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Comparing the Reproductive Success of Yakima River Hatchery- and Wild-Origin Spring Chinook

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Comparing The Reproductive Success of Yakima River Hatchery- And Wild-Origin Spring Chinook

Yakima/Klickitat Fisheries Project Monitoring and Evaluation

Annual Report 2005

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Executive Summary

This year's annual report has been split into two separate parts. Part 1 describes our efforts to link behavioral traits in males to their reproductive success (RS) in the populations that were placed into the observation stream in 2001. The second part is an appendix (Appendix A) that describes similar analyses performed on hatchery and wild males placed into the observation stream in 2004. Below is an abstract that summarizes the results obtained from our comparisons of male RS in the 2001 populations:

Reproductive success in wild- and first generation hatchery-origin spring Chinook males was examined by allowing the fish to compete for spawning opportunities in two sections of an observation stream. Behavioral observations were used to characterize the frequency of aggression and courting activities. Microsatellite DNA from each male and fry collected from the observation stream were used in pedigree analyses to estimate reproductive success. The coefficient of variation in male reproductive success equaled 116 and 86% in the two populations. No differences were detected in reproductive success due to hatchery or wild origin. Nor were any behavioral differences found between hatchery and wild males. Although statistical power was low due to intrinsic variation a great deal of overlap existed in the reproductive success values of hatchery and wild males. Significant disparities existed among the males on their ability to produce offspring. Males achieving high reproductive success mated with numerous females, were socially dominant, aggressive, and tended to stay in localized areas, courting and spawning with females that were adjacent to one another.

Similar analyses were performed on data collected from the males spawning in the observation stream in 2004. Many similarities occurred. For example, the coefficient of variation in male RS in the 2004 population equaled 69% a value that was slightly lower than what had been observed in the 2001 populations but still one indicating that extensive variation in male RS had occurred. Additionally, no difference was seen in the RS of hatchery or wild males. As in the 2001 populations we feel this lack of difference was likely caused by the high degree of overlap occurring in the RS values of hatchery and wild males rather than from a lack of power brought about by high variation. Moreover, as in the 2001 populations, male body size did not strongly affect RS. As suggested above, one possible difference in the 2001 and 2004 populations was the reduced variation in male RS in the 2004 population. We speculate that may have occurred because of the greater area females had to establish nest locations. When females are widely dispersed and simultaneously ready to spawn it makes it difficult for a few males to monopolize most of the spawning opportunities.

Our analyses so far indicate that first generation hatchery spring Chinook males produced by the YKFP are not noticeably impacted by inadvertent domestication. Information from an additional four populations will be used to further test and refine this conclusion and also to examine the effects of hatchery conditions on female RS.

A Comparison Of Reproductive Success In Naturally Spawning Wild- and Hatchery-Origin Male Spring Chinook

Abstract

Reproductive success in wild- and first generation hatchery-origin spring Chinook males was examined by allowing the fish to compete for spawning opportunities in two sections of an observation stream. Behavioral observations were used to characterize the frequency of aggression and courting activities. Microsatellite DNA from each male and fry collected from the observation stream were used in pedigree analyses to estimate reproductive success. The coefficient of variation in male reproductive success equaled 116 and 86% in the two populations. No differences were detected in reproductive success due to hatchery or wild origin. Nor were any behavioral differences found between hatchery and wild males. Although statistical power was low due to intrinsic variation a great deal of overlap existed in the reproductive success values of hatchery and wild males. Significant disparities existed among the males on their ability to produce offspring. Males achieving high reproductive success mated with numerous females, were socially dominant, aggressive, and tended to stay in localized areas, courting and spawning with females that were adjacent to one another.

Introduction

The use of hatcheries as conservation agents for depressed salmonid populations has become a common management strategy in North America. In some cases, naturally produced and local origin fish (wild broodstock) are brought into a hatchery for breeding and subsequent rearing prior to being released into their natural habitat. The concept of using native broodstock and recycling them through artificial culture until abundance increases or becomes stabilized has been referred to as supportive breeding (Laikre and Ryman 1996). It is however, a controversial strategy. Behavioral, morphological, and physiological divergences have been observed between hatchery and wild salmonids (Fleming and Gross 1992; Petersson and Järvi 1993; Lura et al. 1993; Petersson et al. 1996; Fleming et al. 2000; Fleming and Petersson 2001; Knudsen et al. *In press*).

Two paradigms have been proposed to explain why salmonids cultured in hatcheries are genetically and phenotypically different than wild cohorts. The first proposes that natural selection pressures have been significantly relaxed in hatcheries (Einum and Fleming 2001; Lynch and O'Healy 2001). Consequently, fish that normally would have perished because of the possession of unsuitable traits are able to survive. If the traits have a genetic basis, they may become established in a population. The second theorizes that environmental and social conditions in hatcheries are less variable than in the natural environment and that these conditions will remain relatively constant from one generation to the next. In this circumstance, selection for genetic traits that adapt fish to artificial culture will become prevalent. Moreover, characteristics that are favored in a hatchery environment may negatively impact individuals when they reside in natural

environments (Einum and Fleming 1997; McGinnity et al. 1997; Fleming et al. 2000; Einum and Fleming 2001; Dannewitz et al 2004).

A growing body of literature, for example suggests that salmonids produced by artificial culture are not as reproductively successful as wild fish when they spawn under natural conditions (Fleming et al. 1996; Peterssson and Järvi 1997; reviewed in Fleming and Petersson 2001; Hansen et al. 2001; McLean et al. 2004; Weir et al. 2004;). Dannewitz et al. (2004) point out, however, that many of these studies compared the reproductive success (RS) of non-local hatchery fish with native salmonids or with fish that had experienced multiple generations of hatchery exposure. Few efforts have compared RS when both hatchery and wild fish possess a common genetic history (Dannewitz et al. 2004). Several exceptions have occurred. The RS of hatchery and wild Atlantic salmon (*Salmo salar* L.) from the same population were compared by Fleming et al. (1997). They found no differences in the reproductive success of females. Hatchery males, however, were found to be reproductively inferior to their wild counterparts. Dannewitz et al. (2004) performed two similar experiments with brown trout (*S. trutta* L.). In the first one, the RS of seventh generation hatchery fish was compared with wild fish when both were allowed to spawn under natural conditions. No significant differences in RS were found in either males or females. In the second experiment, wild fish were brought into a hatchery and artificially spawned. Their offspring were reared, selectively marked and released. When these first generation hatchery fish returned they were allowed to spawn with seventh generation hatchery fish under natural conditions. In this instance, males that originated from wild parents were more successful at producing

offspring; no differences in RS between first and seventh generation hatchery females were observed (Dannewitz et al. 2004).

Inadvertent domestication caused by hatchery culture appears to affect the reproductive success of males more strongly than it does females (Fleming and Gross 1993; Fleming et al. 1996 and 1997; Fleming et al. 2000; Fleming and Petersson 2001). Because males are reproductively active for longer periods than females the Operational Sex Ratio (OSR, Emlen and Oring 1977) in salmonid spawning communities is often heavily skewed, causing males to compete for relatively few sexually active females. Under such circumstances, the effects of changes in maturation timing, body size and shape, and behavioral traits like aggressiveness on reproductive success can be magnified.

The principal objective of our study was to compare the reproductive success of anadromous hatchery and wild origin spring Chinook (*Oncorhynchus tshawytscha*, Walbaum, 1792) males by allowing them to compete for spawning opportunities under quasi-natural conditions. The hatchery fish had experienced a single generation of artificial culture and were derived from spring Chinook native to the upper Yakima River, Washington State. Spawning occurred in an observation stream that had been built on the grounds of the Cle Elum Supplementation and Research Facility (CESRF). Extensive behavioral observations were made on the fish while they reproduced. In addition, DNA samples were obtained from each adult fish and from a sample of their progeny making it possible to estimate how many offspring each fish had produced. The behavioral data were used to ascertain what factors affected male RS in spring Chinook

while the results of pedigree assignments were employed to compare the RS of hatchery and wild males.

Materials and methods

Origin and collection of wild and hatchery fish

The CESRF began operation in the upper Yakima River in 1997. One purpose of this facility was to examine the effects of inadvertent domestication on wild spring Chinook caused by exposure to hatchery conditions. Prior to the initiation of the CESRF program negligible introductions of hatchery Chinook had occurred in the Upper Yakima River making this a native population with little or no hatchery influence. The hatchery fish used in our study were the progeny of the first wild spring Chinook used as broodstock in the CESRF while the wild fish were free of any known hatchery ancestry.

Both hatchery and wild adults were collected in the upper Yakima River from April through August 2001 at the Roza Adult Monitoring Facility (RAMF). This building is attached to Roza dam (rkm 206) an irrigation diversion structure that all upper Yakima River salmon must pass through before they can reach their spawning grounds. Hatchery fish produced from the CESRF have their adipose fins excised, are tagged with coded wire, marked with elastomer, and 5% also possess PIT tags. These tags and marks made it possible to separate hatchery and wild adults when they were captured at RAMF. A representative sample of adults of each type was collected using methods described by Knudsen et al. (2006). Collected fish were transported 81 kms to CESRF and held in a 30.5 m long x 4.6 m wide x 3 m deep holding pond until reaching maturation.

Beginning in early September 2001, adult Chinook were examined weekly to determine if they had reached maturity. Males were assumed to be mature if pressure on

their posterior lateral surfaces caused them to extrude milt. Maturation status in females was ascertained by assessing the firmness of their ventral surface; those with a soft ventral surface that sagged slightly when the female was pointed head down were regarded as ripe. The fish were also inspected for deformities, skin abrasions and general vigor. Ripe fish with detectable abnormalities or that were in poor condition were not used. Selected fish were sequestered and allowed to recover for 24 h before being introduced into an observation stream.

Prior to placement into the stream each fish was anesthetized in a solution containing one part of MS222 to 19 000 parts of water (Bell 1964). Once docile, the fish were weighed to the nearest gram on an electronic balance, had their fork lengths taken, and were tagged with numbered and color coded 3.8 cm diameter Petersen disks. To facilitate later observations, the sexes received different colored tags and the tags within a sex had unique numbers. While the fish were being tagged, a 4 to 6 mm^2 piece of fin material was taken from the ventral edge of the dorsal fin and placed in 100% ethanol for later microsatellite DNA extraction. After being tagged, the fish were placed into 124 *L* insulated coolers filled with freshwater and transported 200 m to the observation stream where they were released. Two groups of fish were placed into the observation stream in 2001. The first group of hatchery and wild fish were released into the upper part of the stream on Sep 12 while a second group of fish was introduced into the lower part of the stream on Sep 19. The number of hatchery and wild fish placed into the stream on each date, and the range and mean of their body weights is shown in Table 1. Twelve precocious males, seven hatchery and five wild, were also added to the lower population.

Table 1. Number and body weight of the hatchery and wild spring chinook placed into the observation stream

Population	Sex[a]	Origin	No.	Mean Body Weight (kilos)	Range In Body Weight (kilos)
	Male	Hatchery	10	3.24	2.14 – 4.95
	Jack	Hatchery	1	1.45	-
Upper	Male	Wild	12	4.69	2.03 – 7.46
	Jack	Wild	2	1.48	1.42 – 1.53
	Female	Hatchery	10	4.07	3.07 – 4.76
	Female	Wild	10	4.57	2.10 – 6.57
	Male	Hatchery	7	3.54	2.60 – 5.25
	Jack	Hatchery	2	1.25	1.11 – 1.38
Lower	Precocious	Hatchery	7	0.12	0.08 – 0.16
	Male	Wild	11	3.56	1.78 – 4.90
	Precocious	Wild	5	0.01	0.01 – 0.01
	Female	Hatchery	8	3.70	3.11 – 4.28
	Female	Wild	10	3.99	3.18 – 4.88

[a] Jacks are anadromous males that matured at age 3, one year earlier than most (>80%) of the spring chinook that return to the upper Yakima River. Precocious males matured in freshwater at age 1 (hatchery) or age 0 (wild).

Observation Stream

Behavioral observations on spawning hatchery and wild spring Chinook were made in an observation stream that was constructed on the grounds of the CESRF in 2000. The structure is 127 m long x 7.9 m wide and is shaped like a "U". It is subdivided into seven subsections: a curved "elbow" that is 21 m long x 7.9 m wide and six straight sections each measuring 15.2 m x 7.9 m. Each subsection is level but a 30 cm drop occurs between a cross weir and the subsection that lies directly downstream of it. A profile of the stream would resemble a staircase with 15 m long level steps separated from one another by 30 cm risers. The banks of the stream have 2:1 slopes that are armored with river rock 10 to 30 cm in diameter and when in operation its wetted width ranges between 4.3 to 5.5 m. The streambed is lined with geotextile to prevent water loss and is filled with 90 cm of stream gravel that ranges in size from 7.1 to 100 mm in diameter. When the gravel was first placed into the stream in August of 2000 it had a Fredle Index

(Lotspeich and Everest 1981) value of 10.6. Discharge water from the raceways at the CESRF is pumped into the observation stream from September through May and flows are adjusted so that velocities vary from 0.1 to 2.0 m per second and total discharge averaged 0.37 m^{-3}·s. Depth was maintained by stop logs placed into the cross weirs and averaged 0.4 m. These criteria were patterned after the velocities and depths that naturally spawning Chinook have been observed to use (Healey 1991; Bjornn and Reiser 1991). A 2.1 m high wall of camouflage netting was installed on both banks of the stream. Openings at eye level were cut into the netting every 2 m along its length to facilitate fish viewing.

The observation stream was divided into two equal parts referred to as the upper and lower sections. Each part possessed three of the straight subsections and was therefore 45.6 m long x 7.9 m wide. Fish introduced into the upper or lower sections were prevented from migrating into other parts of the stream by pickets, however; they were able to move freely within the three subsections contained in their portion of the stream. Every subsection had a grid system made of 0.6 cm nylon cord that was stretched approximately 30 cm over the surface of the water. The squares in the grid measured 1.5 m wide x 3 m long and each was provided with a unique alphanumeric designation so that fish movements and locations could be recorded.

Behavioral Observations

After fish were placed into the observation stream, up to five observers used audiotape recorders to dictate the activities of individual fish. These observations occurred during daylight hours and lasted for as long as females continued to spawn, usually 48 to 72 h. Individual fish were observed using Focal Animal Sampling methods

(Altmann 1974) from 4 to 10 continuous minutes and then another fish and its activities would be recorded. Consequently, every fish was periodically observed throughout each day. At the beginning of each recorded observation the date, time, and stream location were noted. Four categories of information were recorded for males. Their social status, i.e. were they the primary courting male associated with a female (alpha male), or positioned immediately behind or to one side of a courting pair (satellite male) or an individual that was not associated with a female (wandering). The occurrence of any courting behavior they exhibited. In Chinook this consists of crossing over (swimming back and forth over the caudal peduncle or back of a female), quivering (darting movements toward the female's side accompanied by short and rapid quivering of the head and body) and gentle nudges into the female's side. Additionally, Chinook salmon exhibit an array of color patterns; almost black, light gold or blonde with elliptical black spots, or fish with a single pronounced dark stripe on their lateral sides and white or grey bellies. These three patterns were identified as black, medium, or stripe and the color pattern a male possessed was periodically noted.

Finally, the agonistic interactions a male experienced were described. The sex and tag numbers of the fish he attacked and who attacked him were recorded along with the type of attack. These were categorized as chases, body rams, bites, lateral displays and parallel swimming. Lateral displays occurred when a male swam upstream of an opponent, raised his dorsal fin, turned sideways, and displayed his lateral surface to an opponent while drifting downstream. Parallel swimming attacks occurred when two fish swam parallel to one another with raised dorsal fins and repeatedly rammed their heads against the sides or heads of their opponents while swimming upstream.

The audiotapes containing these observations were transcribed by hand using symbols and normal English to create continuous ethograms that were divided into one minute time periods. These records were used to create two databases; one described the social status, color patterns, and frequency of courting activities of each male. The other dealt with the frequency and type of agonistic interactions individual males experienced. Because data on each male were chronologically organized it was also possible to track the movements of individual males throughout each observation day. Information contained in the databases were use to create nineteen measures of behavior (Table 2). Nine of them described the agonistic interactions a male experienced and another nine summarized the social status of each male. Male social status was divided into two aspects, over all dominance and also the types of associations he had with prospective mates. The last behavioral trait measured male movement and two other attributes were used to characterize the size of each male.

The agonistic traits measured included *Ago+PerObs* that equaled the mean number of attacks per observation period instigated by a male. The percentage of observation periods a male was observed attacking other fish regardless of their gender was represented by the trait *PctObsAgo+*. *AllDom* represents the percentage of the attacks a male was involved with that he instigated while *MxMAgo* and *MxFAgo* were subsets of this variable. The first one, *MxMAgo* equaled the number of attacks a male inflicted on other males divided by the total number of male x male attacks he experienced. Similarly, *MxFAgo* represented the number of attacks made on females

Table 2. Definitions of the traits used to characterize the behavior and size of spring chinook males spawning in the observation stream.

Trait Category	Trait Name	Definition of Trait	Type of Data
Agonistic	AllDom	Percentage of attacks instigated by the observed male on opponents of both sexes	Percentage
	Ago+PerObs	Mean number of attacks instigated by the male on all fish per observation min	Mean
	PctObsAgo+	Percentage of all observation min where a male attacks opponents	Percentage
	Ago-PerObs	Mean number of attacks a male received per observation period	Mean
	PctObsAgo-	Percentage of all observation min where a male is attacked by opponents	Percentage
	MxMAgo	Percentage of all male x male attacks that were instigated by the observed male	Percentage
	MxFAgo	Percentage of all male x female attacks that were instigated by the observed male	Percentage
	FemAgos	Percentage of observation min where the observed male is attacked by female(s)	Percentage
	FemAgoObs	Mean number of attacks a male received from females per observation min	Mean
Social Status Agonistic	AllRivals	Percentage of all the males in a population dominated by a male	Percentage
	DomObsOpp	Percentage of observed opponents a male dominated	Percentage
	SubObsOpp	Percentage of observed opponents that dominated a male	Percentage
	Black	Percentage of observations a male possessed the black color pattern	Percentage
	Stripe	Percentage of observations a male possessed the stripe color pattern	Percentage
Social Status Courting	Court	Percentage of observations where a male performs a courting behavior	Percentage
	LinkFem	Percentage of females in a population male was an alpha or satellite to	Percentage
	Alpha	Percentage of observation min a male is courting and guarding a female (alpha status)	Percentage
	SatAlpha	Percentage of observation min a male is an alpha or satellite (an individual that is positioned behind a pair who is dominated by the alpha male)	Percentage
Size	RelSize	The proportion of the male population that an individual was larger than	Percentage
	Body Wt.	Body weight in kilograms	Weight
Move	Move	The probability of moving from one section to another in the observation stream in a 30 min period.	Percentage

divided by the total number of male x female agonistic interactions a male experienced. Two other variables quantified the percentage of observation periods a male was attacked by one or more females (*FemAgo*) or described the mean number of attacks a male received from females per observation period (*FemAgoObs*). Additionally the percentage of observation periods where a male was attacked by males or females was quantified (*PctObsAgo-*) as well as the mean number of attacks he received from both sexes per observation period (*Ago-PerObs*).

The social status variables that summarized male dominance were *Allrivals*, which equaled the percentage of all potential male rivals in a population a fish dominated. Dominance was determined by examining the agonistic interactions observed between a male and the fish he interacted with. If he attacked an opponent more often than an opponent attacked him he was regarded as dominate. Males did not interact with all the males in their population. Consequently, a companion dominance variable (*DomObsOpp*) was developed that assessed the percentage of males that an individual interacted with that he dominated. The converse of this trait was *SubObsOpp*, which equaled the percentage of observed opponents that dominated a male. *Black* and *Stripe* were variables that represented the percentage of observations that described a male's color pattern that fell into those categories. The black pattern in this species is linked to dominance while the stripe is associated with sub-dominance.

Status traits associated with courting included two variables used to represent the percentage of time a male was classified as an alpha male (*Alpha*) or as an alpha plus satellite male (*SatAlpha*). Another variable called *LinkFem* equaled the percentage of females in a population a male was associated with either as an alpha or satellite male.

Court equaled the percentage of observation periods where a male executed one or more courting activities

Two other variables were use to quantify size, one was the body weight of the fish in grams and was called *BodyWt*. The other *RelSiz* equaled the probability of a male encountering a smaller opponent and was calculated by dividing the number of males smaller than the target male by the total number of potential male rivals in his population. A final trait, referred to as "*Move*" converted movement information collected on males into binomial data. That was done by subdividing each observation day into 30 min increments and then observing if a male moved from a subsection of the observation stream into another one within that time interval. If he did, the time period received a score of one, if he did not the time period was given a score of zero. The movement scores a male received were summed and divided by the total number of time periods he was observed to estimate the probability a male would move from one subsection to another in a 30 min time period.

Pedigree assessment

Modified fyke nets with floating live boxes were installed at the ends of the upper and lower sections of the observation stream in mid January 2002, several weeks prior to fry emergence. The live boxes were checked daily, captured fry were counted and sampled by placing ten percent of the catch into labeled jars containing 100% ethanol. This procedure was continued until fry migration ceased, at that time seines and electro-shocking gear were used to remove all the Chinook fry rearing in the stream so that they could be counted and sampled. Our goal was to obtain a sample of 1000 fry from each portion of the stream. More than this number were collected; consequently the number of

fry analyzed from each day's sample was reduced by a constant percent to produce a representative sample of 1000 fry.

Genomic DNA was extracted by digesting fin tissue in 5% chelex (BioRad Chelex 100 resin) solution containing 0.4 mg proteinase K (Sigma). Following digestion at 65°C for 180 min, the samples were heated for 10 min at 95°C to denature proteins. The DNA extracts were stored at 5°C until all analyses were completed. Adults and fry were genotyped at 11 loci (Table 3). The number of alleles per locus among the candidate parents ranged from five at Ots-1 to 40 at Ots-100. The exclusionary power or the average probability of excluding a single randomly-chosen unrelated individual as a candidate parent for a randomly drawn genotype from the population with neither parent known ranged from 0.183 at Ots-1 to 0.841 at Ots-100. The estimated exclusionary power with neither parent known for the suite of 11 loci was 0.9998.

The polymerase chain reaction mixture contained the following for a 5 µl reaction: approximately 25 ng template DNA, 1X Promega buffer, 1.5 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP, and dTTP, approximately 0.1 µM of each oligonucleotide primer, and 0.05 units *Taq* polymerase (Promega). Amplification was performed using an MJ Research PTC-200 thermocycler. The thermal profile was as follows: an initial denaturation step of 3 minutes at 92°C; 35 cycles of 15 s at 92°C, 30 s at 49-58°C, and 1 min at 72°C; plus a final extension step at 72°C for 30 minutes, followed by a final indefinite holding step at 4°C.

Microsatellite DNA loci were amplified via the polymerase chain reaction (PCR) using fluorescent-labeled primers obtained from Applied Biosystems or Integrated DNA

Table 3. The loci and the number of alleles each possessed that were used to make the pedigree assignments. Cumulative exclusionary power equals the summed probability of excluding a single randomly chosen unrelated individual as a candidate parent, starting from the first listed locus.

Locus	No. Of Alleles	Cumulative Exclusionary Power
Ssa-197[a]	21	0.7830
Ots-101[b]	24	0.9668
One-8[c]	13	0.9898
Ogo-2[d]	10	0.9961
Ogo-4[d]	11	0.9987
Ots-3M[e]	8	0.9993
Ots-1[f]	5	0.9995
Oci-1[f]	7	0.9997
Ots-100[b]	40	0.9999
Ots-2M[e]	8	0.9999
Ots-107[g]	21	0.9999

[a] O'Reilly et al. 1996
[b] Small et al. 1998
[c] Scribner et al. 1996
[d] Olsen et al. 1998
[e] Banks et al. 1999
[f] Condrey and Bentzen 1998
[g] Nelson and Beacham 1999

Technologies. Data were collected using an ABI-3100 Genetic Analyzer. Applied Biosystems Genemapper v.3.0 software was used to collect and analyze the raw data and to determine genotypes at each locus. Allele identification on sampled fry was attempted on all eleven loci. However, in some instances, allele identification at all loci was not possible. Fry, however, had to be genotyped at six or more loci before being assigned to their adult parents.

Pedigree analyses involved comparing genotypes of candidate parental pairs with offspring genotypes. Genotyping errors can produce parent-offspring mismatches and suggest exclusion of true parent-offspring pairings. Alternatively, genotyping errors can lead to failure to exclude parent-offspring pairings that are incorrect. A maximum likelihood procedure in Cervus 2.0 (Marshall et al. 1998) was used to infer parent-

offspring relationships. The procedure uses allele frequency data to assign likelihoods to parent-offspring combinations and allows mismatching genotypic data to be evaluated concurrently with matching genotype data.

Statistical analyses

The pedigree assessments determined how many fry each male fathered out of the juveniles that were sampled from each portion of the observation stream. These numbers were converted into percentages that were normalized by the arc sin square root transformation (Zar 1999). The transformed percentages were used to estimate the RS (the capacity to produce fry) of each male placed into the observation stream. A Model I two-way ANOVA was performed to determine whether hatchery and wild males differed in their ability to produce offspring. The fixed factors in the analysis were population (upper or lower sections) and male type (hatchery or wild). The dependent variables were the estimates of each male's reproductive success.

Backward stepwise multiple regressions (Zar 1999) were used to examine the importance of behavioral traits and body size on male reproductive success. An initial model with all the variables included was produced and then a stepwise process was used to determine which variables should remain in the model. A p-value of ≤ 0.15 was used to determine if a variable should be retained in the model. Prior to performing the stepwise regressions, correlation analyses were performed to determine if collinearity existed between any of the independent variables. Tight correlations among independent variables used in stepwise regression may produce regression coefficients that do not reflect their true values, provide beta coefficients with incorrect slopes and exclude predictors with true significance from a final model (Grapentine 1997; Graham 2003).

Collinearity was discovered among the behavioral traits that were measured and three approaches were used to minimize its effects. First, in a number of instances the independent variables measured had a high degree of redundancy. When that occurred one was chosen to represent a group of variables that were apparently measuring the same general attribute. Second, a method Graham (2003) referred to as residual/sequential regression was employed. In this instance, one of two highly correlated variables was assumed to be the most functionally important. The less important variable was regressed against the more important one and replaced by the residual values produced by the regression procedure. And third summated scale data (Grapentine 1997) were used. These variables were calculated by adding the values of traits that measured male agonistic or social status and then dividing each total by the number of traits that were summed. Once the stepwise regressions were run, outliers and observations identified as having excessive leverage were removed and analyses were repeated. Spurious data were identified by SYSTAT 11 the computer software used to carryout the analyses (SYSTAT Software Inc. 2004).

A Pearson product moment correlation analysis was performed to determine if the factors affecting male reproductive success were similar in the two populations. In this analysis the "r" values obtained from correlating specific traits to male reproductive success in each population were correlated against one another. Ordinary Least Squares regression analyses were used to examine the importance of body size (independent variable) on male reproductive success (dependent variable). Moreover, a series of Model I two-way ANOVAs were employed to investigate whether hatchery- or wild-origin males possessed different behavioral traits.

Similar analyses were performed to examine how specific behavioral traits affected male reproductive success regardless of origin (hatchery or wild). In these analyses, the males in each population were split into three groups based upon their reproductive success scores. One group consisted of individuals that had produced no offspring; another to those that were moderately successful (0.13 to 2.4% of the sampled fry) and the last group consisted of males that had achieved the highest reproductive success values in their population (3.6 to 24.8% of the sampled fry). In these tests the fixed treatments were population (upper or lower section) and male type (low, moderate, and high reproductive success groups). When significant differences occurred, Tukey's multiple comparison tests were used to compare the mean values expressed by each type of male.

Results

The pedigree analyses disclosed the reproductive success (RS) of males placed into the observation stream was quite variable. The coefficient of variation (CV) of male reproductive success, for example, equaled 116% in the 4 and 5-yr-old males placed in the upper section of the stream and 86% in the lower section. Figure 1 shows the percentage of fry in each sample that had been produced by every anadromous male placed into the stream. Out of the 40 males (39 four-year-olds and 1 five-year-old) evaluated 15 or 37.5% of them contributed no progeny to the fry samples. Conversely, three males in the upper section sired over 56% of all the fry sampled and four males in the lower section produced over 52% of the fry that had been sampled from their population. Consequently relatively few males were responsible for most of the progeny

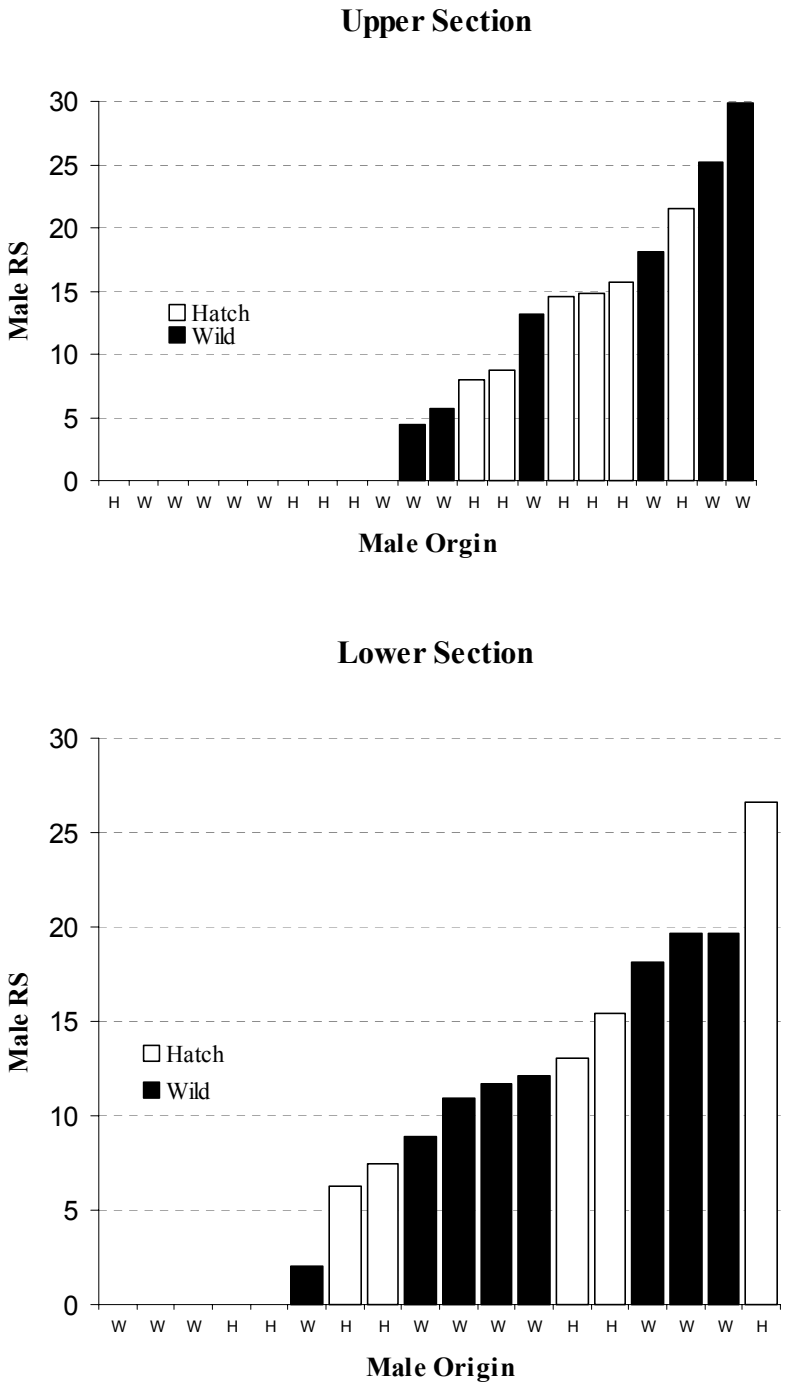

Figure 1. The percentage of fry in the pedigree sample fathered by hatchery- and wild-origin spring Chinook males placed into the upper and lower sections of the observation stream.

produced in each section. The pedigree analysis also identified which females spawned with each male but not the number of times they may have spawned. These data showed that anadromous males that spawned with numerous females produced more offspring than those that had spawned with fewer individual females (Figure 2).

The 12 precocious males in the lower section sired 16% of the fry that were sampled from that population. The coefficient of variation for reproductive success in these fish equaled 100% and was thus comparable to that seen in the anadromous males. Hatchery-origin precocious males were all 1 yr-olds and had an average fork length of 191 mm (range 142 – 227 mm). They were substantially larger ($p < 0.001$) than the wild 0 age precocious fish that averaged 81 mm (range 77 – 87 mm FL). Only one of the five wild precocious males sired any fry while all seven hatchery precocious males accounted for one or more fry sampled from the lower section. This difference in RS between ($p = 0.002$) hatchery and wild precocious males was probably caused by body size as we found a positive relationship between precocious male fork length and RS ($r^2 = 0.70$, $p < 0.001$). Like anadromous males, there was also a positive relationship between male RS and the number of females they spawned with ($r^2 = 0.72$, $p < 0.001$). The maximum number of females that precocious males mated with was four as opposed to ten in the anadromous males.

The two-way ANOVA that examined whether anadromous hatchery and wild males had different RS values failed to reject the null hypothesis that hatchery and wild males were similar ($p = 0.950$). In addition, no section effect was seen ($p = 0.592$) nor was there a significant interaction effect between section and male type ($p = 0.982$). The two-way ANOVAs used to assess whether anadromous hatchery and wild males

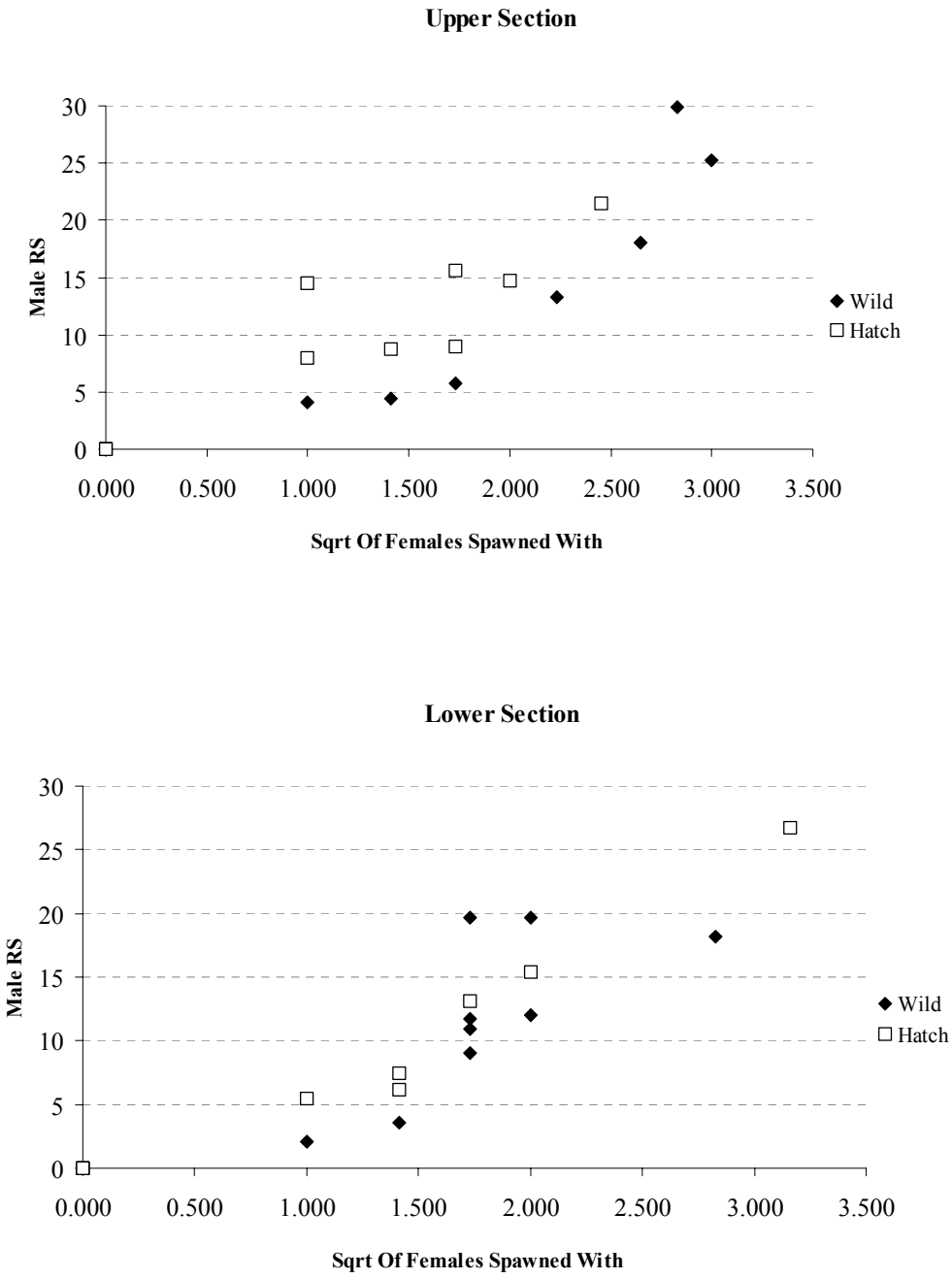

Figure 2. The relationship between male RS and the number of females they spawned with for hatchery- and wild-origin spring Chinook males spawning in the observation stream.

possessed different behavioral traits showed that hatchery and wild males differed from one another in one instance; hatchery males, on average received more attacks from neighboring fish (55% of their observation minutes) than did wild males (36% of their observation minutes). No differences were seen between hatchery and wild males in their ability to court females, to attack rivals, or in their general movement patterns (Table 4).

Prior to performing stepwise regressions, all variables that could potentially be used in the analyses were correlated against one another to test for collinearity. Table 5 presents the r-values of each trait correlated with male RS. In general, traits that impacted RS in one population had similar effects in the other. For instance, a Pearson correlation that matched the r-coefficients obtained by relating traits to male RS in both populations was highly significant ($r = 0.97$, $p < 0.001$). In addition, many of the traits measured were highly correlated with one another indicating that collinearity had to be considered before stepwise regressions could be conducted (Table 6). Traits that measured similar attributes were grouped and then approaches designed to minimize the effects of collinearity were applied.

The stepwise regression model for the Lower section had three variables, *SatAlpha, BlackRes* (the residual values obtained from regressing *Black* onto *SatAlpha*), and *Move*. It had an adjusted multiple r^2 of 94.0% and its overall p-value was <0.001. Initially, the model had an additional variable, *RelSize* but it was subsequently removed during the backward stepwise regression procedure because its p-value (0.220) was greater than the one established for inclusion in the model ($p < 0.150$). Consequently, in this population, males that were alpha or satellites, exhibited high social status, were dominant over rivals, and did not make extensive movements realized high RS values.

Table 4. Results of 2-way ANOVAs that tested whether there were differences between the expression of behavioral traits in males caused by their population origin (upper or lower), type (hatchery or wild) or if an interaction existed between population origin, male type and the expression of a behavioral activity.

Trait Category	Trait Name	N	Pop (Low vs. Up) p	Hatch vs. Wild p	Interaction p	Results of 2-Way ANOVAs (Observed Differences)
Agonistic	AllDom	40	0.591	0.147	0.120	No differences were observed
	Ago+PerObs	40	0.452	0.332	0.237	No differences were observed
	Pct ObsAgo+	40	0.824	0.388	0.211	No differences were observed
	Ago-PerObs	40	0.023*	0.163	0.106	Males in the lower population were attacked more often per observation min than those in the upper population
	PctObsAgo-	40	0.856	0.031*	0.500	Hatchery males received more attacks per observation min than wild males
	MxMAgo	40	0.675	0.138	0.134	No differences were observed
	MxFAgo	40	0.877	0.110	0.192	No differences were observed
	FemAgos	40	0.017*	0.287	0.171	Males regardless of type had a higher incidence of female attacks in the lower population
	FemAgoObs	40	0.013*	0.997	0.124	Males in the lower population had a higher mean number of female attacks per observation min
Social Status Agonistic	AllRivals	40	0.593	0.113	0.252	No differences were observed
	DomObsOpp	40	0.914	0.146	0.101	No differences were observed
	SubObsOpp	40	0.780	0.210	0.240	No differences were observed
	Black	40	0.655	0.217	0.147	No differences were observed
	Stripe	40	0.342	0.307	0.546	No differences were observed
Social Status Courting	Court	40	0.602	0.478	0.498	No differences were observed
	LinkFem	40	0.783	0.540	0.986	No differences were observed
	Alpha	40	0.737	0.328	0.465	No differences were observed
	SatAlpha	40	0.490	0.508	0.802	No differences were observed
Size	RelSize	40	0.966	0.104	0.124	No differences were observed
Movement	Move	40	0.734	0.512	0.856	No differences were observed

Table 5. Results of Pearson correlation analyses between male traits and reproductive success in the lower and upper sections.

Trait Category	Trait Name	N	Lower Population	Upper Population
Agonistic	AllDom	45	0.688	0.709
	Ago+PerObs	45	0.721	0.762
	PctObsAgo+	45	0.713	0.734
	Ago-PerObs	45	-0.505	-0.534
	PctObsAgo-	45	-0.649	-0.558
	MxMAgo	45	0.717	0.706
	MxFAgo	45	0.558	0.639
	FemAgos	45	-0.466	-0.508
	FemAgoObs	45	-0.283	-0.391
Social Status Agonistic	AllRivals	45	0.541	0.747
	DomObsOpp	45	0.599	0.700
	SubObsOpp	45	-0.620	-0.701
	Black	45	0.740	0.704
	Stripe	45	-0.458	-0.442
Social Status Courting	Court	45	0.547	0.643
	LinkFem	45	0.417	0.713
	Alpha	45	0.681	0.738
	SatAlpha	45	0.610	0.763
Size	RelSize	45	0.606	0.511
	Body Weight	45	0.589	0.540
Movement	Move	45	-0.476	-0.535

The same four variables were used to model male reproductive success in the upper section. In this case, the final model had two variables, *SatAlpha* and *Move*. It possessed an overall r^2 value of 62.2% and its p-value was again <0.001. However, a greater amount of variation in male RS in the upper section was explained by using a model that had two summed-scale variables, *Status* and *AgoSum,* along with *RelSize*, and *Move*. The behavioral traits summed and averaged in the *Status* variable included *SatAlpha*, *Alpha*, *AllRivals*, *Black,* and *DomObsOpp*. To create *AgoSum* the following four agonistic traits: *Ago+PerObs, PctObsAgo+, AllDom*, and *MxMAgo*, were summed and averaged. When all four variables were present, the r^2 value of the model equaled 68.2%. After the stepwise procedure was completed only the *Status* variable remained. The p-value of the

Table 6. Correlation coefficients between selected traits used to measure aggression, social status, body size and movement patterns in anadromous spring Chinook males spawning in the upper section of the observation stream.

Traits		Agonistic				Social Status: Agonistic				Social Status: Courting			Size	Move
		All Dom	Ago+ Per Obs	MxM Ago	Fem Ago	All Rivals	Dom Obs Opp	Black	Stripe	Court	Alpha	Sat Alpha	Rel Size	Move
Agnostic	All Dom	-												
	Ago+PerObs	0.960	-											
	MxMAgo	0.991	0.957	-										
	FemAgo	-0.775	-0.704	-0.723	-									
Social Status: Agonistic	AllRivals	0.940	0.891	0.939	-0.744	-								
	DomObsOpp	0.928	0.880	0.935	-0.725	0.981	-							
	Black	0.859	0.837	0.831	-0.723	0.791	0.746	-						
	Stripe	-0.651	-0.562	-0.615	0.603	-0.578	-0.587	-0.629	-					
Social Status Courting	Court	0.887	0.831	0.854	-0.806	0.809	0.794	0.700	-0.723	-				
	Alpha	0.938	0.877	0.915	-0.816	0.878	0.856	0.811	-0.739	0.946	-			
	SatAlpha	0.830	0.793	0.786	-0.772	0.826	0.773	0.775	-0.593	0.863	0.913	-		
Size	Rel Size	0.779	0.718	0.757	-0.844	0.780	0.762	0.634	-0.737	0.770	0.803	0.677	-	
Move	Move	-0.512	-0.553	-0.522	0.426	-0.520	-0.542	-0.603	0.253	-0.331	-0.456	-0.440	-0.379	-

completed model was <0.001 and it explained 66.2% of the variation in male RS. The *Status* variable was an attempt to reify the capacity of a male to gain access to females (*SatAlpha* and *Alpha*) and to defend them by dominating potential rivals (*Allrivals, Black*, and *DomObsOpp*). In the upper section, the ability to perform these behaviors led to high reproductive success.

In past studies, large size (weight or length) has been linked to reproductive success in male salmon (Fleming and Gross 1993; Fleming et al.1996; Fleming et al. 1997). A variable associated with male size (*RelSize*) was incorporated into each of the stepwise regression analyses that were attempted. However, it was never included in any of the final models suggesting that this variable was relatively unimportant in determining RS. To further explore the importance of size two traits, *BodyWt* and *RelSize* were regressed against RS values obtained from the 4- and 5-yr-old anadromous males placed into the upper and lower sections. All four of regressions were significant (p-values ranged from 0.014 to 0.005), yet the regression coefficients were not large (r^2 ranged between 22.9 to 27.7%) when compared with other behavioral traits that were measured.

The ANOVAs used to determine if males achieving high, moderate, or no reproductive success had different behavioral traits corroborated the results of the stepwise regressions. In general, males that sired the most offspring were observed next to females more often than males in the moderate and low categories, plus they were more aggressive and they moved less frequently than those that had sired few or no progeny (Table7).

Table 7. Results of 2-way ANOVAs that tested whether there were differences between the expression of behavioral traits in males caused by their population origin (upper or lower), reproductive success (high, moderate, or low) or if an interaction existed between population origin, male reproductive type and the expression of a behavioral activity. Differences among high, moderate, and low male RS groups were determined by using Tukey's multiple comparison test.

Trait Category	Trait Name	N	Pop (Low vs. Up) p	High, Moderate, vs. Low RS p	Interaction p	Results of 2-Way ANOVAs (Observed Differences)
	AllDom	45	0.225	0.000	0.672	High > Moderate & Low
	Ago+PerObs	45	0.866	0.000	0.845	High > Moderate & Low
	Pct ObsAgo+	45	0.614	0.000	0.619	High > Moderate & Low
	Ago-PerObs	45	0.010	0.013	0.975	The mean number of attacks a male received in the lower population was higher than in the upper population; High Low Low Moderate
Agonistic	PctObsAgo-	45	0.975	0.004	0.782	Moderate Low > High
	MxMAgo	45	0.255	0.000	0.754	High > Moderate & Low
	MxFAgo	45	0.455	0.000	0.269	High > Moderate & Low
	FemAgos	45	0.018	0.016	0.358	In the lower population there were more female attacks on males; High Low Low Moderate
	FemAgoObs	45	0.045	0.195	0.566	On average, males in the lower population received more attacks from females than those in the upper population. No difference among male RS types
	AllRivals	45	0.815	0.000	0.342	High > Moderate & Low
Social	DomObsOpp	45	0.822	0.000	0.592	High > Moderate & Low
Status	SubObsOpp	45	0.932	0.000	0.750	Moderate & Low > High
Agonistic	Black	45	0.883	0.000	0.795	High > Moderate & Low
	Stripe	45	0.400	0.008	0.640	Low Moderate > High

Discussion

Fitness or the ability to move genes through time in male salmonids depends on their capacity to detect when egg deposition occurs, their ability to exclude rivals, and on sperm competition when more than one male spawns simultaneously with a female (Fleming 1998). Offspring survival and reproductive performance is also directly linked to fitness. How breeding abilities and offspring quality are affected by exposure to hatchery environments have become increasingly important questions since hatcheries have the potential to be important tools in salmon conservation and recovery. Previous studies have indicated that significant domestication effects can be induced by hatcheries and that such exposure reduces the ability of both sexes to reproduce under natural conditions.

Much of this work compared wild fish to those that had been cultured for multiple generations, were bred specifically for human consumption, or who had spent their entire lives under artificial culture. Some notable exceptions have occurred, Fleming et al. 1997 compared RS in wild and first generation hatchery Atlantic salmon originating from the same population. Dannewitz (2004) and Petersson and Järvi (1997) made similar comparisons using sea trout except that their hatchery fish had been subjected to multiple generations of culture. The results of these investigations revealed some hatchery effects, they were not, however, as severe as those found in more highly cultured fish. We had the opportunity to evaluate the biological consequences of hatchery exposure on a population of spring Chinook that had experienced a single generation of hatchery life. Not only did we want to determine if these fish would be affected by such exposure but

we also wished to explore relationships between behavior and reproductive success in hatchery and wild males that had originated from the same population. The capacity to make such evaluations was greatly added by the ability to carry out microsatellite-based pedigree assignments as they provided evidence of breeding success that could not be assessed by visual observation (*see* e.g. Garant et al. 2001)

The results of the ANOVA that evaluated whether hatchery and wild males had different mean RS values did not detect any difference between them. However, in ANOVA the ability to discern differences depends on the magnitude of the difference between the means being evaluated, the number of means being compared (power increases as number of groups decreases), the number of replicates or n, and on how much variation exists within the replicates (Zar 1999). In our case, differences in mean male RS values were minimal, 4.7% in the lower section and 3.1% in the upper section. In both cases, hatchery males had higher mean values. Replication averaged 10 males of each type per section, but variance in RS values was high. To discern differences in inherently noisy data like male RS values, large differences have to exist or numerous replicates of each type must be employed.

Only a few other studies have quantified individual variation in male RS in salmonids. Similar high levels were found. The CV in male RS, for instance, averaged 151% (range 60 to 268%) in Atlantic salmon studied by Fleming et al. (1996, 1997). The numbers of fish used in their study populations were comparable (6 to 12) to those we used. Additionally, Garant et al. (2001) reported that male RS had a CV of 77% in the Atlantic salmon males they studied. The capacity of a few dominant males to exclude

sexual rivals and participate in numerous spawning events is the likely cause of high variation in salmonid male RS.

In spite of such variation, previous studies that have examined the effects of hatchery culture on salmonids have shown 35 to 49% reductions in male RS after a single generation of exposure (Jonsson and Fleming 1993; Fleming et al. 1997). We did not find a comparable effect. The above two studies plus observations by Petersson and Järvi (1997) and Weir et al. (2004) found that hatchery-origin males were unsuccessful at maintaining dominance hierarchies around sexually active females. Consequently, when hatchery males did spawn, more than one male often participated. This reduced their breeding success from what it would have been if they had participated in a solo male spawning. Thus, poor defense of gravid females was cited as one of the principal reasons hatchery-origin Atlantic salmon and sea trout had lower RS values than the wild males they were competing with. We observed 23 spawning events in the observation stream. In every instance only one anadromous male was observed spawning with a female. Satellite males were seen in close proximity to pairs, yet alpha males were successful at driving them away just prior to ovideposition. It is possible that multiple male spawnings occurred during darkness when the ability of dominant males to detect rivals was limited by low light levels. If so, hatchery and wild alpha males appeared to have been equally affected.

Five multiple male spawnings were observed in the lower section and they all involved a single anadromous male with one or more precocious males. Initially both anadromous males and females would chase and bite at precocious males that were in the vicinity of a developing nest. In some cases as many as ten chases per minute were seen.

The shorter turning radius and cryptic color patterns possessed by these small males allowed them to escape almost all attacks. As spawning progressed the anadromous fish became habituated to their presence. Clear dominance relationships among the precocious males were observed. Large precocious males were able to drive smaller rivals to the rear or to one side of a nest. In a number of instances, two separate male dominance hierarchies were seen around a developing nest, one among anadromous males and the other among their precocious competitors. We believe the precocious males reduced the variation associated with anadromous male RS in the lower section because they fertilized eggs that would have just been available to an alpha male. This reduction in fertilizations decreased the disparity in offspring numbers produced by anadromous males in the lower part of the observation stream.

Other behavioral differences besides the ability to defend females from rivals have been observed in hatchery and wild salmonids. Salmonid males of hatchery or farmed origin exposed to multiple generations of artificial culture were found to be less aggressive, have reduced frequencies of courting behavior, and to react inappropriately when presented with spawning opportunities (Fleming and Gross 1992, 1993; Fleming et al. 1996; Petersson and Järvi 1997; Fleming et al. 2000; Weir et al. 2004). Conversely, no differences were found in these traits when first generation hatchery fish were compared against wild fish originating from the same population (Jonsson and Fleming 1993; Fleming et al. 1997).

The results of the ANOVAs we performed that examined whether differences in behavioral traits existed first generation hatchery and wild spring Chinook males disclosed only one difference and that was a tendency for hatchery males to be attacked

more often than wild males. The inability to detect behavioral dissimilarities must again be tempered by the power of the tests used. Since these traits were significantly correlated to male RS it was not surprising that their variances were also high. For example, CV values of many of them exceeded 200% in both hatchery and wild males. Consequently, high variances made it difficult to discern any small behavioral differences that may exist between these two types of males.

Three of the behavioral traits: mean number of attacks a male made per min (*Ago+PerOb*); mean number of attacks he received per min (*Ago-PerOb*); and mean number of attacks he received from females per min (*FemAgoObs*) had low variances and possessed CV values that ranged from 5 to 60%. Consequently, they provided us with an opportunity to see if behavioral differences in hatchery and wild males existed when power was relatively robust. Of these traits, *Ago+PerOb* was the one most closely associated with male RS. It explained 57% and 52% of the variation in male RS in the upper and lower sections. It indicated that males that had high attack rates on neighboring fish had greater RS values than those that were less aggressive. We believe it reflects a strategy commonly used by alpha males to attack all fish, regardless of sex that were within 3 to 4 m of a female they were courting. When successfully executed these assaults created a zone around a female where male rivals were excluded and simultaneously protected a courted female from being attacked by neighboring fish. This provided her with an opportunity to prepare and test a nest without interruption. No difference in this trait was observed between hatchery and wild males ($p = 0.332$). Its opposite, *Ago-PerOb* was negatively correlated to male success with r-values equaling –0.53 and –0.51 in the upper and lower sections. Sub-dominate males were often chased,

sometimes on an almost continuous basis, until they could find a refuge by lying along a bank or positioning themselves in areas that were not being used by territorial females. This variable was produced in an attempt to measure sub-dominance, and again no difference was observed between the two types of males ($p = 0.163$). The last trait, *FemAgoObs* was an average of how often females attacked a male during each observation minute. This was the least variable trait we measured (CVs ranged from 4.6 to 16.8%). A moderate relationship between this variable and male RS was observed in the upper section ($r = -0.467$, $p = 0.028$), while a non-significant one was obtained from the lower section ($r = -0.284$, $p = 0.254$). As in the other two traits, no difference was seen between hatchery and wild males ($p = 0.997$).

One additional comparison between hatchery and wild males was made. Garant et al. (2001) and Dannewitz et al. (2004) discovered male RS in Atlantic salmon and sea trout was related to the number of females they spawned with. Our pedigree analyses allowed us to identify the females each male spawned with, but not the number of times they may have spawned together, to create offspring. Regression analyses between male RS and number of females spawned with were highly significant ($p < 0.001$) in both populations and the r^2 values equaled 0.83 in the lower and 0.86 in the upper sections (Figure 2). The 2-way ANOVA used to examine the effects of male origin on the number of females spawned with, failed to reject all three null hypotheses; $p = 0.284$ for population, $p = 0.589$ for male type, and $p = 0.859$ for the interaction effect. This relationship helps explain the tendency for the males we observed to quickly break pair bonds soon after spawning. Generally, this occurred within 20 min or less. The tactic of quickly leaving and searching for other spawning opportunities did not prevent males

from repeatedly spawning with the same female. One individual, for example, was observed spawning with the same female on four different occasions and this same fish also produced offspring with nine other females.

Although no differences were found between hatchery and wild males significant disparities were seen in the RS values of males placed into the observation stream. The stepwise regression analyses indicated that most of the variation in male RS could be explained by just a few variables. In the Lower Population*, SatAlpha, BlackRes* and *Move* explained over 90% of the variation in male RS. *SatAlpha* and *Move* represent two aspects of female accessibility. The former measured the ability of a male to court and defend females while the later measured whether a male could move from one nearby and receptive female to another without being driven off by rivals. Correlation analyses indicated that *Move* was negatively associated with dominance and social status (Table 6). Sub-dominate individuals were forced to move and spend more time searching for receptive females and therefore had less access to them. The stepwise regression model that best explained the factors affecting male RS in the upper section used only one variable referred to as *Status*. The values of *SatAlpha, Alpha, AllRivals, Black*, and *DomObsOpp* were summed and divided to obtain a mean value that reflected the overall social status of each male. As in the model created for the lower section, this variable indicated that male RS was largely driven by access to females and by the capacity to exclude potential rivals from them.

The two-way ANOVAs that examined whether behavioral differences existed between males that had achieved high RS values and those that were less successful corroborated the general findings of the stepwise regression models. Males that had high

RS values were dominate, courted females more often, and moved less than males in the moderate and low RS groups (Table 7). However, male RS did not appear to be linked to how often they received attacks from females. This probably happened because of the diversity of situations where females attack males. During early territory establishment, for example, females were observed to attack fish of either sex at high frequencies (> 1 per min). Additionally, solitary females preparing nests may attack newly arriving males. Weak or sub-dominate males often left after being attacked whereas dominate individuals stayed and attempted to court the female. Furthermore, males that are courting females may receive attacks from neighboring females that are guarding territories. And females will also attack males that have the stripe color pattern; a pattern that is usually adopted by sexually active territorial females but one that also occurs on subdominant males. The occurrence or frequency of female attacks under such a broad range of conditions apparently made males with varying degrees of RS equally susceptible to female aggression.

Male body size was a relatively unimportant factor in determining male RS. This observation differs somewhat from other investigators. Fleming et al. (1996, 1997) discovered that size in Atlantic salmon could account for 23 to 45% of RS in males. Fleming and Gross (1993) also reported that male size in coho salmon (*Oncorhynchus kisutch*) significantly influences which individuals have access to spawning females and a similar result was observed in chum salmon (Schroder 1981). Dannewitz et al. (2004) however, found no evidence that male size affected male RS in spawning brown trout. A similar finding for Atlantic salmon was reported by Garant et al. (2001). Differences in male densities in these experiments may help explain the variation found in the

importance of relative size on male RS (Dannewitz et al.2004). Data from Schroder (1981) on chum salmon (*O. keta*) corroborate that idea. In this species, Operational Sex Ratios (OSRs) or the number of sexually active males per active female had profound effects on the types of males that had access to females. When OSR values were around 1, every male regardless of his relative body size was able to pair with females. When an OSR exceeded 1.5, the smallest males were excluded from females and when OSRs rose to 3 and above intra-sexual competition among males became intense and relative size became even more important. Thus, localized differences in OSR values in breeding aggregations of chum salmon affected which males had access to females. Quinn and Foote (1994) also found that considerable variance in dominance status may occur among salmonid males of similar size. The capacity to dominate opponents in the populations we studied could explain 30 to 56% of the variation seen in male RS. The reason it does not account for more variation is that sub-dominate males utilize reproductive strategies (e.g. the satellite strategy) designed to circumvent intra-sexual competition. Therefore, the capacity to produce offspring is not directly related to being dominant. It is also linked to how successful a male has been in utilizing alternative breeding strategies such as a satellite or sneaker strategy.

The adult Chinook salmon placed into the observation stream exhibited a variety of color patterns that ranged from being almost entirely black to fish with light green or gold backs that possessed a distinctive dark purple stripe on their sides. The overall background color of the fish was typically brown or yellow but it could shift and become gray blue depending on incidental light conditions. Some of the fish also had gray to snow-white bellies. These color patterns could change within seconds and were very

variable. Our behavioral observations disclosed that males possessing the black color pattern were the most aggressive, socially dominant individuals in their populations and that alpha males often possessed this pattern (Table 6). Results of the pedigree analyses also indicated that about 50% of the variation associated with male RS was explained by this single trait (Table 5). A practical consequence of the tight relationships existing between the black color pattern and dominance, aggression, and social status is that this color pattern can be used as a surrogate variable that accurately reflects these traits in male spring Chinook.

In conclusion, the tests used to compare the RS of hatchery and wild spring chinook males did not detect any difference between them. However, the power of the tests used was low due to the large variation inherent in male RS and the traits we measured that were closely allied to RS. Yet inspection of the information collected illustrated that extensive overlaps occurred in data collected on male RS and the behavioral correlates that were measured. Consequently, any reduction in masculine reproductive competence associated with a single generational exposure to hatchery conditions in this population appear to be low. Clearly hatchery males from the CESRF facility produced offspring in the observation stream and they appear to be appropriate fish to use in a supplementation effort. Whether their reproductive competency will remain as robust once they have been exposed to multiple generations of hatchery culture remains to be seen and is one of the research objectives of the Yakima/Klickitat Fisheries Project.

The analyses that examined whether behavioral traits in males obtaining high RS differed from those that had achieved moderate or low success showed clear disparities.

Males with high RS values were socially dominant, aggressive, and tended to stay in localized areas, courting and spawning with females that were adjacent to one another. As females finished their spawning activities, all males regardless of their RS status increased their movement patterns in an attempt to locate new potential mates. The behavioral and body size information obtained from the males showed that how a male behaved had a greater influence on his RS than his absolute or relative size.

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APPENDIX A:

Comparing the reproductive success of hatchery- and wild origin spring chinook placed into the observation stream in 2004

Introduction

One of the research objectives of the Yakima Klickitat Fisheries Project (YKFP) is to determine if first-generation hatchery spring chinook originating from native Yakima River fish can successfully reproduce under natural conditions. In 2000, an observation stream was constructed on the grounds of the Cle Elum Supplementation Research Facility (CESRF) to allow comparisons between the reproductive success (RS) of hatchery- and wild-origin spring chinook under quasi-natural conditions. In 2001 and 2002, mixtures of hatchery and wild fish were placed into two equally sized parts of the stream and allowed to spawn naturally. Their ability to produce offspring was estimated by performing pedigree assessments on samples of fry collected from the stream. These assessments, based on variation in microsatellite DNA, allowed us to estimate the number of offspring each adult fish placed into the observation stream had produced.

The spawner densities and degree of intrasexual competition present in the 2001 and 2002 populations was purposively made high so that behavioral differences in the ability to acquire territories, defend nest sites (for females) and find and defend sexually active females (for males) could be expressed and quantified. In 2003, 2004, and again in 2005, the entire observation stream was used by a mixed population of first-generation hatchery and wild spring chinook. Slightly more adult fish were placed into the stream but the amount of space available to them was more than doubled. The goal was to create

spawning communities that had intrasexual competition levels similar to those occurring in salmon populations that were depressed and in need of supplementation.

In this appendix, we examine the effects of this more spacious and less populated spawning community on the RS values of hatchery- and wild males placed into the stream in 2004. As in Part 1, we directly compare the RS of hatchery- and wild-origin males. In addition we examine the relationship between male RS and body size and the number of different females a male is able to spawn with. In addition, the RS of hatchery and wild precocious males placed into the stream in 2004 is described and compared. Finally we offer some speculative comments about the overall effect of a larger spawning area and the influence of precocious males on variation in male RS in the observation stream. Comprehensive behavioral analyses on the fish have not been completed so the affect of behavioral traits on male RS is not discussed. Until final analyses have been completed these results should be regarded as preliminary.

Materials and Methods

Origin and collection of wild and hatchery fish in 2004

The same methods employed to collect adult spring Chinook in 2001 (*see* Part 1 of this report) were used to obtain fish placed in the observation stream in 2004. Briefly, upstream migrating spring Chinook salmon adults were collected in the upper Yakima River from April through August at the Roza Adult Monitoring Facility. Representative samples of hatchery and wild fish were collected using the methods described by Knudsen et al. (*in press*). Selected fish were PIT tagged at the Roza trap and then trucked 81 kms to CESRF where they were held in a 30.5 m x 4.6 wide x 3 m deep holding pond until reaching maturity.

On September 14, fish in the holding pond were inspected for maturity. Mature individuals that were free of deformities, skin abrasions, and were judged to be vigorous were set aside and allowed to recover for 24 hrs before being placed into the observation stream. On September 15, 21 females (10 hatchery and 11 wild), 29 males (19 hatchery and 10 wild), 4 jacks (2 hatchery and 2 wild) and 14 precocious males (7 each of hatchery and wild origin) were introduced into the observation stream (Table 1). Prior to being placed into the stream each fish was anesthetized in MS222, weighed to the nearest gram, measured to the nearest mm, and tagged with 3.8 cm Petersen disks using methods previously described in Part 1. The precocious males were not tagged due to their small size. Approximately 4 – 6 mm^2 of material was also removed from the posterior trailing edge of the dorsal fin of each fish and placed in 100% ethanol for later microsatellite DNA extraction. After being processed the fish were transported 200 m and released into the observation stream.

Table 1. Number and body weight of the hatchery and wild spring Chinook placed into the observation stream in 2004

Sex[a]	Origin	No.	Mean Body Weight (kilos)	Range in Body Weight (kilos)
Male	Hatchery	19	3.49	1.76 – 5.81
Jack	Hatchery	2	1.04	0.70 – 1.39
Precocious Male	Hatchery	7	0.10	0.07 – 0.14
Male	Wild	10	4.26	3.00 – 6.62
Jack	Wild	2	1.07	0.88 – 1.26
Precocious Male	Wild	7	0.04	0.02 – 0.07
Female	Hatchery	10	3.92	2.54 – 5.00
Female	Wild	11	3.73	3.12 – 4.57

[a] Jacks are anadromous males that matured at age 3, one year earlier than most (>80%) of the spring Chinook that return to the upper Yakima River. Precocious males matured in freshwater at age 1

Observation Steam

As indicated in Part 1, the observation stream is a "U" shaped structure that is 127 m long by 7.9 m wide and is lined with geotextile to prevent water loss. Its banks have 2:1 slopes that are armored with river rock 10 to 30 cm in diameter. It is filled with 7.1 to 100 mm in diameter stream gravel that has a Fredle Index (Lotspeich and Everest 1981) value of 7 to 8 and total gravel depth is 90 cm. Discharge water from the raceways at the CESRF is pumped into the stream from September through May and water velocities in the stream can range from almost zero to over 2.0 m per second. Total discharge is approximately 0.37 cubic meters per second and water depth, which is regulated by stop logs, averages 0.4 meters. To facilitate behavioral observations a 2.1 m high wall of camouflage netting was installed on both banks of the stream (for a more detailed description of the stream *see* Part 1).

In the studies described in Part 1 the stream was subdivided into two parts, each being 45.6 m long by 7.9 m wide. In 2004, however, a single population consisting of both hatchery and wild Chinook was allowed to utilize the entire observation stream. This

was done to lower intrasexual competition in both sexes and to produce a spawning community with a density reminiscent of what might occur in depressed populations. It therefore provided an opportunity to determine if a greater amounts of space would alter the relative reproductive success of hatchery and wild-origin males.

Behavioral Observations

As in previous years, multiple observers made comprehensive behavioral observations on the fish as they spawned in the observation stream by using audiotape recorders (*see* Part 1 for further details). Focal Animal Sampling methods (Altmann 1974) were employed with observers shifting their attention from one focal fish to another after 4 to 10 continuous minutes of observation. In this manner the behavioral activities of each fish were recorded throughout every observation day. Agonistic and courting activities, nuptial color patterns, fish locations and movements were recorded. As in previous years, most spawning activity was completed 72 hours after fish introduction into the stream. These observations are currently being transcribed into continuous ethograms and no analyses or summations have been completed on them. Consequently, the importance of the male behavioral traits examined in Part 1 will not be described here.

Pedigree assessment

Modified fyke nets with floating live boxes were installed at the end of the observation stream in December 2004 prior to fry emergence. The live boxes were checked daily until late April 2005. On each collection day ten percent of the captured fry were randomly removed and preserved in 100% ethanol. When fry emergence ceased, the observation stream was electroshocked and seined to capture any spring chinook that may

have established residency in the stream. Daily catches of fry were counted and a ten percent sample was also collected and preserved in 100 % ethanol for later microsatellite DNA extraction and analysis. A sample goal of 3000 fry was established. More fry than this were collected and thus a constant percentage of fry was removed from each day's sample to obtain a representative sample of 3000 fish.

Microsatellite DNA was extracted from the fin tissue samples collected from the adults and precocious males placed into the observation stream. Microsatellite DNA was also obtained from 3000 putative offspring making it possible to determine how many progeny each adult placed into the observation stream in 2004 had produced. Kassler (2006) describes how the DNA extraction and amplifications took place and the methods that were used to perform the pedigree assignments.

Statistical analyses

The pedigree assignments presented by Kassler (2006—*see* his Table 4) indicated how many fry each male sired out of the juveniles that had been sampled. These values were converted into percentages by dividing the number of fry sired by a male by the total number of fry that had been used in the pedigree evaluation. These percentages were normalized by the arc sin square root transformation (Zar 1999). As in Part 1, the transformed percentages were used to estimate RS (the capacity to produce offspring) of each male placed into the stream. A two-sample t-Test was performed to determine if RS values of anadromous 4- and 5-yr-old hatchery- and wild-origin spring chinook males differed from one another. Two additional t-tests were used to analyze data collected on precocious males. The first one evaluated whether RS values of wild- and hatchery-origin

precocious males were dissimilar from one another. The second, assessed whether a difference existed in the body lengths of hatchery and wild precocious males.

Linear regression methods (Ordinary Least Squares) were used to examine the relationship between male RS and the number of females he spawned with. As mentioned in Part 1, the pedigree assignments make it possible to determine how many females a males spawns with, but not the number of times he may have spawned with an individual female. Two such regressions were performed, one for data collected on 4- and 5-yr-old anadromous males and another for the precocious males. Data collected on jacks (3-yr-olds) were not examined due to the small number of these fish. Two additional regression analyses were performed to ascertain the importance of male size on RS of anadromous males and on the RS of precocious males. In each of these analyses the natural log of the weight or length of a male was the independent variable while the dependent variable was the estimated RS value of each male.

Results & Discussion

The pedigree analysis disclosed that five males or 15% of the males sired just over 50% of all the fry used in the pedigree analysis. The coefficient of variation (CV) in RS for the anadromous males, excluding jacks, equaled 69%, which is less than what was observed in the smaller sections used in 2001. In that year, male RS in the upper section where precocious males were not present had a CV value of 116%. In the lower section where precocious males were present, the CV equaled 86% (*see* Part 1 for further details). In the 2004 population the amount space available for females was more than doubled and precocious males were also present. These two factors may have reduced variation in male RS in 2004 through two avenues. First, the area available to females for

redds was greater in the 2004 population than it was in the 2001 populations. This made it difficult for a few dominant males to monopolize most of the spawnings as females close to spawning could be widely dispersed throughout the stream. Second, as speculated in Part 1, precocious males fertilize eggs that would normally be fertilized by an alpha male and thus reduce the RS values of dominant fish. However, in the 2004 population, the 14 precocious males fathered slightly less than 4% of the 2,892 fry that were part of the pedigree analysis. It is likely then that distribution patterns and synchronous maturation of the females played a more important role in reducing variation in male RS in this population than did egg fertilizations by precocious males.

As indicated above, the pedigree analysis also identified which females a male had spawned with. These data once again showed that males spawning with numerous females produce more offspring than those that had spawned with fewer females (Figure 1). The linear regression that examined this relationship was significant ($p < 0.001$) and had an r^2 value of 72%. The two-sample t-Test that evaluated whether a difference existed between the RS of hatchery- and wild-males failed to reject the null hypothesis that both types of males had similar mean RS values (p 0.976). The RS values of the anadromous males placed into the stream are shown in Figure 2. This figure also illustrates the high degree of overlap that exists in hatchery- and wild-origin RS values.

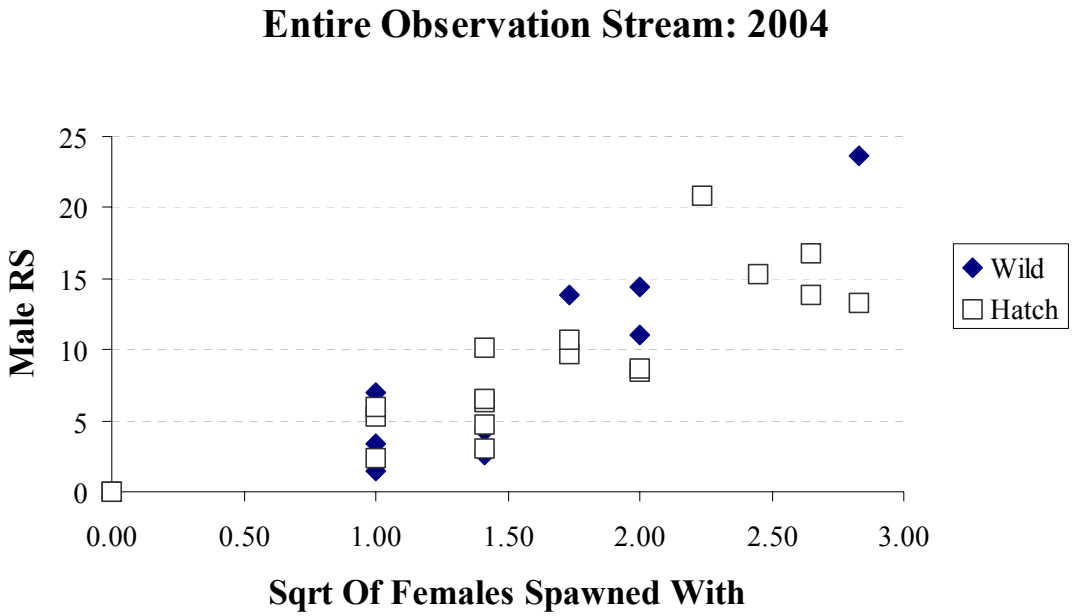

Figure 1. The relationship between male RS in hatchery- and wild-origin spring chinook and the number of females he spawns with in the 2004 population.

The coefficient of variation for reproductive success in the precocious males equaled 102%, a value that was almost identical to the one observed for precocious males spawning in the lower section of the observation stream in 2001. All the wild and hatchery-origin precocious males placed into the observation stream in 2004 were 1-yr fish. The average FL of the hatchery precocious males was 199 mm, which was significantly larger than the mean size of the wild fish that equaled 138 mm (two-sample t-test, $p < 0.001$). No difference, however was seen in the RS values of wild and hatchery precocious males (two-sample t-test, $p = 0.466$; Figure 3) nor was there a relationship between size (natural log FL) and RS ($p = 0.321$ and $r^2 = 0.5\%$). Like the larger anadromous males, there was a positive relationship between the RS of precocious males and the number of females they spawned with ($r^2 = 76\%$, $p < 0.001$). The maximum

number of females that a precocious male spawned with equaled five compared to eight for anadromous males.

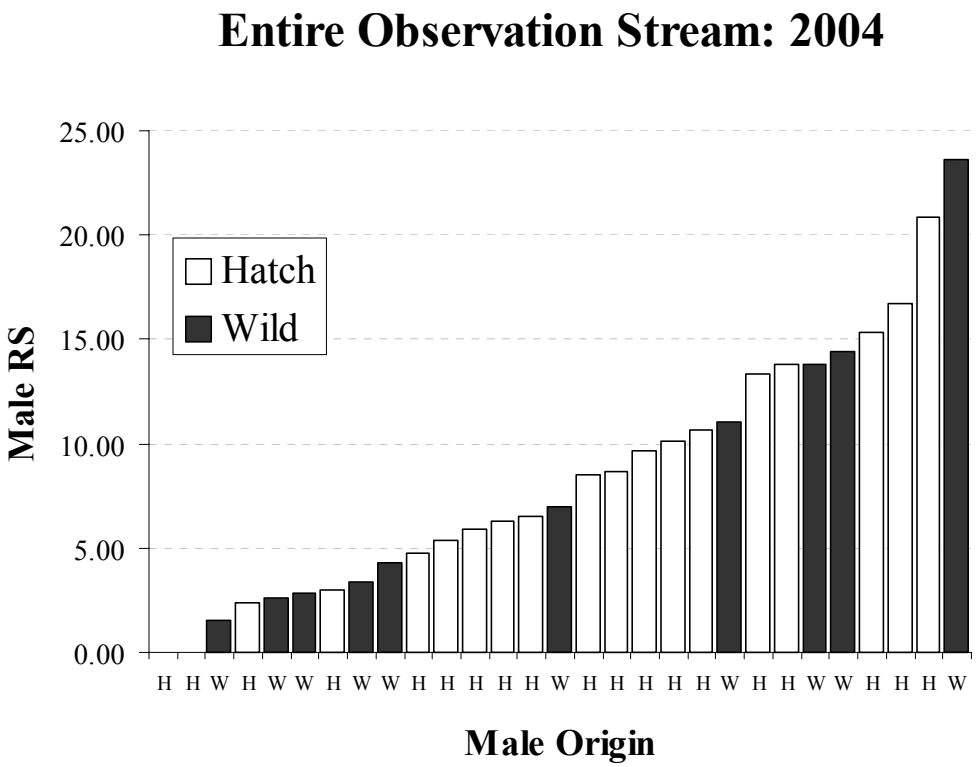

Figure 2. The reproductive success values of the hatchery- and wild 4- and 5-yr-old anadromous spring chinook males placed into the observation stream in 2004.

The affect of body weight and length on RS values in anadromous males was examined using linear regression methods. These analyses showed that significant relationships existed between body size and male RS but neither had high explanatory power ($p = 0.036$, $r^2 = 11\%$ for fork length; $p = 0.031$, $r^2 = 11\%$ for body weight).

In summary, many similarities exist in the males examined in the observation stream in 2001 and 2004. Extensive variation in male RS was found, no difference was seen in the RS of hatchery or wild males and as Figures 2 and 3 illustrate this was likely

caused by the high degree of overlap occurring in these values rather than from a lack of power brought about by high variation. Moreover, as in the 2001 populations, body size

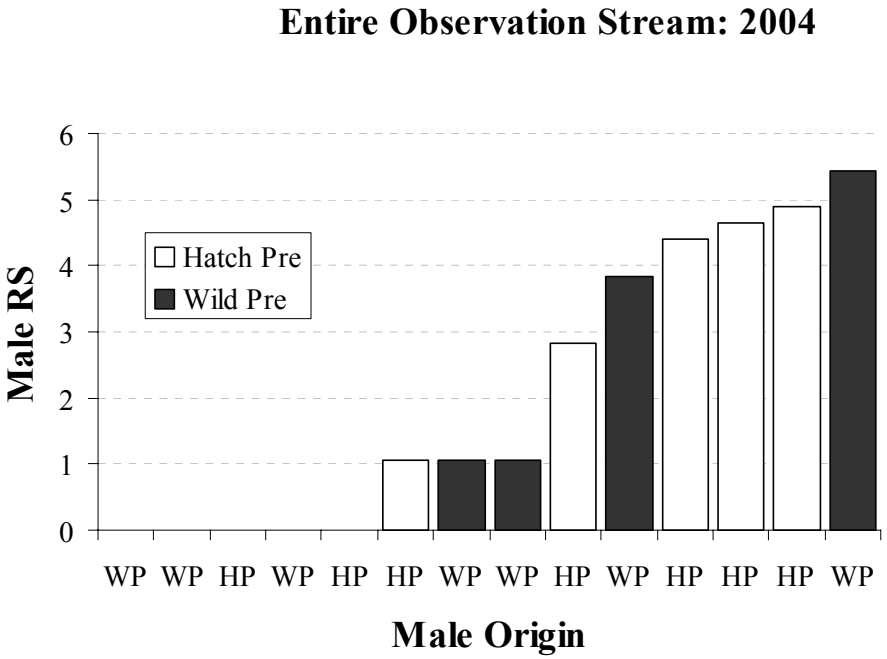

Figure 3. The reproductive success of the hatchery and wild precocious spring chinook males placed into the observation stream in 2004.

did not strongly affect RS in the males we examined. One possible difference in these populations was the reduced variation in male RS in the 2004 population. We speculate that may have occurred because of the greater area females had to establish nest locations. Two other populations, one in 2003 and another in 2005 were also allowed to spawn throughout the entire observation stream. Both had comparable densities of fish as the 2004 population. Information from them will further our efforts to determine if significant differences occur in the capacity of first generation hatchery- and wild-origin spring chinook to produce unfed fry.

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