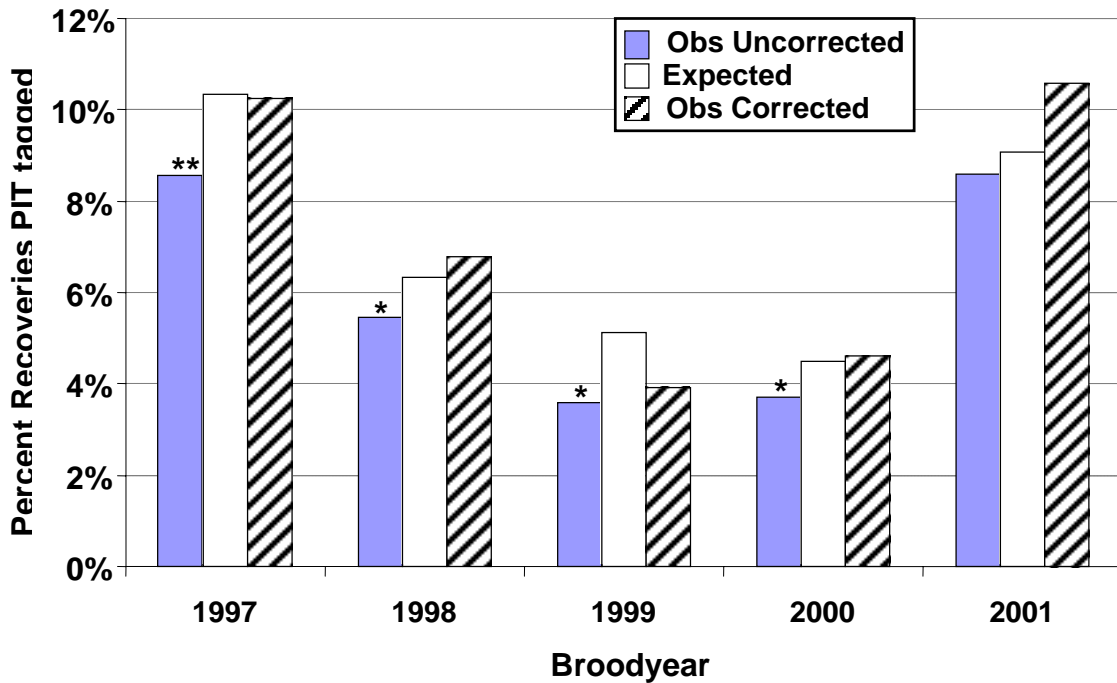
Figure 3. Percentage of recoveries made up of PIT tagged fish by broodyear. "Obs Uncorrected" (dark bars) represents the observed number of PIT tagged fish. "Expected" (white bars) represents the expected number of PIT tag recoveries based on the proportion of PIT tagged fish released. "Obs Corrected" (hatched bars) represents the observed number of PIT tagged fish corrected for PIT tag loss. Significance between uncorrected observed recoveries and expected recoveries are indicated above the respective columns ("*" $p \leq 0.05$ and "**" $p \leq 0.01$).

A) Age 3
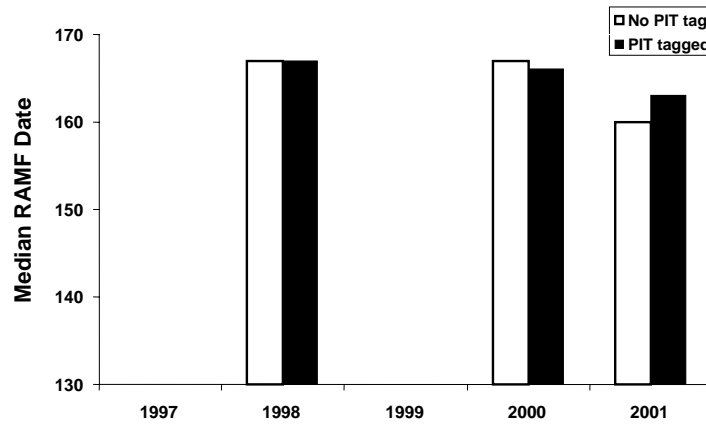
B) Age 4
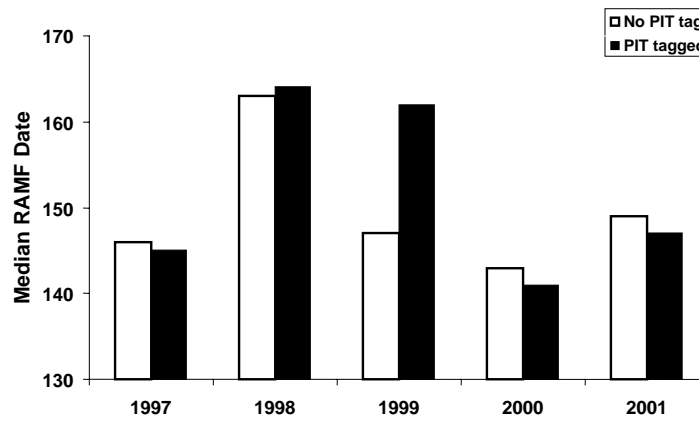
C) Age 5
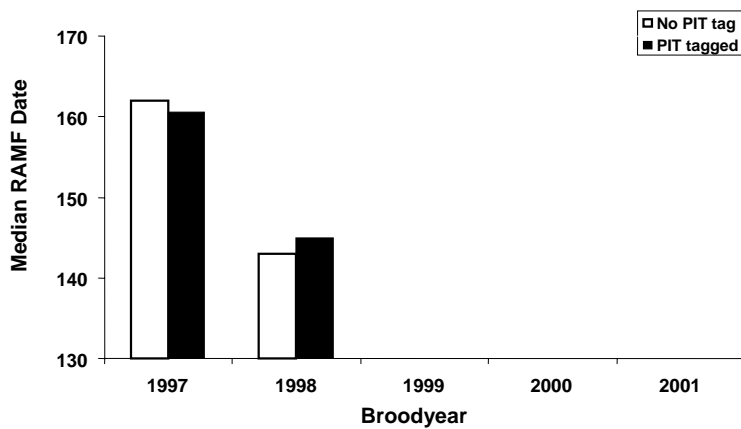
Figure 4. Median ordinal date of passage at RAMF of adult age 3, 4 and 5 spring chinook with (dark bars) and without (white bars) PIT tags returning over broodyears 1997 to 2001.

Body Size Comparisons

In all comparisons between PIT tagged and non-PIT tagged fish by age and broodyear, PIT tagged fish were smaller than non-PIT tagged fish (Figure 5). Analyses of POHP length and body weight distributions using 2-way ANOVA (Tag type and Broodyear effects) found significant differences in age 4 returns (Tag type effect POHP p=0.024; Body weight Tag type effect p=0.043). However not in age 3 (Tag type effect POHP p=0.174; Body weight Tag type effect p=0.601) or age 5 (Tag type effect POHP p=0.203; Body weight Tag type effect p=0.429) returns. There were no significant Tag type x Broodyear interactions in any ANOVA (all interaction p-values>0.43).
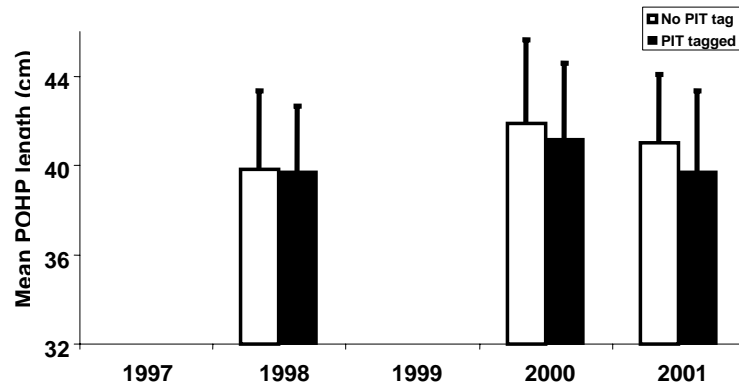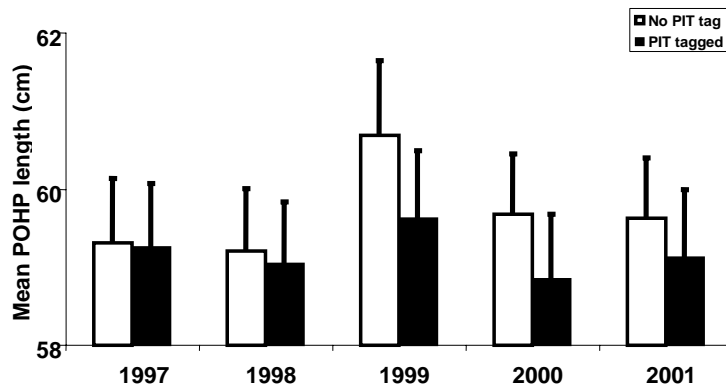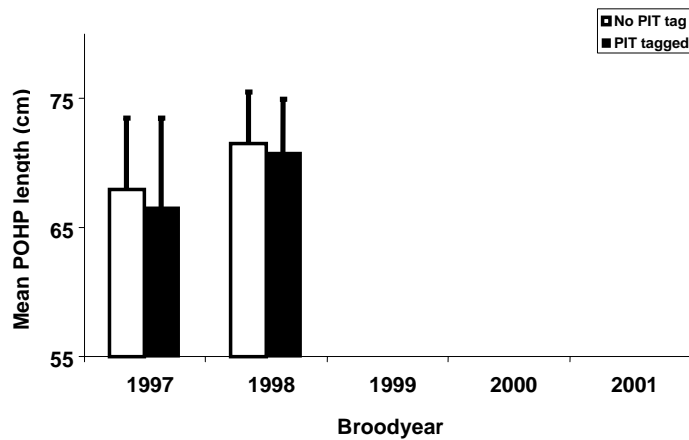
Figure 5. Mean POHP lenght (+1 sd) of adult age 3, 4 and 5 spring chinook with (dark bars) and without (white bars) PIT tags returning over broodyears 1997 to 2001.

Discussion

Recently, remote monitoring of PIT tagged adults returning to the Columbia River has become possible and analysis of PIT tags was used to examine impacts of flow on juvenile-to-adult chinook salmon survival in the Columbia River system (Berggren et al. 2003; 2005). Berggren et al. (2005) extrapolated their results to the untagged portions of study populations and in response the Independent Science Advisory Board (ISAB) noted, "the apparent documentation that PIT-tagged fish do not survive as well as untagged fish. This point has major implications for all uses of PIT-tagged fish as surrogates for untagged fish" (ISAB 2006). The difference in survival between PIT tagged and untagged chinook noted by the ISAB could be due to either survival differences or to PIT tag loss (tagged fish missing their tag would not be recovered and would be included as mortalities) or some combination of both.

After accounting for PIT tag loss, the percentage of PIT tag recoveries was not significantly different than expected based on juvenile releases. This argues strongly that post-release mortality due to PIT tags was not significant in upper Yakima River hatchery spring chinook tagged as pre-smolts and allowed to fully recover from tagging stress (Sharpe et al. 1998) for between 2-3 months prior to release. Our study should be considered a "best case" scenario in terms of the impacts of tagging on post-release fish stress because 1) tagged fish were allowed to recover from the stresses of tagging for 70 or more days prior to being allowed to volitionally emigrate from their raceways, 2) tagging occurred during late fall when water temperatures were relatively low, 3) fish averaged 14 g or greater at tagging, and 4) fish were not experiencing the physiological challenges of smoltification during tagging. Our results likely do not represent the "worst case" scenario of wild and hatchery origin, actively migrating smolts captured *in situ*, often tagged at fork lengths as small as 60 mm when water temperatures are elevated, and then released less than 24 h after tagging. Under these conditions post-release mortality due to PIT tagging could be a problem and is not the situation we tested.

Prentice et al. (1994) collected five weekly samples of PIT tagged coho adults as they reached full maturity. Unfortunately, they presented the data as cumulative counts. We reconstructed their figure using the proportion of fish sampled each week with lost tags and estimated 95% confidence intervals assuming a binomial distribution (Figure 6) and their data demonstrate no clear temporal trend in PIT tag loss. However, the size of the weekly samples were very small, especially the later collections, resulting in nearly 0-100% confidence intervals. Their results do point out the potential for males and females to experience different tag loss rates. In their study, mid-term PIT tag loss (eight months after tagging) was estimated to be 1% (Prentice et al. (1993). Thus, nearly all of the tag loss documented in Prentice et al. (1994) occurred after release and sometime during the 12 months prior to spawning. We did not estimate PIT tag losses for males and females separately because our ability to identify the sexes at RAMF is poor. Based on fish sexed at RAMF and subsequently examined post mortem at CESRF during spawning, we identified males and females with approximately 70% and 90% accuracy, respectively.
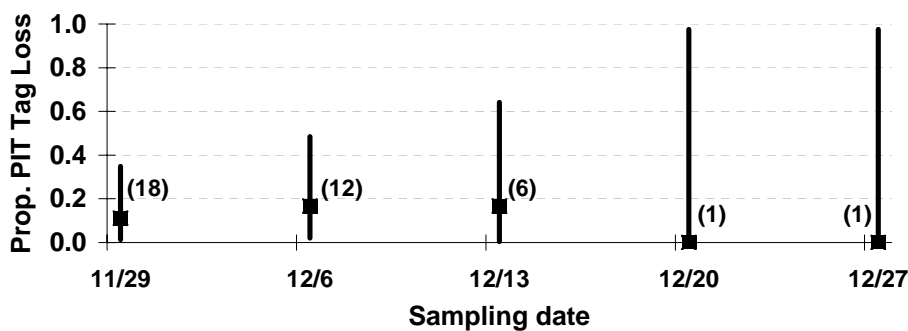
A) Females
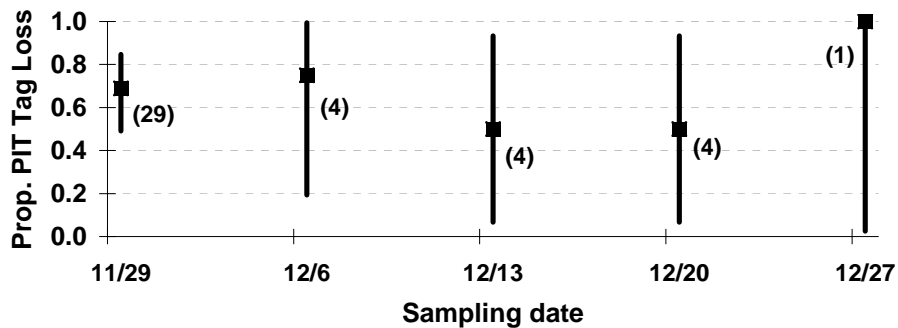
B) Males

Figure 6. The proportion of A) female and B) male PIT tags lost over time in Skagit Hatchery adult coho salmon (adapted from Figure 15 in Prentice et al. (1994)). Weekly proportions with 95% binomial confidence intervals are shown with sample sizes in parentheses.

In our study we did not dissect fish that were determined to have lost their PIT tag, and thus we cannot rule out that in some cases the PIT tag was actually present, but not functioning. However, earlier work by 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. Thus, our detections of PIT tag codes as fish volitionally exited their raceways more than 70 days after being placed there should be indicative of viable tags. We have no reason to believe that PIT tag failure was greater than average in our releases and thus likely contributed 1% or less to the overall observed PIT tag loss.

While we found that PIT tagged fish were smaller on average than non-PIT tagged fish, the body size differences despite being statistically significant in age 4 returns was on average 1.1 cm and 0.1 kg. Since our results showing no significant effect on actual post-release survival of PIT tags once corrected for tag loss, this suggests that these body size differences are relatively small in terms of biological significance and impacts on survival. We are aware of only one other study comparing the effects of PIT tags on adult body size of free ranging adult Pacific salmon. Prentice et al. (1994) compared PIT and snout-CW tagged adult coho salmon and found that the fork length of PIT tagged adults was significantly smaller by 2.0 cm. Prentice et al. (1993) also

compared PIT tagged and control (untagged) chinook salmon reared in net pens for 18 months and found that PIT tagged fish were 2 cm smaller.

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. 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 result should be a valid relative survival comparison. Caution should be exercised when making extrapolations to the un-PIT tagged portion of the population unless the effects of PIT tags on study fish have been investigated and taken into consideration.

Our results demonstrate that PIT tags are shed in hatchery spring chinook post-release at rates averaging 17% and there was no indication that tag shedding increased significantly with age. Thus, most tag loss occurred within the first 6 months after release. Without correcting for tag loss, estimates of survival based on actual PIT tag recoveries were significantly underestimated. After correcting for PIT tag loss, PIT tag fish survivals were comparable to untagged fish. PIT tagged adults were smaller in length and body weight than fish not PIT tagged and migration timing within the upper Yakima River was not significantly affected by the presence of PIT tags.

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