DOCUMENT ID #P113087
Report covers work performed under BPA contract #36957
Report was completed under BPA contract #41659

**PROJECT 1993-056-00 PROGRESS REPORT (PERFORMANCE PERIOD: 1
JUNE 2008 THROUGH 31 MAY 2009)**

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Funded by

US Department of Energy
Bonneville Power Administration
Environment, Fish and Wildlife
P.O. Box 3621
Portland, Oregon 97208-3621

Project No. 1993-056-00
Contract 36957

15 July 2009

PROJECT 1993-056-00 PROGRESS REPORT (PERFORMANCE PERIOD: 1 JUNE 2008 THROUGH 31 MAY 2009)	1
ABSTRACT (600 WORD LIMIT)	3
LIST OF AUTHOR AFFILIATIONS (ALPHABETICAL).....	5
OBJECTIVE 1: MAINTAIN ADAPTIVE LIFE HISTORY CHARACTERISTICS IN CHINOOK SALMON	6
INTRODUCTION	6
MATERIALS, METHODS, AND DESCRIPTION OF STUDY AREA	7
RESULTS	10
<i>Courtships</i>	10
<i>Participation in spawning events</i>	10
<i>Relative reproductive success</i>	11
DISCUSSION	11
SUMMARY AND CONCLUSIONS	12
DATA MANAGEMENT ACTIVITIES	13
ACKNOWLEDGEMENTS	13
REFERENCES.....	13
OBJECTIVE 2 - DETERMINE CRITICAL IMPRINTING PERIODS FOR SOCKEYE SALMON	23
INTRODUCTION	23
MATERIALS, METHODS AND DESCRIPTION OF STUDY AREA	25
<i>Experimental Design</i>	25
<i>Behavioral testing</i>	27
<i>Sampling for olfactory tissue, hormones, morphometrics, gonadal histology</i>	27
<i>Molecular testing</i>	27
<i>Maturation physiology, gonadal histology and morphometrics</i>	28
<i>Statistical analyses</i>	28
RESULTS AND DISCUSSION OF RESULTS	29
<i>Behavioral testing</i>	29
<i>Molecular assessments of imprinting</i>	29
<i>Maturation physiology, gonadal histology and morphometrics</i>	30
SUMMARY AND CONCLUSIONS	30
DATA MANAGEMENT ACTIVITIES	31
REFERENCES.....	31
OBJECTIVE 3 - USE ENVIRONMENTAL FACTORS TO MATCH WILD PHENOTYPES IN CHINOOK AND SOCKEYE SALMON REARED IN HATCHERY SUPPLEMENTATION PROGRAMS.....	45
INTRODUCTION	45
MATERIALS AND METHODS, AND DESCRIPTION OF PROJECT AREA.....	46
<i>Sampling and Laboratory Methods</i>	46
RESULTS AND DISCUSSION	47
SUMMARY AND CONCLUSIONS	48
DATA MANAGEMENT ACTIVITIES	48
REFERENCES.....	49

Abstract (600 word limit)

This project was developed to conduct research to improve the efficacy of captive broodstock programs and advance hatchery reform throughout the Columbia River Basin. The project has three objectives: 1) maintain adaptive life history characteristics in Chinook salmon, 2) improve imprinting in juvenile sockeye salmon, and 3) match wild phenotypes in Chinook and sockeye salmon reared in hatcheries. A summary of the results are as follows:

Objective 1: The ratio of jack to adult male Chinook salmon were varied in experimental breeding populations to test the hypothesis that reproductive success of the two male phenotypes would vary with their relative frequency in the population. Adult Chinook salmon males nearly always obtained primary access to nesting females and were first to enter the nest at the time of spawning. Jack male spawning occurred primarily by establishing satellite positions downstream of the courting pair, and ‘sneaking’ into the nest at the time of spawning. Male dominance hierarchies were fairly stable and strongly correlated with the order of nest entry at the time of spawning. Observed participation in spawning events and adult-to-fry reproductive success of jack and adult males was consistent with a negative frequency-dependent selection model. Overall, jack males sired an average of 21% of the offspring produced across a range of jack male frequencies. Implications of these and additional findings on Chinook salmon hatchery broodstock management will be presented in the FY 2009 Annual Report.

Objective 2: To determine the critical period(s) for imprinting for sockeye salmon, juvenile salmon were exposed to known odorants at key developmental stages. Molecular assessments of imprinting-induced changes in odorant receptor gene expression indicated that regulation of odorant expression is influenced by developmental status and odor exposure history. Expression levels of basic amino acid receptor (BAAR) mRNA in the olfactory epithelium increased dramatically during final maturation in both Stanley Basin and Okanogan River sockeye. These increases appeared to be independent of odor-exposure history, rising significantly in both arginine-naïve and arginine-exposed fish. However, sockeye exposed to arginine during smolting demonstrated a larger increase in BAAR mRNA than arginine-naïve fish. These results are consistent with the hypothesis that odorant receptors sensitive to home stream waters may be upregulated at the time of the homing migration and may afford opportunities to exploit this system to experimentally characterize imprinting success and ultimately identify hatchery practices that will minimize straying of artificially produced salmonids. Additional analysis of Sockeye salmon imprinting and further implications of these findings will be presented in the FY 2009 Annual Report.

Objective 3: Objective 3: Photoperiod at emergence and ration after ponding were varied in Yakima River spring Chinook salmon to test the hypothesis that seasonal timing of emergence and growth during early stages of development alter seasonal timing of smoltification and age of male maturation. Fish reared under conditions to advance fry emergence and accelerate growth had the greatest variation in seasonal timing of smolting (fall, spring and summer) and highest rates of early male maturation with most

males maturing at age 1 (35-40%). In contrast, fish with delayed emergence and slow growth had the least variation in phenotypes with most fish smolting as yearlings in the spring and no age-1 male maturation. Growth (not emergence timing) altered rates of age-2 male maturation. Results of this study demonstrate that altering fry development, as is often done in hatcheries, can profoundly affect later life history transitions and the range of phenotypes within a spring Chinook salmon population. Additional work in the next funding period will determine if these rearing regimes affected other aspects of smolt quality, which may affect ultimate survival upon ocean entry.

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OBJECTIVE 1: MAINTAIN ADAPTIVE LIFE HISTORY CHARACTERISTICS IN CHINOOK SALMON

By

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Introduction

Pacific salmon (*Oncorhynchus* spp) males exhibit alternative reproductive phenotypes. In several species (sockeye: *O. nerka*; coho: *O. kisutch*; and Chinook: *O. tshawytscha*), males that mature one year earlier than the youngest females in a population, termed ‘jacks’, are substantially smaller and less sexually dimorphic than older males (hereafter ‘adults’). The persistence of both forms is presumably important to the sustainability of natural populations. Understanding the evolutionary mechanisms that maintain the alternative male reproductive phenotypes should provide for informed fisheries management. For example, habitat can influence the breeding success of male fishes adopting different breeding tactics, size selective salmon harvest may favor one phenotype over the other (Hankin et al. 1993), and there is wide disagreement regarding the appropriate use of jacks in hatchery broodstock management.

Currently, jack and adult male phenotypes probably represent two ‘tactics’ that are part of a conditional ‘strategy’ as described by Fleming (1996). This is in part because they are not genetically distinct and because the interplay between genetic and environmental factors determines the ‘decision’ whether or not an individual will mature early. Evidence for strong heritability in age-at-maturity comes from quantitative genetic studies (Hankin et al. 1993; Heath et al. 1994; Heat et al. 2002) and measures at the population level (Hard et al. 1985, Appleby et al. 2003). Physiological traits mediated by environmental conditions (e.g., body size, fatness), can trigger early male maturation (Vøllestad et al. 2004; Shearer et al. 2008). Early male maturation may represent the default developmental pathway, with the slower growing males repressing maturation until the following year (Aubin-Horth 2005).

Once salmon physiologically ‘decide’ whether or not to mature, trade-offs between ecological and sexual selection may shape the frequency of alternative phenotypes in a population. Jack males benefit from higher survival to maturity owing to their reduced time spent at sea. They may benefit from reduced predation or other forms of ecological selection during reproduction (Quinn et al. 2001). Nevertheless, jack males often represent a small portion of breeding populations of sockeye, coho and Chinook salmon (Carlson et al. 2004, Appleby et al. 2003, Myers et al. 1998). This may partly reflect disadvantages of small size and reduced access to females during reproduction (Carlson et al. 2004), although the natural relative breeding success of jack and adult male Pacific salmon has received very little attention. Gross (1985) indirectly estimated breeding

success in coho salmon jacks at 66% that of older males. The only direct evaluation in Pacific salmon (*Oncorhynchus* spp) comes from Foote et al. (1997), who used genetic markers and quantified the relative success of just seven jack sockeye salmon males in 3.5 m by 3.5 m cages and found highly variable breeding success (3% to 97%; average = 42%) relative to that of older males.

Pacific salmon adult males fight for primary access to females and thus the ability to engage in courtship and spawn with them. Losers often establish ‘satellite’ positions downstream of the courting pair, from which they attempt to ‘sneak’ spawn, or are excluded from breeding opportunities altogether. The smaller jacks very rarely challenge larger males for courtship opportunities, and instead compete for satellite positions as well. Just as adults are not obligate consorts, jacks are not obligate sneakers. Jack male Chinook salmon will court and spawn with females in the absence of older males, and exhibit high fertilization success (Berejikian et al. 2000, 2005).

Gross (1985) proposed that co-existence of the two life history phenotypes in coho salmon is maintained through negative frequency-dependent selection. Negative frequency dependent selection favors the rare form, and the fitness of one tactic decreases as it becomes relatively more frequent in the population. In Gross (1985), intermediate-sized coho salmon males (mainly adults) maintained distances further from nesting females than did larger adult males and smaller jack males. He assumed proximity to the female was negatively related to fertilization success, (citing work on chum salmon by Schroder et al. 1982), and concluded that this was evidence of disruptive selection on male size. Foote et al. (1997) detected no relationship between proximity to the female prior to spawning and fertilization success, contrary to a key assumption in Gross’s work. Finally, Gross assumed that the addition of more jack males would reduce proximity of jack males to the female and decrease their fitness. In the present study, we directly measure adult-to-fry reproductive success of jack and adult males and make detailed observations of actual spawning events.

This study had the following objectives: 1) describe the breeding behavior and quantify reproductive success of jack and adult Chinook salmon males, 2) test the hypothesis of negative frequency-dependent selection, 3) quantify the mean and total contribution of the two life history forms at different frequencies to provide a template for hatchery broodstock management. We varied the ratio of jack and adult males in eight separate breeding groups to quantify the effects of jack and adult male frequency on access to breeding opportunities, participation in observed spawning events, and individual reproductive success (adult-to-fry). Male density was held constant to avoid confounding frequency-dependence with density-dependence.

Materials, Methods, and Description of Study Area

Breeding groups and spawning observations

Adult spring Chinook salmon were obtained from the Trask River Hatchery, located on the Trask River, OR). Twelve females and 32 males were collected and transported on

each of two dates (5 September and 12 September 2007) in an oxygenated transport tank to the Oregon Hatchery Research Center (OHRC), located on Fall Creek approximately 3 hrs from the Trask River Hatchery. Upon arrival, the fish were held overnight in 12-m diameter tanks supplied with Fall Creek water. The following day, each adult Chinook salmon was anesthetized (MS-222), weighed (nearest g), and measured (nearest mm FL), and externally tagged with a uniquely numbered Petersen disc tag. A portion of the anal fin was removed and preserved in 100% non-denatured alcohol for genetic analysis. This procedure was repeated on 3 and 10 September 2008.

Six female and 16 male Chinook salmon were stocked into each of two replicate spawning channels on 6 and 13 September 2007 and 4 and 11 September 2008. Males less than 545 mm FL were presumed to be ‘jacks’ (age-2) and those larger than 600 mm FL were presumed to be adults (age-3 or age-4); scales were removed from each fish for subsequent ageing. In both years, the number of jack and adult males was varied among the four channels (Table 1).

Each meandering spawning channel measured 8 m wide by 65 m long, and contained a mixture of substrates ranging from coarse sand to large cobbles. Each channel contained several meanders, pool-riffle sequences, and woody debris, placed in such manner that each channel was a very close replicate of the others. Shade cloth was suspended above the streams. Water was diverted from Fall Creek, through a settling pond to each channel to provide approximately 0.043 m³/s per channel. Airlift pumps were used to partially re-circulate water through the channels to create a total flow of approximately 0.057 m³/s in each.

Behavioral observations were conducted from dawn to dusk in each channel from the time the fish were introduced until the last female in each channel was no longer sexually active (i.e., no longer nest building). We scanned each of the stream channels multiple times each day to document the activity of each female, and we conducted focal sampling of females that were constructing nests and preparing to spawn (see Berejikian et al. 2001). When a female was determined to be constructing a nest, we characterized the male dominance hierarchy surrounding the female. The ‘dominant’ male had primary access to the female, exhibited courtship behaviors (crossing over and quivering) and chased off other males positioned either downstream or upstream of the female. ‘Satellite’ males held positions typically downstream of the courting pair, were frequently chased by the dominant male, and would occasionally sneak into the nest pocket prior to and during spawning. The satellite maintaining closest proximity to the female was designated ‘S1’, the second closest, ‘S2’ and so on. Dominant and satellite males were determined to have participated in a ‘courtship’, and the number of courtships was tabulated for each male over the course of the spawning season.

We attempted to observe as many actual spawning events as possible and record the males that participated in each event and their order of nest entry at the time of spawning. Only instances in which females crouched in the nest pocket, gaped, were accompanied by at least one gaping male, and covered their eggs with a rapidly increased rate of digging (Berejikian et al. 2000) were considered actual spawning events. These were

discriminated from false spawning events in which females do not engage in egg covering. We tabulated the frequency of spawning participations for each male and categorized them by rank order of nest entry (1 = first to enter, 2 = second to enter, etc.).

In 2007, Channels C and D were stocked and monitored first. The pace of spawning was quicker than we had anticipated and we did not continuously observe nesting females for a long enough period of time and as result we missed observing some spawning events. However, in channels A and B (i.e., the second group of fish stocked on 13 September 2007), and in all channels in 2008, we are fairly certain we observed all spawning events that occurred between dawn and dusk because no nesting females were subsequently observed cover digging or constructing new nests without a spawning event being observed. Much spawning occurred at night in both channels that was not observed.

Fry collections

To quantify individual reproductive success of Chinook salmon spawning in 2007, emergent fry were removed from the stream channels by seining and electroshocking on 29 and 30 January 2008. All channels were sampled again on 25 February 2008, and the channels were de-watered to ensure that all fry were collected. We counted the total number of fry from each channel and sub-sampled at a similar rate among channels (Table 2). Sub-sampled fry received a lethal dose of MS-222 and their caudal fins were preserved in 100% non-denatured alcohol for DNA pedigree analyses. Fry offspring were not collected from Chinook salmon spawning in 2008 because eggs from individual nests were intentionally excavated when the eggs were at the eyed stage of development as part of a related evaluation to estimate fertilization success of males at different positions in the hierarchy. Thus, we have adult-to-fry reproductive success estimates for four separate breeding groups at four relative jack:adult ratios from the 2007 spawning season, but not from 2008 (Table 1).

DNA pedigree analyses of collected fry

Genomic DNA was extracted from the adult and offspring tissue samples, and subjected to polymerase chain reactions (PCR) to amplify 6-12 loci that are known to exhibit microsatellite polymorphisms for Chinook salmon. A fragment analysis was conducted on the PCR products using an Applied Biosystems 310 genetic analyzer to determine the genotypes of every individual for each locus. The genotypes of the fry have been compared to those of the adults using the computer program CERVUS to determine the parentage of each fry.

Eyed egg collections from 2008 spawning season

The FY 2009 statement of work for this project covers the genetic analysis of the hydraulic removed eggs to estimate and describe status-dependent male fertilization success. Under the FY 2008 statement of work, eyed eggs were collected from a total of 19 individual nests in which least two males were observed to have participated in the

spawning event. At the time of spawning a weighted, numbered flag was placed in the center of each nest pocket within a few seconds after spawning. Temperature units were monitored to estimate dates during which the eggs would be at the eyed stage of development. In October 2008, we dewatered the spawning channels and removed a sample of eyed eggs from each nest. The eggs were incubated in Heath tray isollettes until the button up stage of development. They were euthanized with an overdose of MS-222 and preserved in 100% non-denatured ethanol for DNA analysis (Table 2).

Results

Courtships

We conducted a total of 878 focal observations of male hierarchies associated with 46 of the 48 females stocked into the spawning channels in 2007 and 2008 (two females died prior to spawning).

Adult males competed primarily among themselves to establish dominance and attain courtships with nesting females. Adult males also competed among themselves and with jacks for satellite positions in the hierarchies. Adult males more frequently attained the dominant ($P < 0.001$) and S1 positions ($P = 0.013$) than jack males. Jack and adult males did not differ in their ability to attain S2 positions ($P = 0.463$). Jack males attained S3 positions more frequently than adult males ($P = 0.003$).

Jack males used a number of different tactics to maintain positions in hierarchies and position themselves for spawning. Jack males hid under logs or in side channel pools, held positions in very shallow water in tailouts where larger males appeared reluctant to chase them, and held positions alongside or downstream of larger males. Jack males often mimicked female coloration patterns (dark lateral stripe) and held positions upstream of courting pairs while periodically sneaking into the nest prior to spawning. Jack males did court females, but it was very infrequent and typically occurred shortly after females had spawned, or were still hours away from their next spawning event.

Participation in spawning events

We observed a total of 67 spawning events (Table 1). The number of males participating in each of those events ranged between one and five (Figure 1). Participation in male hierarchies as captured through focal sampling was a significant predictor of participation in observed spawning events (Figure 2). Adult males entered the nest first during spawning more frequently than jack males ($P = 0.001$). There was no difference in frequency of second to enter the nest ($P > 0.05$), and jack males were more frequently observed as the third male to enter the nest ($P = 0.003$). Consequently, adult males had a significantly mean lower rank order of nest entry than jack males, and mean rank order of nest entry appeared unaffected by the ratio of jack to adult males (Figure 3). Only twice were jack males first to enter the nest at the time of spawning. In one case a jack male was the lone courting male and in the other, the dominant adult male was chasing another satellite when the female he was courting spawned with a satellite jack male.

The proportion of adult males participating in a spawning event was inversely correlated with their frequency in the breeding population ($R^2 = 0.81$, $P = 0.05$). The same was not evident for jack males ($R^2 = 0.02$, $P > 0.50$; Figure 4). There was a large discrepancy between the lowest jack ratio in 2007, where all four males participated in at least one spawning event, and the same ratio in 2008. In 2008, two of the four jack males in channel 2008 B died before 80% of the spawning events were observed and the other two males died the following day before 50% of the spawning events were observed. Thus, the low proportion of jack males observed participating in at least one spawning event (1 of 4, 25%) probably reflects reduced jack male opportunities because of their early death. This is the only spawning channel in which the longevity of jack males was significantly shorter than for adult males ($P = 0.003$).

Relative reproductive success

The reproductive success of jack males spawning in 2007 was similar to that of adult males in the channels with the two lowest jack to adult ratios and was substantially lower than adult males at the two highest jack to adult ratios (Figure 5). At the lowest jack ratio, each of the four jacks produced at least one offspring. At the highest jack ratios, the number of jacks that produced at least one offspring ranged between 65 and 70% (Figure 5).

The average reproductive success of adult males decreased with increasing adult frequency (Figure 5), consistent with the negative frequency-dependent selection model. Nevertheless, adult males collectively sired 78 percent of the fry at the highest adult frequency (12 adults) because there were more of them, and a surprisingly similar percentage (81 percent) at the lowest adult frequency (8 adults). Jack and adult males mated with a similar number of females across all breeding groups (2.0 mates per adult male and 2.1 mates per jack male), but the number of offspring produced per mating was substantially less (10.2 per female) for jack males than for adult males (23.6 per female).

Discussion

The salient findings of this study to date are as follows. Adult males obtained primary access to females and are first to enter the nest at the time of spawning. Jack male spawning occurred primarily by establishing satellite positions downstream of the courting pair, and 'sneaking' into the nest at the time of spawning. Male dominance hierarchies were fairly stable and strongly correlated with the order of nest entry at the time of spawning. Observed participation in spawning events and adult-to-fry reproductive success of jack and adult males was consistent with a negative frequency-dependent selection model.

Both jack and adult males competed for access to nesting females. The dominant position next to the female was nearly always occupied by an adult male. However, adult males also competed vigorously for satellite positions. Jack male competition was primarily for the 2nd, 3rd or later position in the hierarchy, and they were rarely observed in the dominant position. Position in the male hierarchy prior to spawning was consistent

with male order of nest entry during spawning. The dominant male was always the first to enter the nest at the time of spawning, except for one case. The preliminary results suggest that adult males benefit from attaining higher status in male hierarchies and are able to participate in a greater number of courtships and spawning events. Eyed eggs sampled from individual nests with multiple known male participants will be analyzed during the FY09 funding cycle and will help determine the importance of male order of nest entry on fertilization success.

The finding that adult males dominated access to nesting females is not surprising given the large body of literature indicating that larger males tend to have a breeding advantage over smaller males in other salmonids (reviewed by Esteve 2007). Nest building females were nearly always courted by a dominant adult male. In the only spawning event in which a jack male was first to enter the nest, spawning occurred when the adult male had gone to attack another male and was not present. Satellite positions downstream from the courting pair were held by both adult and jack males, similar to the reproductive behavior of male coho salmon described by Prince and Healey (1998). Foote et al. (1997) similarly found that large (adult) sockeye salmon males were always closest to the female prior to spawning. The dominance hierarchies established around a nesting female, typically within one hour prior to spawning, predicted the order of nest entry at the time of spawning. It is unclear whether the first male to enter the nest had a sperm precedence advantage over the second or later males to enter. In most cases, the multiple male ejaculations appeared nearly synchronous, and the order could not be determined. In multiple male spawning events, dominant males attain an apparent fertilization advantage in chum salmon (*O. keta*: Schroder 1981), sockeye salmon (Chebanov et al. 1983) Atlantic salmon (Mjølnerod et al. 1998), and brook trout (Blanchfield et al. 2003) that is presumably an result of rank order of nest entry and sperm precedence. In our study the number of offspring per mate was greater for adult males, suggesting that sperm precedence was an important factor.

Summary and Conclusions

The results of this study support the hypothesis that negative frequency-dependent selection acts to maintain some stability in the ratio of jack to adult male Chinook salmon. Evidence for negative frequency dependent selection had previously been demonstrated in lizards (Bleay et al. 2007), and inferred in bluegill sunfish (Gross et al. 1991), but no studies on salmonids have been conducted or published to our knowledge. The behavioral observation data and the reproductive success together provide important insights into the maintenance of the of the two primary life history strategies of Chinook salmon males. Evidence for negative frequency-dependent selection in the Chinook salmon we studied came from both behavioral observations and adult-to-fry reproductive success estimates. For example, at low adult male frequencies, a greater proportion of adult males were observed participating in spawning events and their mean relative reproductive success was greater. Jack male reproductive success was likewise greatest at the two lowest jack male frequencies and lowest at the two highest jack male frequencies. The presence of negative frequency dependence in Chinook salmon mating systems would suggest that hatchery broodstock management should be sensitive to changes in

the relative abundance of jack and adult males where mimicking natural life history patterns and selection processes is an objective of the program. For example, mimicking intra-sexual selection on life history type during breeding may require that proportion of jacks used in a particular spawning year be inversely related to their frequency in the population. We will provide more specific recommendations regarding broodstock management in the FY 2009 Annual Report.

Data Management Activities

Data were collected by NOAA and Pacific States Marine Fisheries Commission researchers and entered onto preformatted data sheets or directly into electronic spreadsheets or text files. Data were entered and summarized on personal computers operated by researchers; primary software used for these procedures includes Microsoft Excel 2003 and Word 2003. Systat was used for statistical analyses. All data are checked for quality and accuracy before analysis. Analytical processes are described in the text of the annual report under Materials and Methods. Data analyses are reported in the Results section of the annual report. Final data analyses will be included in the FY 2009 Annual Report.

Acknowledgements

We gratefully acknowledge the assistance of the Oregon Hatchery Research Center's Advisory Committee and staff for providing both the opportunity and logistical support necessary to conduct this study. We also appreciate the cooperation of the Oregon Department of Fish and Wildlife, Trask River Hatchery staff for providing adult Chinook salmon for this study.

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Table 1. Stocking date, number, and fork length (mm) of Chinook salmon males stocked into each of the four stream channels at the Oregon Hatchery Research Center in 2007 and 2008. The total number of spawning events observed and the number of fry sampled for DNA pedigree analyses are also shown.

2007 Channel	Stocking date	Adults: Jacks	Mean fork length (s.d.)		Spawn events observed	Fry sampled
			Adult	Jack		
A	12 Sept	8:8	683 (56)	476 (25)	9	301
B	12 Sept	12:4	728 (58)	497 (33)	12	597
C	5 Sept	10:6	705 (88)	500 (48)	2	520
D	5 Sept	11:5	777 (60)	498 (31)	3	368
2008						
Channel						
A	10 Sept	12:4	738 (47)	507 (27)	10	--
B	10 Sept	10:6	773 (53)	493 (20)	6	--
C	3 Sept	6:10	722 (47)	500 (24)	11	--
D	3 Sept	8:8	776 (53)	500 (25)	14	--

Table 2. Eyed embryos were excavated from 19 individual nests constructed in 2008. The number of males participating in each spawning event are shown along with the number of eggs collected, the number preserved (difference reflects incubation mortality in heath trays) for genotyping during the FY 2009 funding cycle

Spawn date	Channel	Female	# males	Eggs collected	Fry preserved
9/4/08	D	W36	5	73	69
9/5/08	D	W95	2	80	80
9/5/08	D	W36	2	99	94
9/5/08	D	W88	2	96	90
9/6/08	D	W88	2	103	95
9/7/08	D	W36	2	94	89
9/7/08	D	W37	5	74	61
9/8/08	C	W54	2	72	69
9/8/08	C	W33	3	45	42
9/8/08	C	W33	5	65	64
9/9/08	C	W54	2	76	74
9/9/08	D	W99	4	53	52
9/10/08	B	W78	2	131	128
9/11/08	A	W71	3	92	92
9/11/08	B	W97	4	97	81
9/11/08	D	W99	5	66	63
9/13/08	A	W71	2	94	92
9/14/08	B	W43	3	72	69
9/16/08	A	W65	3	48	41

Figure 1. The percentage of spawning events in which at least one jack male participated. The numbers above the bars represent the total number of spawning events in each category.

Figure 2. Correlations between the frequency of participations in male hierarchies surrounding nesting females and the frequency of participations in spawning events. Each spawning group is indicated by a different symbol (number of jacks in the channel followed by the year in parentheses). R^2 values range from 0.50 to 0.75 for channel-specific regressions; $P < 0.05$ in all cases.

Figure 3. Mean rank order of nest entry at the time of spawning for jack and adult males (1 = first to enter, 2 = second to enter, etc.). Means were calculated across separate spawning events and in some cases represent multiple participations by the same male. However, data are consistent with ANOVA results that analyze spawning frequency within each rank (see text).

Figure 4. The proportion of adult (squares) and jack (circles) males observed participating in at least one spawning event at different adult to jack male ratios (number of jack males shown on the x-axis). Adult male $R^2 = 0.81$, $P = 0.05$; jack male $R^2 = 0.02$, $P > 0.50$.

Figure 5. Percent of males siring one or more offspring (top graph) and the mean percent of offspring sired (bottom graph) for jack and adult males at each of the four jack to adult ratios.

OBJECTIVE 2 - DETERMINE CRITICAL IMPRINTING PERIODS FOR SOCKEYE SALMON

Andrew H. Dittman, Darran May, Michelle Havey, and Larissa Felli

Introduction

Snake River sockeye salmon were listed as endangered by NOAA Fisheries in 1991, and in that same year IDFG initiated a captive broodstock program with the ultimate goal of re-establishing sustainable sockeye runs to Stanley Basin waters (Kline and Heindel 1999). During the program's initial years all returning wild anadromous adults (16), residual sockeye adults and wild juvenile out-migrants were captured to establish a captive broodstock. Captive rearing dramatically increased juvenile-to-adult survival and the population numbers have increased to the point that since 1993 captively-reared fish have been re-introduced annually into the Stanley Basin. To avoid unanticipated negative consequences of any one reintroduction approach, the IDFG, in conjunction with the Stanley Basin Sockeye Technical Oversight Committee (SBSTOC), has adopted a "spread-the-risk" strategy for reintroducing sockeye back into the wild that includes planting of eyed eggs, net pen and direct lake releases of pre-smolts, smolt releases, and releasing captively-reared adults to spawn naturally (Kline and Heindel 1999).

Despite successful out-migrations of smolts from all the different release strategies, prior to 1999 none of these strategies successfully produced adult sockeye salmon back to Idaho. Fish for these releases were reared at several out-of-basin facilities (NOAA Fisheries hatchery at Big Beef Creek, Washington; IDFG hatchery at Eagle, Idaho, ODFW Bonneville hatchery) because there were no appropriate Stanley Basin facilities, and to avoid the risk of cataclysmic events at a single facility. In some instances fish were transferred several times at different life stages between facilities, and some groups did not experience Stanley Basin waters until they were released as smolts. Earlier studies with coho salmon have indicated that fish released as smolts tended to stray more than fish released as fingerlings a year prior to smolting (McHenry 1981).

Concerned that the lack of adults returning to Stanley Basin may be due to unsuccessful imprinting and straying, the sponsors of the captive broodstock Projects #199107200 and #199204000, and the SBSTOC recommended that Project #9305600 initiate research on the timing of imprinting and environmental factors that influence imprinting, especially in sockeye salmon. Research to examine the timing of imprinting in Columbia Basin sockeye salmon was initiated in 2001. In 1999, the first hatchery-produced adults returned to the Stanley Basin and in recent years a large number of the returning adults have been from the smolt-release group of fish that only briefly experienced Stanley Basin water before emigration. These results appear to indicate that sockeye salmon are capable of imprinting as smolts, but the numbers of returning fish relative to other release groups may be more a reflection of the number of fish released and smolt-to-adult survival than homing success. The stray rates of these smolt release groups are unknown. Homing to the Snake River appears to be largely successful, however, homing success

within the Snake River system is not known. Over the first ten years that adults from the Captive Rearing program have returned to the Snake River only 64.2 % of the adults passing Lower Granite Dam successfully returned to the Stanley Basin (Pacific States Marine Fisheries Commission PTAGIS database; Willard et al 2004). If the successful 2000 return year is excluded, the percentage of fish passing Lower Granite Dam that have returned to Stanley Basin falls to only 36.2 %. Low percentages of successful migrations from Lower Granite Dam to the Stanley Basin continues to be a major confounding factor for recovery of this population. Even in the successful 2008 return year, only 66.4% of the fish that reached Lower Granite Dam successfully returned to the Stanley Basin. While the ultimate fate of these unsuccessful fish is not known, earlier radio telemetry data indicated that a high percentage of fish that migrated past Lower Granite Dam were last identified in locations outside the Stanley Basin watershed (Paul Kline, IDFG, personal communication), suggesting that they are either straying or suffering pre-spawn mortality within the Snake River.

The spread-the- risk-strategy for reintroduction of Stanley Basin Sockeye is necessitated in part by the lack of knowledge about the physiological and developmental processes underlying olfactory imprinting and the ecological factors that facilitate successful homing. For sockeye salmon reintroductions to be successful in the Stanley Basin (and throughout the Columbia Basin (e.g., BPA Project # 200001300; 29016) salmon must be released at appropriate juvenile stages for successful imprinting. Empirical studies have provided some general rules regarding the effect of hatchery rearing and release strategies on straying (Quinn 1993), but in many cases differences between species, watersheds, physical environment of the hatchery, release timing and location, and even basic assumptions about what should be regarded as successful homing may mask the underlying processes that are critical for imprinting and homing. Determining the critical development periods and environmental conditions for imprinting for the different salmon species will be crucial for the development and implementation of rearing and release strategies that will maximize survival without increasing straying.

Most early research on the timing of olfactory imprinting has focused on coho salmon because of their relatively simple life histories. Juvenile coho salmon generally rear in their natal stream until they smolt and migrate to sea during the second or third spring after hatching. Experiments with hatchery-reared coho salmon indicated that this smolting period is the critical period for olfactory imprinting (Hasler and Scholz 1983, Dittman et al. 1996). Unfortunately, the understanding of imprinting inferred from studies of hatchery-reared coho salmon underestimates the complexity and temporal plasticity of the imprinting process in the wild and in other salmonid species (Dittman and Quinn 1996, Nevitt and Dittman 1998). For example, sockeye salmon fry typically emerge from their natal gravel and immediately migrate to rearing areas within a lake where they live for 1 or 2 years before smolting and migrating to sea. During their homing migration, adults migrate past the outlet stream and lake where they smolted and return to their natal area to spawn, suggesting that olfactory imprinting must also occur prior to or during emergence from the gravel. Studies to determine the critical period(s) for imprinting for sockeye salmon were initiated in fall 2000 and the outcome of these experiments will help captive broodstock biologists develop and prioritize future rearing and release plans to minimize straying.

Results from our earlier studies under Project 199305600 demonstrated experimentally for the first time that there are multiple critical periods for imprinting for juvenile sockeye salmon and, specifically, that the alevin and smolt stages are both important developmental periods for successful olfactory imprinting (Dittman et al. 2004). Furthermore, the period of time that fish are exposed to imprinting odors may be important for successful imprinting. Experimental fish exposed to imprinting odors as smolts for six or one week successfully imprinted to these odors but imprinting could not be demonstrated in smolts exposed to odors for only one day. The current experiments focus on long duration odor exposures that parallel rearing and release strategies being tested as part of the Stanley Basin Sockeye Salmon Captive Broodstock program (BPA Project # 199107200).

The overall goal of the research conducted under this objective is to identify hatchery practices that influence olfactory imprinting and thereby develop strategies to minimize straying of artificially produced salmonids. The imprinting process is critical for successful completion of the spawning migration, and salmon that do not experience their natal water during appropriate juvenile stages are more likely to stray to non-natal sites. Reintroduction of captively-reared fish into the wild at inappropriate developmental periods or insufficient periods of exposure to appropriate olfactory cues may result in elevated levels of straying. Results from these studies will help develop captive broodstock reintroduction and hatchery release strategies that will minimize straying.

Materials, Methods and Description of Study Area

Determination of successful imprinting involves correlated assessments of odor attraction and heightened olfactory sensitivity in odorant-exposed fish relative to odorant-naïve controls. For the purposes of these studies, we assume that behavioral attraction (relative to odorant-naïve fish) of maturing adults to odors they have not experienced since smolting equates to successful imprinting. Furthermore, we hypothesize that heightened sensitivity to imprinted odorants as measured by increased levels of specific odorant receptor mRNA relative to control fish also reflect successful imprinting. As part of these studies, we are assessing this hypothesis and these tools by correlating changes in olfactory sensitivity with behavioral responses of fish from the different odor exposure treatment groups. The ultimate goal of these studies is to utilize these tools to define the release strategies that will contribute most to homing success.

Rearing and behavioral experiments were conducted at the University of Washington's Big Beef Creek Research facility in Seabeck, Washington. Laboratory studies were conducted at the Northwest Fisheries Science Center in Seattle Washington.

Experimental Design

Our previous studies demonstrated that sockeye salmon are able to learn odors at distinct developmental stages and odor exposure duration is critical for successful imprinting in sockeye salmon. In Fall 2004, experimental groups of odorant-exposed fish were established with particular emphasis on long duration odor exposures that parallel rearing and release strategies being tested as part of the Stanley Basin Sockeye Salmon Captive Broodstock program (BPA Project # 199107200). Two populations of fish were used for these studies: 1) F1 offspring of captively-reared Okanogan River sockeye salmon

originally obtained from the Colville Tribe Cassimer Bar Salmon Hatchery and 2) Stanley Basin sockeye obtained from the NMFS Redfish Lake Sockeye Salmon Captive Broodstock program at Burley Creek, Washington. Okanogan River fish have been used as a surrogate upper Columbia River population for our studies because it was originally not possible to conduct experiments with endangered Stanley Basin sockeye. For these experiments a limited number of Stanley Basin sockeye were also available. Eyed eggs from both populations were transferred to the Big Beef Creek field station (BBC) in December 2004 and reared in constant 10°C well water.

Okanogan River sockeye were divided into six treatment groups (200 Fish/treatment) (see Figure 1): (i) odorant naïve; (ii) alevin to smolt exposure, L -Arginine (January 2005 to May 2006) - odor exposure similar to eyed egg plants and naturally produced fish; (iii) pre-smolt to smolt exposure, L -Arginine (October 2005 to May 2006) - odor exposure similar to fall pre-smolt releases into Stanley Basin lakes from Eagle and Sawtooth hatchery; (iv) smolt exposure, L -Arginine (May-June 2006) - odor exposure similar to Oxbow/Eagle/Sawtooth hatchery rearing and smolt release (v) pre-smolt only exposure, L -Arginine (February to March 2006) – odor exposure during presumed “non-sensitive period” for imprinting; (vi) smolt exposure, L -Leucine (May 2006) - odor exposure to unrelated amino acid odorant. To examine population differences in imprinting and olfactory physiology and to establish imprinting patterns of Snake R. sockeye, Stanley Basin sockeye were divided into two treatment groups (200 fish/treatment): (i) Control 1 – odorant naïve; (ii) smolt exposure, L -Arginine (May-June 2006).

Amino acid odorants are being used for these studies because they have previously been used successfully as imprinting odorants (Morin et al. 1989; Dittman et al. 2004); the olfactory physiology of amino acid detection by salmon is well characterized (Hara 1992), and recent studies suggest that amino acids may be homing cues in natural waters (Shoji et al. 2003). Arginine was chosen as the primary imprinting odorant because our previous studies demonstrated that salmon imprint successfully to this amino acid and the receptor for this odorant has been identified, thus facilitating molecular assays of imprinting (see below). Leucine was chosen as the second odorant because it does not activate the arginine receptor (Hara 1992; Specia et al 1999) and sockeye salmon demonstrate no innate behavioral attraction to or avoidance of this amino acid (Dittman, unpublished). During the designated periods, odorants were continuously metered into tanks to maintain a final concentration of 100nM odorant. Prior to and during the period of the parr-smolt transformation (February - June 2006), 10 fish/treatment from each exposure group were sacrificed every three weeks for physiological sampling of gill Na^+/K^+ ATPase activity (McCormick 1993) and plasma thyroxine levels (Dickhoff et al 1982) to assess smolting and olfactory rosettes were collected for molecular analysis of imprinting. All groups were maintained separately until after the parr-smolt transformation (July 2006), then marked by treatment group and are being reared communally as a population to maturity at BBC. During the 2006-2008 period, both Okanogan and Stanley Basin arginine-naïve and arginine smolt-exposed fish (8-10 fish/treatment) were sacrificed to assess maturation-associated changes in olfactory function and imprinting. Fish were sampled in December 2006, March 2007, and then monthly from May 2007 until final maturation (September-October 2007). Fish were selected randomly from odor-exposed (n=8-10) and odor naïve (n=8-10) groups and euthanized in MS-222 using IUCAC-approved methods.

Under this Objective we are testing the following null hypotheses:

H01: Timing and duration of odor exposures that parallel Stanley Basin juvenile release strategies has no effect on imprinting success as measured by behavioral, physiological and molecular assay.

H02: Maturational physiology does not correlate with ultimate imprinting success.

H03: Maturational profiles (physiology, morphology, timing) do not differ between Okanogan River and Stanley Basin sockeye salmon.

H04: Imprinting success and timing (as measured by behavioral, physiological and molecular assay) does not differ between Okanogan River and Stanley Basin sockeye salmon.

Hypothesis H01 was tested by physiological, molecular and behavioral assessments of imprinting during 2008--2009 in which data collected on behavioral attraction and olfactory sensitivity to arginine are compared between arginine-naïve fish and arginine-exposed treatment groups. Relative importance of exposure duration will be tested by comparisons between exposure groups. Hypothesis H02 and H03 will be tested by comparing the maturational physiology (plasma FSH, LH, steroids, morphology, timing), arginine receptor expression and attraction to arginine in arginine-naïve and smolt-exposed fish from Stanley Basin and Okanogan River. Hypothesis H04 was tested by comparing the odor exposure groups and timing that produce successful imprinting in each population.

Behavioral testing

Behavioral responses of maturing sockeye salmon to imprinting odorants were tested August-September 2007 in three 2-choice mazes similar to those used in previous studies at the BBC facility (Havey and Dittman unpublished). Data collection and statistical analysis of behavioral attraction was completed in 2008 (Dittman et al. 2008).

Sampling for olfactory tissue, hormones, morphometrics, gonadal histology

Beginning in March 2007, Okanogan River (n=16) and Stanley Basin (n=20) sockeye salmon were sampled monthly until final maturation to assess imprinting success and correlative maturational status. Fish were selected randomly from odor-exposed (n=8-10) and odor naïve (n=8-10) groups and euthanized in MS-222 using IUCAC-approved methods. Fish were measured for length and weight and approximately 3-5 ml of blood was collected from the caudal vein using a heparinized syringe. Blood was centrifuged at 1000 x g for 15 min and plasma was stored at -70 °C for later hormone analysis. Each fish was photographed for subsequent morphometric analysis. Sex and gonad weight (prior to final maturation) was determined for each fish. GSI was calculated as (gonad weight/body weight) x100. Olfactory rosettes were removed and frozen directly in liquid nitrogen and stored at -70°C for subsequent analysis of gene expression of odor receptors. A small piece of gonadal tissue was placed in Bouin's fixative for 48 hours at 4° C and stored in 70% ethanol for subsequent histological analysis.

Molecular testing

The initial events in odor (e.g., homestream water) recognition are mediated via binding of odorant molecules to specific receptor proteins expressed in the olfactory receptor

neurons (ORNs) of the salmon olfactory rosette. Each of the millions of ORNs in the rosette expresses one of a family of approximately 100 distinct odorant receptors. For one of these receptors, we have identified the odorant ligand that binds and activates the receptor. In goldfish this receptor is a basic amino acid (arginine and lysine) receptor and in our ongoing work we have identified the coho and sockeye salmon orthologue of the goldfish receptor gene. Furthermore, we have developed a real-time PCR assay for quantifying the expression of the arginine odorant receptor (AOR) mRNA levels in the salmon olfactory rosette. During imprinting, the number of olfactory neurons that are responsive to an imprinting odorant increases and the sensitivity of those neurons to that odorant is heightened. We hypothesize that this heightened cellular sensitivity is due to increased numbers of cells expressing a given receptor protein and increased expression of the receptor within responsive neurons. Utilizing the AOR we have attempted to test this hypothesis by exposing coho salmon to arginine during sensitive periods for imprinting and measuring the levels of AOR mRNA in arginine-exposed vs. arginine naïve fish. Juvenile coho salmon exposed to arginine during smolting demonstrated heightened sensitivity of the olfactory epithelium to this odorant and increased expression of AOR mRNA relative to odorant-naïve controls.

Molecular assessments of imprinting involved quantifying changes in the AOR mRNA levels in the salmon olfactory rosette in response to odorant exposures using a sockeye salmon-specific quantitative AOR PCR assay (Dittman et al 2005). RNA was isolated from individual rosettes of salmon collected from each treatment group during maturation (Tireagent, MRC) and qPCR assays were conducted using an ABI Prism 7700 real time thermocycler. For molecular analysis, treatment effects on gene expression level in olfactory tissue were analyzed by ANOVA. Final analysis of Okanogan River sockeye mRNA expression is completed but not fully analyzed and will be included in the next annual report.

Maturation physiology, gonadal histology and morphometrics.

Plasma levels of FSH and/or LH were measured in all samples using the methods of Swanson et al. (1989). In females, plasma estradiol-17 β (E₂) will be measured with a commercially available radioimmunoassay kit (ICN Diagnostic Products, Irvine, CA). Plasma levels 11-KT levels in males will be measured as described in Larsen et al. 2004. Histological processing and developmental staging of gonadal tissues was conducted in 2008-2009. Fixed gonadal tissues was processed and embedded in paraffin. Sections were cut on a microtome, stained with hematoxylin and eosin, and photographed with a digital camera. The degree of maturation was determined by the proportion of germ cells at each stage of gametogenesis according to methods described in Campbell et al. (2003, 2006). Morphometric analysis will be conducted in 2009-2010 using the methods of Winans et al. (2003).

Statistical analyses

Once all physiological and morphometric measures have been compiled, we will use multivariate analysis to test for correlations between factors (population, sex, maturation status, plasma hormone levels, smolting indicators) regulating imprinting success (attraction to imprinting odor, odor receptor expression). We will then use Akaike's Information Criteria to select the most parsimonious model to explain the data.

Significant effects may be further characterized by Kruskal-Wallis analysis followed by pair-wise comparisons between treatments using Wilcoxon sign rank tests. All analyses will be performed using JMP (SAS, Carey, NC) and S-Plus.

Results and discussion of results

Behavioral testing

Maturing fish from both populations (n=342) were tested for attraction to imprinting odors by measuring the time fish spent in the arm of the maze scented with odors. On average, fish from all groups that experienced arginine as juveniles spent more time per entry in the odor arm than control fish (Figure 2). The pre-smolt exposure group spent significantly greater time per entry in the Arginine arm than control fish ($P=0.015$) and the smolt exposure group from both the Okanogan and RFL population were also attracted to the arginine relative to controls but these effects were marginally non significant due to the unexpectedly low numbers of fish making choices in the maze ($P=0.076$, and $P=0.187$, respectively). A tendency to prefer the arginine-scented arm was also seen with the Okanogan fish exposed to arginine throughout the juvenile rearing period (egg-smolt) ($P=0.33$).

Taken together, these results suggest that sockeye salmon are capable of imprinting to homing cues during the developmental periods that correspond to several of current release strategies employed as part of the Captive Broodstock program (specifically, planting eyed eggs, fall and smolt releases into the lake) appear to be appropriate for successful homing of sockeye in Redfish Lake. Also, our findings indicated that sockeye salmon were capable of olfactory imprinting at multiple life stages and over varying exposure durations. Fish exposed to L-Arginine for any duration as juveniles (i.e., all treatment groups and both populations) spent longer, on average, in the odor arm per entry than the control arm. Interestingly, fish exposed to odors just prior to smolting showed the strongest attraction to the imprinting odor arginine and this period corresponds to the period of highest plasma thyroxine levels and increased BAAR receptor mRNA in juveniles.

Molecular assessments of imprinting

Expression levels of BAAR mRNA in the olfactory epithelium increased dramatically during final maturation in both Stanley Basin (Figure 3) and Okanogan River sockeye (data not shown). These increases appeared to be independent of odor-exposure history, rising significantly in both arginine-naïve and arginine-exposed fish. However, sockeye exposed to 10^{-7} M arginine during smolting demonstrated a larger increase in BAAR mRNA than arginine-naïve fish. These results are consistent with the hypothesis that odorant receptors sensitive to home stream waters may be upregulated at the time of the homing migration and may afford opportunities to exploit this system to experimentally characterize imprinting success. Further analysis of physiological correlates of maturation (reproductive hormones, gonadal histology, morphometrics) should shed further light on these processes.

Maturation physiology, gonadal histology and morphometrics.

Approximately 95% of the Stanley Basin sockeye and 68% of the Okanogan River sockeye matured as three-year olds in Fall 2007. Both populations grew rapidly during their final spring and then grew relatively little in length from July until final maturation (Figures 4 and 5). Males were slightly larger (fork length) than females but there was no difference between populations. Okanogan River sockeye matured earlier than Stanley Basin sockeye and this was reflected in an increased GSI in Okanogan sockeye relative Stanley Basin sockeye as much as four months prior to final maturation (Figures 6 and 7).

Temporal patterns of plasma gonadotropins (FSH and LH) levels were similar for both populations but varied considerably between sexes. For females, plasma FSH levels rose dramatically in July, peaking in late September and then dropping back to prematuration levels just before final maturation (Figure 8). Males experienced an earlier surge in FSH levels that remained elevated until just before final maturation (Figure 9). Just prior to final maturation, plasma LH levels in females surged dramatically (Figure 10) while males experienced more modest increases in plasma LH at maturation (Figure 11). The general patterns of gonadotropin levels are consistent with previous studies of sockeye salmon (Swanson et al. 1995).

Initial correlative analysis suggests that developmental increases in BAAR mRNA expression occurred at the time most fish were approaching final maturation but determination of direct correlations will require final compilation of data and multivariate analysis to test for correlations between factors (population, sex, maturation status, plasma hormone levels, smolting indicators). Differences in BAAR expression levels between imprinted and unimprinted fish were apparent prior to the surge in LH and final maturation and were consistent with increased sensitivity to imprinted odors during the period salmon would be homing to their natal streams. These differences might be exploited to determine environmental and rearing conditions that facilitate successful imprinting. Once all physiological and morphometric measures have been compiled, we will use multivariate analysis to test for correlations between factors (population, sex, maturation status, plasma hormone levels, smolting indicators) regulating imprinting success (attraction to imprinting odor, odor receptor expression).

Summary and Conclusions

Reintroduction of captive-reared fish into the wild at inappropriate developmental periods or insufficient periods of exposure to appropriate olfactory cues may result in elevated levels of straying. The overall goal of this research is to identify hatchery practices which influence olfactory imprinting, thereby develop strategies to minimize straying of artificially produced salmonids. The primary focus of these efforts is to develop and utilize imprinting assessments tools to identify developmental periods that are important for olfactory imprinting and thereby define release strategies that will contribute to homing success. To determine the critical period(s) for imprinting for sockeye salmon, juvenile salmon were exposed to known odorants at key developmental stages. Molecular assessments of imprinting-induced changes in odorant receptor gene expression indicated that regulation of odorant expression is influenced by developmental status and odor exposure history.

Data Management Activities

Data have been collected by research staff from NMFS and the University of Washington, and onto preformatted data sheets and entered directly into their PCs. All data are checked for quality and accuracy before analysis. Analytical processes are described in the text of the annual report.

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Figure 1. Experimental design for odorant exposures and testing for imprinting success. Salmon were exposed to odorants at developmental stages and for durations that parallel Stanley Basin sockeye reintroduction strategies.

Figure 2. Average time per entry, in seconds, for each fish. Values for both the L-Arginine (A) and L-Leucine (L) arms are given for each group as the mean time per entry $\pm$ S.E.M.

Figure 3. Changes in expression levels of BAAR mRNA during maturation in Stanley Basin sockeye salmon.

Figure 4. Changes in length during final year prior to maturation in experimental Okanogan River (OK) and Red Fish Lake (RFL) sockeye salmon females.

Figure 5. Changes in length during final year prior to maturation in experimental Okanogan River (OK) and Red Fish Lake (RFL) sockeye salmon males.

Figure 6. Changes in GSI during final year of maturation in experimental Okanogan River (OK) and Red Fish Lake (RFL) sockeye salmon females.

Figure 7. Changes in GSI during final year of maturation in experimental Okanogan River (OK) and Red Fish Lake (RFL) sockeye salmon males.

Figure 8. Changes in plasma FSH during final year of maturation in experimental Okanogan River (OK) and Red Fish Lake (RFL) sockeye salmon females.

Figure 9. Changes in plasma FSH during final year of maturation in experimental Okanogan River (OK) and Red Fish Lake (RFL) sockeye salmon males.

Figure 10. Changes in plasma LH during final year of maturation in experimental Okanogan River (OK) and Red Fish Lake (RFL) sockeye salmon females.

Figure 11. Changes in plasma LH during final year of maturation in experimental Okanogan River (OK) and Red Fish Lake (RFL) sockeye salmon males.

OBJECTIVE 3 - USE ENVIRONMENTAL FACTORS TO MATCH WILD PHENOTYPES IN CHINOOK AND SOCKEYE SALMON REARED IN HATCHERY SUPPLEMENTATION PROGRAMS

by

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Introduction

In supplementation programs for Columbia River Basin steelhead trout and Chinook salmon, juveniles may be reared for at least a year in hatcheries prior to release as smolts. Early life histories of both species are variable and influenced by an interaction between the genetic composition of the animals and environmental factors, particularly those that affect growth (c.f. Hankin et al. 1993, Healey 1994; Heath et al. 1994). For example, smoltification can occur at different sizes, seasons (fall or spring) and ages, and the proportions of males maturing at age 1 and 2 can vary (Metcalf and Thorpe, 1992; Mullen et al., 1992; Rowe and Thorpe 1990a,b; Rowe et al. 1991; Silverstein et al. 1998; Shearer and Swanson 2000; Swanson et al. 2004; Shearer et al. 2006). Because of this plasticity, the early life history of steelhead and Chinook salmon reared in supplementation hatcheries may depart considerably from the pattern exhibited in wild fish. Environmental conditions during hatchery rearing (temperature and feeding rates) may alter life history characters at release (size, degree of smolting, age of maturation, fecundity, egg size) and inevitably lead to differing patterns of mortality and reproductive success (Beckman et al. 1999; Zabel and Achord 2004; Connor et al. 2005). This ultimately alters the genetic structure of populations.

The long term aim of research under Objective 3 is to improve hatchery-rearing protocols to produce fish with life history characters similar to wild fish, and minimize artificial selection due to altered phenotypes of juvenile fish at release. The focus of our efforts has been to understand how hatchery rearing practices alter smoltification and age of male maturity because high rates of maturation of male spring Chinook salmon at age 2 have been documented for a variety of hatcheries in the Columbia River Basin (Larsen et al. 2004; Pearsons et al., 2004; Beckman and Larsen 2005). In this study the effects of two environmental factors, photoperiod (at emergence) and food availability (growth during early rearing), on seasonal timing of smolting, smolt quality, and age of male maturity are being examined in spring Chinook salmon. We chose to examine these factors because previous studies have shown that emergence timing (Beckman et al. 2007) and growth during critical periods of the life cycle affects the proportion of males maturing at early ages relative to females (Rowe and Thorpe 1990a,b; Rowe et al. 1991; Silverstein et al. 1998; Shearer and Swanson 2000; Campbell et al. 2003; Swanson et al. 2004, 2005; Vøllestad et al., 2004; Shearer et al. 2006).

Materials and Methods, and Description of Project Area

Experimental design and fish rearing

This experiment tests the following hypotheses:

H₀₁: Photoperiod at emergence does not alter seasonal timing of smoltification or age of male maturation in Yakima River spring Chinook salmon.

H₀₂: Growth (ration) after ponding does not alter seasonal timing of smoltification or age of male maturation in Yakima River spring Chinook

The independent and interactive effects of photoperiod at emergence (3 different photo regimes) and growth (2 rations) post-ponding on age of maturity in spring Chinook salmon males are being examined. Buttoned-up fry (Yakima River spring Chinook salmon) were placed into each of three different photoperiods: Early Emergence (EE, photoperiod equivalent to that found on 1 December), Middle Emergence (ME, photoperiod equivalent to that found on 15 February), and Late Emergence (LE, photoperiod equivalent to that found on 1 May). Throughout the rest of this report calendar day (day #) will refer to when events take place, months will refer to seasonal photoperiod (for each calendar day there are 3 different photoperiods). Early emergence represents hatchery/captive broodstock programs that accelerate emergence by incubating eggs in relatively warm water (~ 10°C) and late emergence represents conditions found in very cold, high elevation Snake River tributaries. We anticipated that this range of photoperiods would provoke the entire range of phenotypic responses found for spring Chinook salmon under current environmental conditions.

Ponding occurred on day # 45 (mid-February). Because of this design fish in all treatments were the same age throughout the experiment even though they are exposed to different photoperiods. Within each photoperiod treatment, fish were fed under two feeding regimes: Hi Feed and Lo Feed. Target sizes after first year were 10 and 25g on day # 270 (1 October) for Lo Feed and Hi Feed treatments, respectively. Target sizes in spring of the second year (at 1+ age smolting) were 25 and 50g on day # 485 (1 May) for Lo Feed and Hi Feed treatments, respectively. Feeding rate was adjusted so that fish from a given feeding treatment (Hi Feed or Lo Feed) were the same size regardless of photoperiod treatment. A third feeding regime (satiation) was tested, but rearing of fish was terminated early because of constraints with tank size for the rapidly growing fish. This group was monitored for growth and smolting along with other ration treatments.

Sampling and Laboratory Methods

At approximately 4-week intervals after ponding, size was assessed with batch weights (3 batches of 50 fish) and sub-samples of individual fish (8 fish/tank, 16 fish/treatment). Individual fish were euthanized to obtain gill tissue, blood plasma and gonad tissue. Gill Na-K ATPase activity was measured as an index of smolting (McCormick et al. 1993). Seawater challenges were conducted monthly (72 hours in 37.5 ppt seawater) as another assessment of smolting. In the next funding period, plasma levels of a variety of metabolic hormones (Shimizu et al. 2000) and body fat levels will be analyzed to assess growth and metabolic status to discern any effects of rearing regimes on other aspects of

smolt quality. Plasma 11-ketotestosterone (11-KT) will be measured to determine treatment effects on the timing of onset of maturation (Cuisset et al. 1994, Larsen et al. 2004). At the end of the experiment, maturing males were readily identified by gonadosomatic indices (GSIs), however plasma 11-ketotestosterone levels in the immature males will aid in determining whether some males are maturing in the subsequent year (age 3).

Results and Discussion

The experiment was terminated in August 2008. Sample analyses for characterization of smoltification have been completed. This report describes data on male maturation rates at age 1 (Fall 2007) and age 2 (Fall 2008), and seasonal timing of smoltification. Data on growth and age 1 male maturation were included in the previous annual report. Analyses of plasma levels of metabolic hormones and body composition, and final statistical analyses of all data will take place during fiscal year 2009.

Both emergence timing and ration affected the percent of early maturing males, but the proportion of males maturing at age 1 versus age 2 differed according to timing of emergence (Figure 1). In the early emergence groups, 35-40% of males matured at age 1, whereas less than 5% of males in the middle emergence groups and almost none of the males in the late emergence groups matured after the first year (Figure 1B). However, the middle and late emergence groups had a higher proportion (30-40%) of males maturing at age 2 than the early emergence groups (20-25%; Figure 1C). This was not due to any treatment effect on sex ratios since 50% of the fish in all groups were females (Figure 1A). The proportion of males maturing at either age 1 or 2 was also affected by ration with the groups on higher ration having higher rates of male maturation than those on low ration irrespective of emergence timing (Figures 1 and 2). The highest rates of early male maturation were observed in groups that emerged early and had high ration. In this group, 60% of the males had matured by the second year compared to 30% in the late emergence and low ration group (Figure 2). The total proportion of males maturing by the second year (age 1 and age 2) was negatively related to day of emergence (Figure 3). The effect of emergence timing on male maturation was most pronounced during the first year (Figure 1B). If the mature yearling males are removed from the data analysis and rates of age 2 maturation are calculated on the basis of number of males that did not mature at age 1, it is apparent that ration and not emergence timing affected the decision of remaining yearling fish to mature at age 2 (Figure 4). In this case, the effects of ration on age 2 maturation appeared to be most evident in the early emergence groups. This may be because fish with lower size/growth rate thresholds for maturation were removed since they matured at age 1. These data indicate that in the Yakima River spring Chinook salmon population, both emergence timing and ration can drastically alter the proportion of males maturing at age 1 versus age 2, while ration (growth) can affect the propensity of a male that did not mature at age 1 to mature the following year.

In addition to age of male maturity, the seasonal timing of smolting (as measured by changes in gill $\text{Na}^+\text{-K}^+$ ATPase activity) was also affected by emergence timing (Figure 5). Fish from the early emergence group appeared to smolt during three periods; prior to the summer solstice, and at the autumn and spring equinox. In the middle emergence group, fish smolted at the summer solstice and spring equinox. Smolting was also observed in two periods (spring and fall equinox) in the fish from the late emergence group although the fall smolting was dampened. This pattern was also somewhat

reflected in the results of saltwater challenges (Figure 6). In all groups, survival after the saltwater challenge was highest during the second spring (Mar-May). However, the fish from the early emergence group also had high survival after saltwater challenges in their first fall. Ration also affected survival after saltwater challenges in the early emergence group; nearly 50% of fish on the early emergence and satiation treatment survived the seawater challenge in the first fall, indicating that a large proportion of fish in this group had smolted. Within the early emergence group, ration appeared to affect the duration of the summer and fall smoltification periods (Figure 6A) with the satiation fed fish having high survival post-seawater challenge and high gill $\text{Na}^+\text{-K}^+$ ATPase activity throughout the summer into fall. In contrast, fish exposed to the late emergence photoperiod had 100% mortality after the seawater challenge in the first fall, but high survival in the spring challenge test, irrespective of ration treatment. The photoperiod at emergence appeared to affect the size threshold for smolting in the fall with more of the smaller fish (80-100 mm length) in the early emergence group (Figure 7) showing elevated gill $\text{Na}^+\text{-K}^+$ ATPase activity in the fall (August-September 2007) than those in the middle or late emergence groups (Figures 8-9). This is evident in the relationship between body length and gill $\text{Na}^+\text{-K}^+$ ATPase activity at various times of year (Figures 7-9). Thus, the early emerging fish smolted over a broader body size range and seasonal period.

Summary and Conclusions

The results of this study indicate that alterations in the seasonal timing of fry emergence and growth after ponding of spring Chinook salmon can have profound effects on later life history events; age of male maturity and smoltification. Another study using Sacramento River winter run Chinook salmon showed similar effects of emergence timing and growth on the life history phenotypes (Beckman et al. 2007) suggesting this may be a more general pattern in Chinook salmon. Many hatcheries manipulate emergence timing and growth to more efficiently utilize facilities that often accommodate different species and to achieve a critical size for tagging prior to release. However, the results of this study indicate that the practice of altering the emergence timing can have profound effects on later life history events which depart considerably from fish reared in natural stream conditions. Results from this study also show that photoperiod at emergence and feeding regimes can alter the range of phenotypes within a population. For example, fish reared under conditions to delay emergence and slow growth had the least variation in phenotypes with most fish smolting as yearlings in the spring and no age 1 male maturation. In contrast, early emerging fish raised on high growth conditions had the greatest variation in seasonal timing of smolting and highest rates of early male maturation with most males maturing at age 1. Work in the next funding period will determine if these rearing regimes affected other aspects of smolt quality (e.g. body energy stores and metabolism), which may affect ultimate survival upon ocean entry.

Data Management Activities

Data are being collected by NOAA and University of Washington researchers onto preformatted data sheets or directly into electronic spreadsheets or text files. Data are entered and summarized on personal computers operated by researchers; primary software used for these procedures includes Microsoft Excel 2000 and Word 2000. JMP or Prism Graphpad are used for statistical analyses. All data are checked for quality and accuracy before analysis. Analytical processes are described in the text of the annual

report under Materials and Methods. Data analyses are reported in the Results section of the annual report. Final data analyses will be included in the FY 2009 Annual Report.

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Figure 1. Percent of females (A), males that matured at age 1 (B), and males that matured at age 2 (C) in groups of fish reared on early, middle and late emergence photo regimes, and either Lo Feed (striped box) or Hi Feed (solid box) treatments.

Figure 2. Percent of males that matured at age 1 and 2 combined in groups of fish reared on early, middle and late emergence photo regimes, and either Lo Feed (striped box) or Hi Feed (solid box) treatments.

Figure 3. Relationship between percent of males that matured at age 1 and 2 combined and day of emergence in groups of fish reared on early (blue), middle (green) and late (orange) emergence photo regimes, and either Lo Feed (striped circles) or Hi Feed (solid circles) treatments.

Figure 4. Percent of yearling males that matured at 2 (calculated from number of males maturing at age one removed) in groups of fish reared on early, middle and late emergence photo regimes, and either Lo Feed (striped box) or Hi Feed (solid box) treatments.

Figure 5. Gill Na⁺-K⁺ ATPase levels in nonmaturing fish from groups reared on early (A), middle (B) or late (C) emergence photo regimes and either satiation (S, orange triangles), Lo Feed (LF, blue solid circles) or Hi Feed (HF, green open squares) ration treatments. Solstice: dotted vertical lines, Equinox: solid grey vertical lines. Data points are mean $\pm$ standard errors of 6-12 replicates.

Figure 6. Percent survival after 24 hour seawater (SW) challenge tests for in nonmaturing fish from groups reared on early (A), middle (B) or late (C) emergence photo regimes and either satiation (S, orange triangles), Lo Feed (LF, blue solid circles) or Hi Feed (HF, green open squares) ration treatments. Solstice: dotted vertical lines, Equinox: solid grey vertical lines)

Figure 7. Relationship between body size (length) and gill Na⁺-K⁺ ATPase levels in all nonmaturing fish reared on the early emergence photo regime (all ration treatments combined). Data are color coded by photoperiod at time of sampling. Months shaded in grey indicate months with > 3 fish with ATPase levels that were > 1 standard deviation from the mean. Horizontal light blue rectangle indicates lowest mean value +/- 1 standard deviation of that mean (n=12).

Figure 8. Relationship between body size (length) and gill Na⁺-K⁺ ATPase levels in all nonmaturing fish reared on the middle emergence photo regime (all ration treatments combined). Data are color coded by photoperiod at time of sampling. Months shaded in grey indicate months with > 3 fish with ATPase levels that were > 1 standard deviation from the mean. Horizontal light blue rectangle indicates lowest mean value +/- 1 standard deviation of that mean (n=12).

Figure 9. . Relationship between body size (length) and gill Na⁺-K⁺ ATPase levels in all nonmaturing fish reared on the late emergence photo regime (all ration treatments combined). Data are color coded by photoperiod at time of sampling. Months shaded in grey indicate months with > 3 fish with ATPase levels that were > 1 standard deviation from the mean. Horizontal light blue rectangle indicates lowest mean value +/- 1 standard deviation of that mean (n=12).