DOCUMENT ID #P111274

PROJECT 1993-056-00 PROGRESS REPORT (PERFORMANCE PERIOD: 1 JUNE 2007 THROUGH 31 MAY 2008)

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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 32125

15 July 2008

Abstract

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: Adult and jack Chinook salmon males were stocked into four replicate spawning channels at a constant density (N = 16 per breeding group), but different ratios, and were left to spawn naturally with a fixed number of females (N = 6 per breeding group). Adult males obtained primary access to 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. Spawning participation by jack and adult males is consistent with a negative frequency dependent selection model, which means that selection during spawning favors the rarer life history form. Results of DNA parentage assignments will be analyzed to estimate adult-to-fry fitness of each male.

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. The 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 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.

Objective 3: Spring Chinook salmon were exposed to three different photoperiods and three feed rations at the button-up stage of development. Both photoperiod at emergence and ration post-ponding affected the number of males maturing at age one. Nearly 70% of the males in the early emergence and satiation fed group matured after the first year of rearing, while none of the fish reared on late emergence photoperiod (equivalent to emergence on May 1) matured during this time irrespective of ration treatment. Within the early emergence groups, reducing growth using ration (low or high) appeared to reduce the number of males maturing at age one from 70% to 40-50%. Maturation rates of fish that emerged in a photoperiod equivalent to mid-February (middle emergence) ranged from 10-25%.

Together these data indicate that the seasonal timing of fry emergence and growth after ponding can alter life history patterns in spring Chinook salmon. The results imply that hatchery rearing practices that alter seasonal timing of fry emergence can have drastic effects on life history patterns in juvenile Chinook salmon.

All three objectives are on-going and will result in recommendations (at the end of the FY 2009 performance period) to advance hatchery reforms in conventional and captive broodstock programs.

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OBJECTIVE 1: THE ADULT-TO-FRY REPRODUCTIVE SUCCESS OF JACK AND ADULT MALE CHINOOK SALMON SPAWNING UNDER DIFFERENT RELATIVE FREQUENCIES.

By

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Introduction

One of the major concerns with the use of captive broodstocks and conventional hatchery programs for supplementing endemic populations is the relaxation of selection pressures or unintentional directional selection during reproduction (Waples 1999). While recognizing that hatchery spawning practices cannot exactly mimic the sexual and ecological selection that occurs leading up to and during natural spawning, much debate has focused on whether broodstocks in conservation programs should be selected to maximize genetic variability or manage to maximize adaptive genetic variation (genetic quality) (Wedekind 2002). Hatchery and Genetic Management Plans (HGMP) now frequently emphasize maintaining natural run timing and body size in hatchery broodstocks and implementing spawning practices (e.g., one-to-one matings) to maintain a high level of genetic variability in the offspring. Nevertheless, there is a general consensus that some divergence of hatchery populations from the wild state is inevitable and that all possible measures should be taken to minimize it, particularly for ESA-listed populations (Flagg et al. 2000). The Hatchery Scientific Review Group in Washington State has concluded that a detailed genetic management plan is the “first step” towards hatchery reform (HSRG 2005).

Perhaps the most notable, complex and evolutionarily significant life history strategy in Chinook salmon is the co-existence of males that mature one year earlier than the youngest females in a population; that is age-3 ‘jack’ males and older (age-4 and -5) males. In Pacific salmon, the presence of jack and older males has been explained as a conditional reproductive strategy with two alternative tactics: older males who fight for access to females, and much smaller sneakers (usually jacks) who do not challenge fighters, but instead position themselves downstream of nesting females, and dart into the nest during spawning to fertilize eggs (Gross 1985). Jacks are not obligate sneakers, however. Jack male Chinook salmon will court females in the absence of older males, and females readily spawn with them without compromising fertilization success (Berejikian et al. 2000, 2005). This life history strategy allows for gene flow between brood years and probably buffers populations from negative genetic consequences of small population sizes or catastrophic mortality in any given year (Healy 1991).

The existence of these two major life history patterns in Chinook salmon represents a trade-off between ecological and sexual selection. Jack males benefit from higher survival to maturity owing to their reduced time spent at sea. They may also be favored by other forms of ecological selection during reproduction (Futuyama 1998), such as

reduced predation by avian or terrestrial predators. Based on results from DNA-microarray analysis of jack and non-maturing male Atlantic salmon, Aubin-Horth (2005) has proposed that early male maturation may actually represent the default developmental pathway, and the slower growing males repress maturation until the following year. Nevertheless, jack males often represent a very small portion of breeding populations of sockeye, coho and Chinook salmon (Carlson et al. 2004, Appleby et al. 2003, Myers et al. 1998), suggesting that their low frequency in the population reflects a significant genetic component coupled with poor breeding success (because they are small and relegated to sneaking, Carlson et al. 2004). 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 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%) relative to that of older males. The present study provides the first such information for the Chinook salmon.

Jacking in Pacific salmon is mediated both by genetic and environmental factors. Body size, fatness, and other environmentally mediated traits explain a significant portion of the variability in jack rates (Vøllestad et al. 2004; Shearer et al. in press). Nevertheless, several studies have identified that jacking is heritable (Hankin et al. 1993; Heath et al. 1994). Heath et al. (2002) estimated a high heritability coefficient for the sire component of the additive genetic variance ($h^2_{sire} = 0.62$), which indicated a strong sex-linked component (Y-chromosome) to jacking in chinook salmon, although evidence for at least some autosomal contribution was also observed. Significant differences in jacking rates between Chinook salmon stocks, and higher jack returns from coho salmon offspring sired by jack fathers indicates that early male maturity has a strong heritable component (Hard et al. 1985, Appleby et al. 2003).

We conducted two experiments. The first experiment evaluated the reproductive behavior and success of jack and adult male Chinook salmon spawning under different ratios. The second experiment evaluated the growth and survival of jack and adult male offspring. We used a paired spawning design test for male genetic effects by accounting for variation in offspring behavior and survival associated with maternal effects, which can be quite significant.

Materials, Methods, and Description of Study Area

Experiment 1: Reproductive behavior and success

Adult Chinook salmon were obtained from the Trask River Hatchery and transported to the Oregon Hatchery Research Center (OHRC), located on Fall Creek. Six female and 16 male Chinook salmon were stocked into each of four replicate spawning channels. The spawning channels measured 8 m wide by 65 m long, and contained a mixture of substrates ranging from course 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 Falls Creek, through a settling pond to each channel to provide approximately 1.5 ft^3/s per channel. Airlift pumps were used to partially re-circulate water through the channels to create a total flow of approximately 2 ft^3/s in each.

Prior to being stocked into the spawning channels, 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. Two of the Channels (C and D) were stocked on 6 September 2007. The other two channels (A and B) were stocked on 13 September 2007. At the time of stocking, 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. The number of jack and adult males varied among the four channels (A: 12 adults and 4 jacks, B: 8 adults and 8 jacks, C: 10 adults and 6 jacks, D: 5 adults and 11 jacks) although the total number of males was fixed at 16.

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 nest building, 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. 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.

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 subsampled at a similar rate among channels. Subsampled fry received a lethal dose of MS-222 and their caudal fins were preserved in 100% non-denatured alcohol for DNA pedigree analyses.

Experiment 2: Offspring growth and survival

On 20 September 2007, the eggs of 12 sexually mature Trask River Chinook salmon females were manually spawned into a 4 L bucket. For each female, two 300 ml subsamples of the eggs (approximately 500 to 800 eggs depending on egg size) were removed and placed into separate 1 L plastic bags. Three milliliters of semen from either a jack or adult male were added to each bag and the eggs and sperm were swirled in the bag for 1 min prior to adding 0.5 L of water. The eggs were then placed in replicate 10-cm diameter pvc isollettes situated in Heath incubation trays (six isollettes per tray), where the eggs were incubated to the eyed stage of embryonic development. This resulted in 24 full-sibling families (12 maternal half-sibling families). The location of each family's isollettes was randomly distributed throughout the incubation unit to minimize the potential for confounding environmental variables to influence survival to the eyed stage of embryonic development.

All eyed embryos were transported to the NOAA Fisheries, Northwest Fisheries Science Center's Manchester Research Station. On 25 October, 2008, eggs from 10 maternal half-sib families (20 full-sib families) were stocked into Jordan-Scotty egg incubators (http://www.scotty.com/marine/incubator.html). Each full-sib family was placed into a separate, labeled row in each of 16 incubators and the row location for each family was randomized among the incubators. Two incubators were buried into artificially constructed redds in each 10 m long x 3 m wide section of the experimental stream channel. Thus, each full-sib family was represented in each of two incubators in each of the eight sections. The stream channel provided a flow of 6,800 $L \cdot min^{-1}$ of re-cirulated fresh water with 60 $L \cdot min^{-1}$ of water continuously flowing through the system. The stream channel provided quasi-natural incubation and rearing habitat (e.g., gravel substrate, natural aquatic and terrestrial invertebrate production, fluctuating temperature, natural light, and stream-like flow conditions; see Berejikian et al. xxx for details).

The eggs were left to incubate and the fry allowed to emerge naturally from the gravel and compete for food resources in the absence of any predators until 27 May 2008, when all fish were removed by seining and electroshocking. At the time of removal, nearly all fish showed signs of smoltification, including, silvering, loss of prominent parr marks, and loosening of scales.

A total of 100 fish from each of the eight sections was subsampled and individual fork lengths (mm) and weights (nearest 0.1 g) were recorded before preserving each uniquely identified fish separately in 95% ethanol for DNA pedigree analyses.

For experiments 1 and 2, Genomic DNA was extracted from the adult and fry 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. Final determinations were being made at the time this report was written.

Results

Experiment 1: Reproductive behavior and success

We observed a total of 26 female spawning events in all four channels combined. The number of males participating in each of those events ranged between one and five (Figure 1). Adult males nearly completely dominated access to nest building females and jack males took up positions downstream of courting male-female pairs. Jack males were observed courting females, but this typically occurred during times when the females had recently spawned and were hours from the subsequent spawning event.

The majority of observations came from channels A (9) and B (12), and far fewer from C (3) and D (2). 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 hence missed some spawning events. However, in channels A and B (i.e., the second group of fish stocked on 13 September) 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. Cleary much spawning occurred at night in both channels that was not observed.

Courtship frequency was significantly and positively correlated with the number of spawning participations in channel A ($r^2 = 0.53$, $p < 0.01$) and channel B ($r^2 = 0.65$, $p < 0.01$; Figure 2), suggesting that establishing a position within a male dominance hierarchy strongly predicted a behavioral measure of breeding success. In channel A, with highest number of jack males, body size was significantly and positively correlated with courting success ($r^2 = 0.55$, $p < 0.01$). Male body size was not significantly correlated ($P < 0.05$) with courting frequency in the channels B ($r^2 = 0.01$), C ($r^2 = 0.05$), and D ($r^2 = 0.03$) with the three lowest number of jack males (Figure 2).

In channel A, three of the eight jacks (38%) and seven of the eight adults (88%) were observed spawning at least once in the nine observed spawning events. In channel B (lowest number of jacks), all four jacks and 5 of 12 adults were observed spawning at least once in the 12 observed spawning events. Thus, in these two channels a similar total number of males (9 in channel A and 10 in channel B) participated in spawning but the proportion of males contributing from each life history type was inversely proportional to their abundance within each channel. Male rank order of nest entry during spawning was greater for adult males than for jack males (Figure 3)

We collected 3,010 fry from channel A, 5,972 from channel B, 5,198 from channel C and 3,682 from channel D. We conducted DNA pedigree analyses on 301 from channel A,

597 from channel B, 520 from channel C and 368 from channel D. Final pedigree assignments, results and interpretation will be provided in the FY 08 Annual Report.

Experiment 2: Offspring growth and survival

Survival from eyed egg to juvenile collection on 27 May 2008 averaged 78% among the eight sections and ranged between 76% and 83%. Although the density of fish rearing in each of the eight sections remained fairly similar by the end of the study, the average length and weight of fish in the four sections on the south side of the channel were significantly smaller than those on the north side ($P < 0.05$; Table 1). Fourty-eight fry from each of the 8 stream channel sections were genotyped for assignment. Final pedigree assignments, results, and interpretation will be provided in the FY 08 Annual Report.

Discussion

The salient findings of this study to date are as follows. First, adult males obtain primary access to females and are first to enter the nest at the time of spawning. Second, jack male spawning occurred primarily by establishing satellite positions downstream of the courting pair, and 'sneaking' into the nest at the time of spawning. Third, male dominance hierarchies were fairly stable and strongly correlated with the order of nest entry at the time of spawning. Fourth, spawning participation by jack and adult males is consistent with a negative frequency dependent selection model.

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 (Mjölnerod et al. 1998), and brook trout (Blanchfield et el. 2003) that is presumably an result of rank order of nest entry and sperm precedence. We anticipate a similar result when the results of our pedigree analyses are finalized.

Eyed egg to juvenile survival in the offspring growth and survival experiment was consistently high for all groups. Therefore, we do not expect to find a significant difference in among-family survival. Growth rates on the two sides of the channel were substantially different, which will allow for a comparison of jack and adult-sired offspring growth rates under high and low growth conditions.

The existence of alternative male phenotypes (jacks and adults) in Chinook salmon may result from negative frequency-dependent selection (NFDS), in which the rarer form has a fitness advantage over the more abundant form. Evidence for NDFS has 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 relative abundance of jack and adult Chinook salmon males can vary widely among spawning populations and among years within populations. The behavioral assessment breeding success of jack and adult Chinook salmon males in channels A and B of this study provide evidence of NFDS. The presence of NFDS 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. Following analysis of the pedigree data and a second year of experimentation, we will provide recommendations for broodstock management.

Data Management Activities

Data were collected manually by NOAA and PSMFC researchers onto preformatted data sheets or directly into computers. Data were entered and summarized on personal computers operated by researchers using Microsoft Excel. Statistical analyses were conducted using Systat. Backup copies of data were saved on external hard drives. All data are checked for quality and accuracy before analysis. Analytical processes are described in the text of the 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. The number of eyed Chinook salmon eggs stocked into eight stream channel sections and their survival to the smolt stage (collected on 27 May 2008). Non-viable eggs were recovered from the Jordan-Scotty incubators and the channel mortalities were those fry collected from screens in the channels. The missing fish were presumed mortalities because the stream channels were eventually de-watered to remove all fry.

Section	Stocked eggs	Non-viable eggs	Channel mortality	Live count	Grand total	Missing	Survival	Mean FL (mm)	Mean Wt (g)
A1-2	288	28	3	238	269	19	83%	68	3.4
A3-4	288	24	6	219	249	39	76%	69	3.7
A5-6	288	19	7	214	240	48	74%	70	3.9
A7-8	288	9	11	235	255	33	82%	70	3.6
B1-2	288	30	6	235	271	17	82%	80	5.7
B3-4	288	26	9	226	261	27	78%	76	4.8
B5-6	288	29	6	218	253	35	76%	77	5.3
B7-8	288	32	15	222	269	19	77%	79	4.9

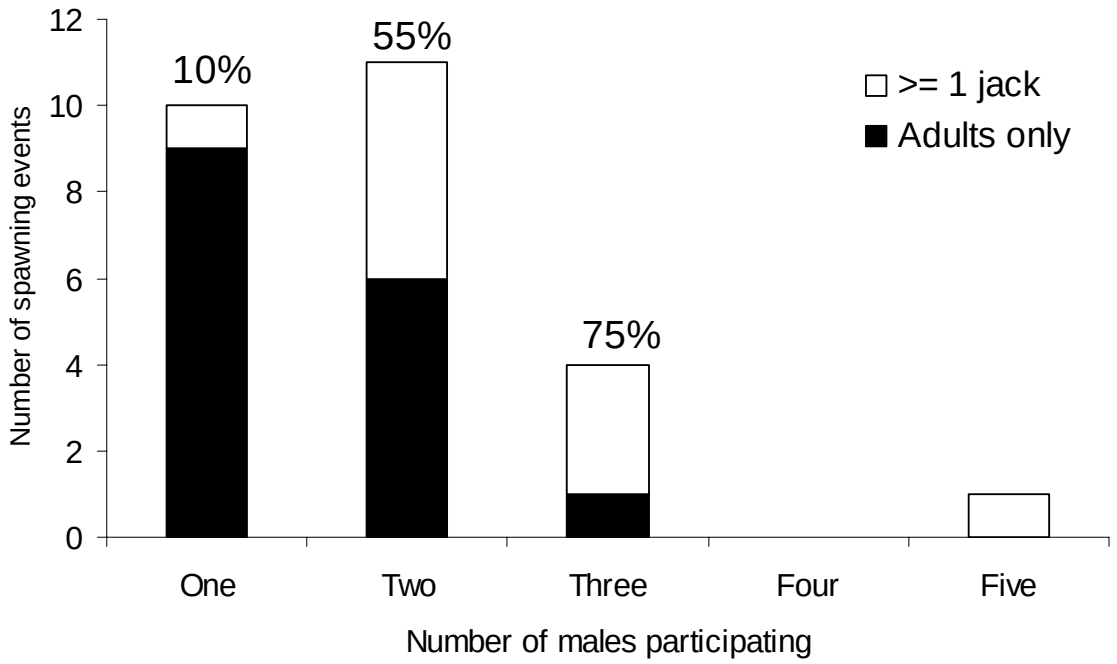

Figure 1. The number of spawning events including one to five male participations. Solid bars show the number of spawning events in which only adults participated and open bars show the number that included at least one jack male.

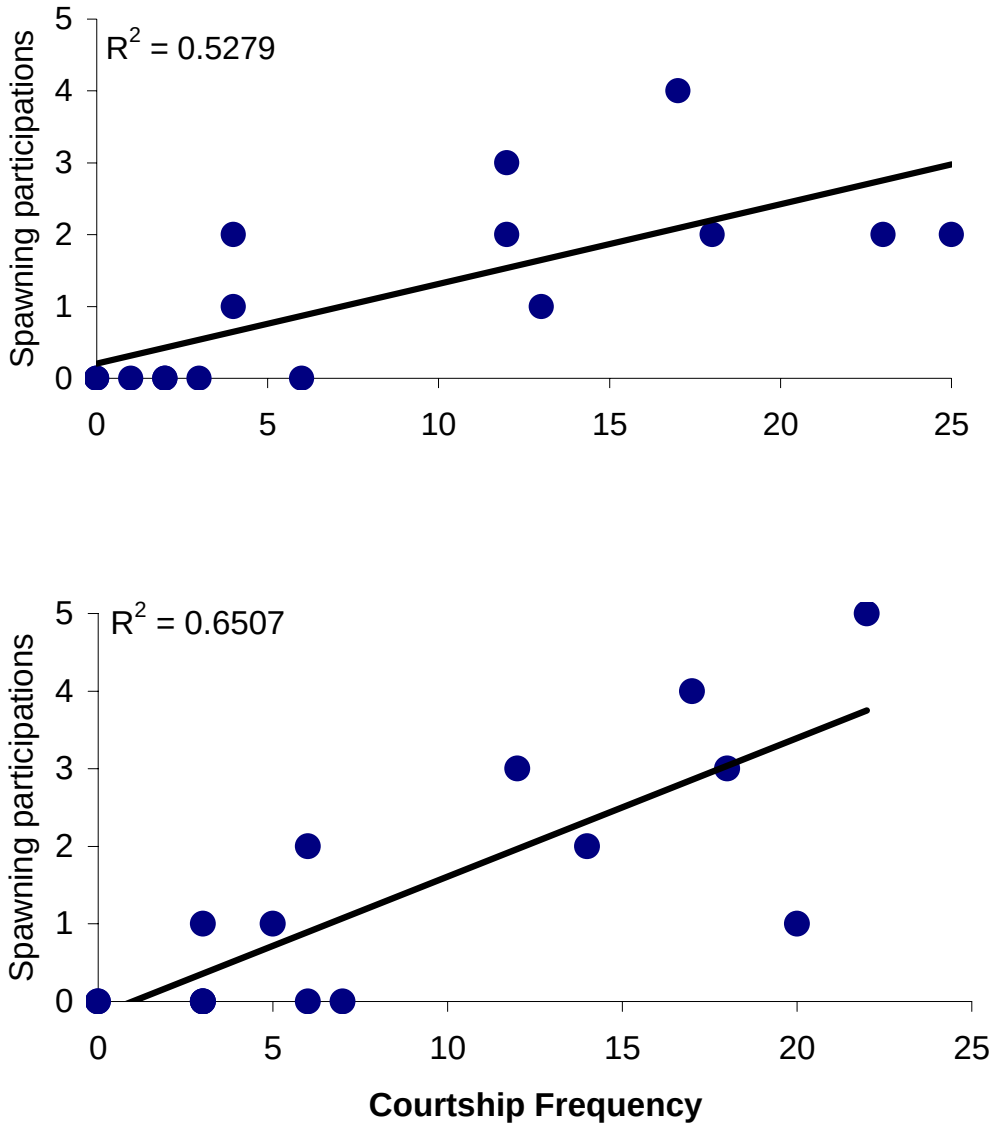

Figure 2. The relationship between courting frequency and spawning frequency for individual male Chinook salmon of different sizes. Jacks were those smaller than 545 mm and adults were those greater than 600 mm. Data are from Channel A (top panel) and Channel B (bottom panel).

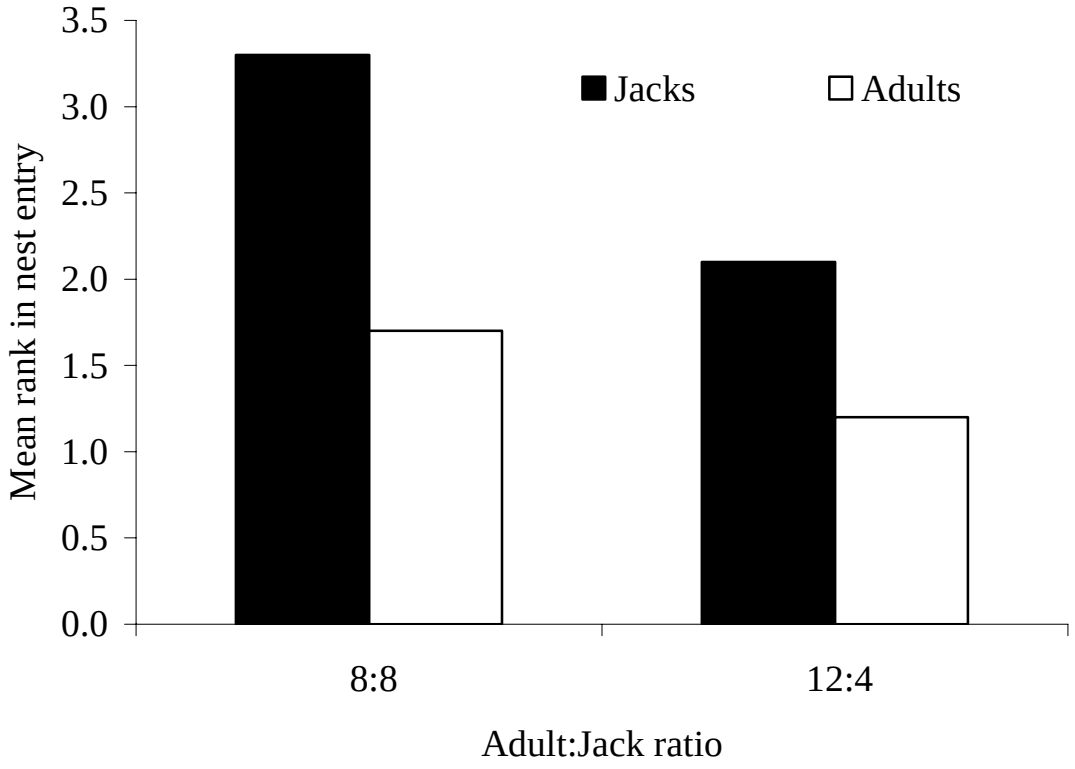

Figure 3. The mean male rank order of nest entry during spawning in Channel A (8 adults and 8 jacks) and Channel B (12 adults and 4 jacks). The first male to enter the nest during spawning was given a rank of 1, the second a rank of 2, etc.

OBJECTIVE 2: IMPROVE OLFACTORY IMPRINTING - DETERMINE CRITICAL IMPRINTING PERIODS FOR SOCKEYE SALMON

By

Andrew H. Dittman, Michelle Havey, and Darran May

Introduction

Over the last several decades, many of the distinct salmon populations in the Columbia River have experienced a steady decline due to habitat loss, dams, and over fishing (NRC 1996, NMFS 2000a, 2000b). In response to these declines, a number of captive propagation and conservation hatchery programs have been initiated to preserve the genetic resources associated with these population, and to re-introduce and restore these populations as environmental conditions associated with the original declines are mitigated (NWPPC 1999). Several Columbia River salmon populations and ESUs have reached critically low levels, and NOAA Fisheries (NMFS 2000a), the Northwest Power Planning Council's Columbia River Basin Fish and Wildlife Program (NWPPC 2000), and several state and tribal agencies, have endorsed and implemented captive broodstock programs as a safety net for endangered populations.

The initial focus of most of these programs has been to capture wild fish from imperiled populations to rear them in captivity, thereby increasing the juvenile-to-adult survival. Ultimately, as their numbers increase, these fish or their progeny are reintroduced into their natal environment. While captive rearing may be necessary to preserve these populations, there are several potential problems that must be addressed in developing successful captive broodstock programs. For example, artificial propagation removes salmon from their natural environment and hatchery rearing and release practices can have profound effects on the development, physiology, behavior and ecological interactions of fish when they are released back into their native environment. One area of particular concern is the effect of artificial propagation and inappropriate reintroduction strategies on the subsequent homing ability of released fish (Grant 1997, NWPPC 1999). Concerns about excessive straying of hatchery-reared fish have led to a call for the re-evaluation of hatchery programs to "avoid unnatural patterns of straying by adult returns" (NRC 1996) and for further research on the causes and consequences of homing and straying of hatchery-reared fish (Grant 1997, Flagg and Nash 1999).

The tendency to home to the natal stream to spawn is fundamental to the unique biology and management of Pacific salmon. Homing results in genetic isolation of populations of salmon uniquely adapted for conditions in their natal streams (Ricker 1972, Taylor 1991). The final freshwater stages of these homing migrations are governed by the olfactory discrimination of home-stream water. Prior to their seaward migration, juvenile salmon learn (imprint on) site-specific odors associated with their home stream, and later use these retained odor memories to guide the final phases of their homing migration (Hasler

and Scholz 1983). This imprinting process is critical for the 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 (Quinn 1993). While low levels of straying from the natal site are normal in the wild, inappropriate hatchery rearing conditions and juvenile release procedures can dramatically increase the level of straying by adult fish (Grant 1997, Pascual and Quinn 1994, Pascual et al.1995). Reintroduction of captively-reared fish into the wild at inappropriate developmental periods, or after insufficient periods of exposure to appropriate olfactory cues, may result in elevated levels of straying (Quinn 1993, Nevitt and Dittman 1998). Straying by captively-reared salmon can jeopardize efforts to enhance endangered populations by either lowering the effective number of spawning adults in a captively-reared target population (USFWS 1996), or via competition and interbreeding of hatchery salmon with endangered wild populations (Crateau 1997, Carmichel 1997).

Hatchery rearing does not necessarily result in increased levels of straying (Labelle 1992, Quinn et al. 1989, Hard and Heard 1999). However, rearing wild fish for even short periods in a hatchery can increase straying and certain hatchery practices do clearly increase stray rates (Quinn 1993). For example, while salmon typically return to the site from which they were released (Donaldson and Allen 1958, Ricker 1972), fish reared and released from a single site generally stray less than fish transported and released off-site (Reisenbichler 1988, Boydstun et al. 1992 reviewed in Quinn 1993). In general, the closer the rearing and release sites are to each other, the more likely adults will return to the rearing site (Lister et al. 1981, Johnson et al. 1990, Slaney et al. 1993). Another important factor that may influence homing fidelity in hatchery fish is the timing of releases and reintroductions into the wild. A number of studies have identified the parr-smolt transformation as a critical period for olfactory imprinting for some species (Hasler and Scholz 1983, Dittman et al. 1996) and chinook salmon released during smolting strayed less than fish released before or after the smolting period (Pascual et al. 1995, Unwin and Quinn 1993). Finally, the length of time fish are exposed (acclimated) to water at the release site prior to release may influence subsequent homing to that site (Johnson et al. 1990, Savitz et al. 1993, Kenaston et al. 2001).

Captive rearing programs for endangered wild salmon require special considerations and have unique constraints not normally required in production hatcheries. The primary goals of most programs are to, (i) increase the number of individuals within a population; and (ii) maintain the genetic and phenotypic integrity and complexity of the wild population until reintroduction (Flagg et al. 2000). The strategies typically employed to achieve one goal often have negative consequences for the other. The longer fish are maintained in captivity the greater the immediate increases in survival, but also the greater the risk of genetic and phenotypic changes in the population (Waples and Do 1994). This is also true for reintroduction strategies. In general, the earlier in the life cycle a fish is released into its ancestral environment (or at least experiences its natal water for imprinting), the better the opportunity for proper imprinting and successful homing. However, re-introduction at these early times carries with it the risk of lower survival. These two competing concerns force managers of captive rearing programs to weigh the likely tradeoffs and benefits of different release strategies to maximize survival

but minimize straying. These problems are further compounded by the need for large and expensive culture facilities to maintain captive broodstocks through all phases of their life cycle. In most cases, these appropriate rearing facilities are not located in the ancestral watershed, and *in situ* rearing of endangered populations is not practical. Fish are typically reared offsite in non-homestream water and then transferred back for reintroduction (Kline and Heindel 1999).

One example that illustrates some of these challenges for a captive broodstock/ conservation hatchery program is the Redfish Lake sockeye salmon captive rearing program (BPA Projects #199107200 and #199204000). 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 as of fall 2001, 290 adults had returned to the Stanley Basin. The majority of adult fish recovered to date have come 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 have 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. While the ultimate fate of these unsuccessful fish is not known, 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. 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.

Experimental Group 1: Odor exposures 2004-2006

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 are being 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) Control 1 – 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) Control 2 - pre-smolt only exposure, L -Arginine (February to March 2006) – odor exposure during presumed "non-sensitive period" for imprinting; (vi) Control 3 - 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, they 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; Speca 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). For each fish, length and weight were measured, plasma was collected to determine maturational hormone levels, gonads were weighed and fixed for later histological assessment of maturation, and olfactory rosettes were collected for molecular analysis of imprinting. Histological and hormonal assessments of maturation will be completed in 2008-2009. Molecular assessments of imprinting were all completed in the 2007-2008 work period but final data analysis will be completed in 2008-2009. Behavioral testing was completed for all 8 treatment groups in Fall 2007.

Experimental Group 2: odor exposures 2006-2009

In Fall 2006, Stanley Basin sockeye were obtained from the NMFS Redfish Lake Sockeye Salmon Captive Broodstock program at Burley Creek, Washington to establish experimental lines of fish to test the effects of rearing environment on reproductive performance of captively reared adults. Two environmental variables were to be tested: 1) does rearing in artificial light vs., natural light influence reproductive performance of captively-reared sockeye salmon 2) is spawning behavior and reproductive performance influenced by the presence of imprinted homestream vs. non-natal unfamiliar water. The basic design for the home water/unfamiliar water experiment involved rearing fish in one of two different water sources (BBC well water and Manchester Research Station (MRS) spawning channel water) through smolting, transferring the MRS fish to BBC for communal rearing to maturation, and then transfer and testing of both groups for reproductive behaviors in the MRS spawning channel. In December 2006, eyed Stanley Basin sockeye eggs were split into two equal groups of 500 fish and transferred to either the MRS or BBC hatchery facilities. Fish were ponded and were reared in 4-foot circular tanks on a ration that produced 25-30 g smolts by June 1, 2008. Originally we planned to rear these fish to maturity to complete the proposed studies.

BPA-dictated budget cuts will result in termination of this experiment by July 2008. While we cannot complete the study as originally designed, we will assess imprinting in the two groups after smolting so that efforts to date on this experiment are not wasted. To assess imprinting success to two different water sources and the environmental effects (i.e. different water chemistry) on the olfactory system, we will examine differences in odorant receptor gene expression in the two experimental groups. At the end of smolting (July 2008), olfactory rosettes from a subset of fish from both groups will be collected and expression levels of representative odor receptors will be measured to assess differences in the olfactory system of salmon imprinted to different water sources.

Experimental assessments of imprinting

H_{01}: Timing and duration of odor exposures (that parallel Stanley Basin juvenile release strategies) has no effect on imprinting success as measured by behavioral and molecular assay.

Determination of successful imprinting involves correlated assessments of odor attraction and heightened olfactory sensitivity in odorant-exposed fish relative to odorant-naïve controls. Hypothesis H_{01} was tested by molecular and behavioral assessments of imprinting during 2005-2007 in which behavioral attraction to arginine and arginine odor receptor expression are compared between arginine-naïve fish and arginine-exposed treatment groups. Relative importance of exposure duration was tested by comparisons between exposure groups. Our previous studies indicated that sockeye salmon are able to imprint as alevin and smolts but no developmental periods in between were tested (Dittman et al 2004).

Behavioral testing

Behavioral responses of maturing sockeye salmon to imprinting odors were tested in three two-choice mazes at the BBC facility. Each maze consists of two arms (3.05 x 0.5 x 0.5 m tanks) flowing in to the main are of the tank (3.05 x 1.22 x 1.22 m). A plastic mesh divider separated the main tank from the arms to limit access to each arm before the start of a trial (Figure 1).

To provide novel background water (i.e., different from rearing water) for testing, water was pumped from a side channel of BBC into each arm and the flow maintained at approximately 15 L/min/arm. Flows into each arm were checked each day, as was the flow rate of the peristaltic pump delivering odor to each arm. Odors (10^{-3} M solutions of L-Arginine and L-Leucine) were metered into the arms of the maze using a peristaltic pump to deliver a final concentration of 100 nM/arm. Fish were acclimated for 1-2 days in holding tanks supplied with channel water to account for differing temperatures between rearing (well) water and testing (channel) water.

To start a trial, a mature fish was moved from the holding tank into the downstream section of the main area of the tank and allowed to acclimate for 30 min. The experimental group to which the fish belonged was unknown to the observer during the trial. A removable mesh screen was placed across the main raceway at the upstream end to prevent the fish from swimming in the arms before the start of a trial. During the last 5 min of the acclimation period and for the remainder of the trial, one odorant (L-Arginine or L-Leucine) was pumped into each arm of the y-maze to ensure that the odors reached the main tank by the start of the trial. After the acclimation period, the screen was lifted and the fish swam freely for 15 min. Each trial was recorded using a digital video recorder and viewed later. At the end of each trial, the peristaltic pump delivering the odor mixture was turned off, the fish removed, and its fin clip recorded to determine the

experimental group. Each maze was flushed with channel water for 5-10 min between trials to remove residual odors. The mazes were drained and scrubbed each day, and the odor delivery tubes switched between arms daily to eliminate any inherent arm preference. There were six possible combinations of y-maze (3) and odor arm (2) testing scenarios (e.g., Maze 1 with L-Arginine in arm A and L-Leucine in arm B, and vice versa). Approximately equal numbers of fish from each treatment group were tested in all y-maze combinations to remove any inherent y-maze or arm bias. The sexual maturity of each fish was assessed at the end of the trial and only data from maturing fish were retained for analysis.

Data analysis

Videos were reviewed without prior knowledge of treatment group to prevent any biased observations. For all analyses, the odor arm refers to the arm scented with the odor to which the fish was imprinted as a juvenile. Responses were only compared within population or within treatment group.

The data were examined in several different ways to ascertain whether the fish's choice of arm and water source differed from random movement in the tanks. We first examined the first arm the fish entered during the trial, the last arm the fish entered, and the arm in which it spent the majority ($\geq$50%) of its time. These data were compiled by treatment and population and analyzed by a Z-test. In addition, the total amount of time spent in the imprinted odor arm, and the average time per entry were log-transformed. Both populations (except the L-Leucine smolt exposed group) were averaged across treatment groups and compared to the appropriate control using two-sample, one-tailed t-tests with unequal variances. Differences in proportion of time spent in the odor arm were compared between experimental groups and the control group using a two-sample one-tailed t-test after the proportions were normalized using an arcsine square root transformation. The frequency of entries into each arm was compared within each treatment group using a paired t-test. In all cases, responses were tested at the P=0.05 significance level. Fish that did not enter either arm during a trial are referred to as "no choice" fish and were removed from further analysis.

Molecular assays

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 (for review see Hara 1992). Each of the millions of ORNs in the rosette expresses one of a family of approximately 100 distinct odorant receptors (Ngai et al. 1993). For one of these receptors, we have identified the odorant ligand that binds and activates the receptor (Speca et al. 1999). 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 (Nevitt et al. 1994, Nevitt and Dittman 2004). 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 previously demonstrated that juvenile coho salmon exposed to arginine during smolting have increased expression of AOR mRNA relative to odorant-naïve controls consistent with imprinting. Molecular assessments of imprinting utilized the sockeye salmon-specific quantitative PCR assay for BAAR RNA (Dittman et al 2005). RNA was isolated from individual rosettes of salmon collected from each treatment group during the parr smolt transformation and during maturation (Trireagent, MRC) and qPCR assays were conducted using an ABI Prism 7700 real time thermocycler. Final analysis of Okanogan River sockeye mRNA expression is not completed.

Results and Discussion

Behavioral testing

Of the 342 maturing fish from both populations that were tested, 126 made a "choice" by swimming into one or both arms during the trial (Figure 3). Far fewer RFL fish made choices in the maze (16%) compared to Okanogan fish (51%) suggesting that there are behavioral differences between the populations. The strongest response variable for odorant attraction was the average time fish spent in the odor arm per entry (Figure 4). On average, fish from all groups that experienced arginine as juveniles spent more time per entry in the odor arm than control fish (Figure 4). 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 not 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) ($P=0.33$). For all treatment groups, a second measure of odor attraction (total time spent in the arginine-scented arm) also suggested that arginine exposure as a juvenile resulted in increased attraction to this odor as adults (Figure 5).

Previous studies have utilized frequency of entries into control vs. odor arms (e.g., Yambe et al. 1999; Yambe and Yamazaki 2000) and the first and last arm choices of each fish (Yambe and Yamazaki 2001) as another measure to assess choice in y-maze studies We observed no significant differences between the treatment and control groups for any of these measures.

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 6) 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 Okanogan River sockeye and analysis of physiological correlates of maturation (reproductive hormones, gonadal histology, morphometrics) should shed further light on these processes.

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.

Summary and Conclusions

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. 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.

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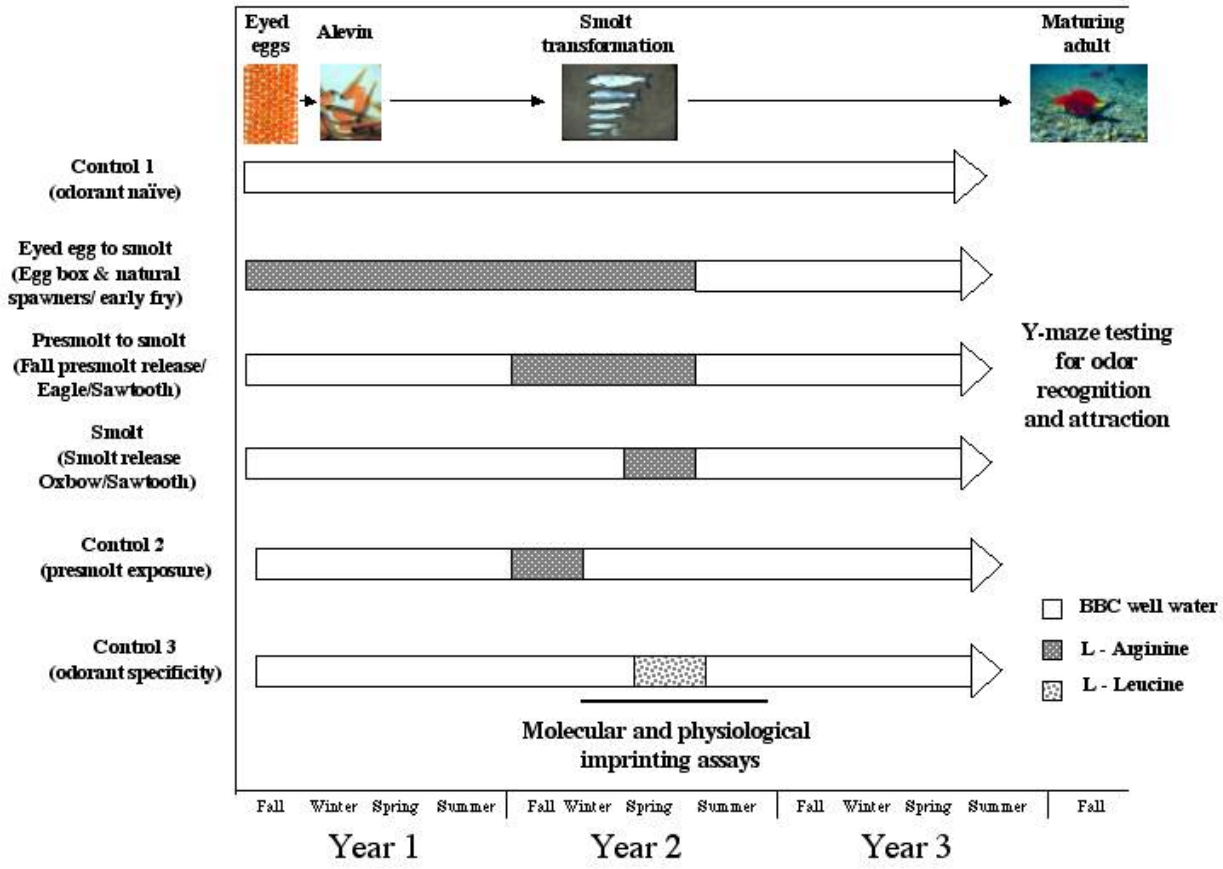

Figure 1. Experimental design for 2004-2006 odorant exposures and testing for imprinting success. Salmon were exposed to odorants at developmental stages and for durations that parallel Stanley Basin sockeye reintroduction strategies.

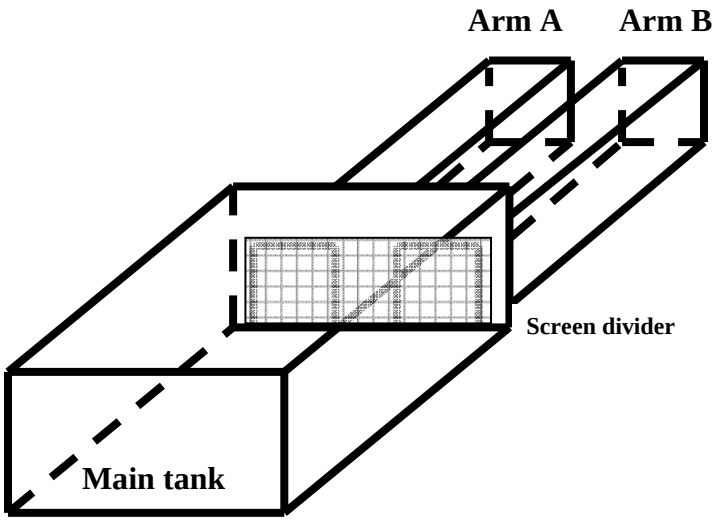

Figure 2. Schematic of a Y-maze for testing adult sockeye salmon. The two arms flow directly into the main tank and the mesh divider prevents upstream access to the arms until the trial begins. A camera was placed directly above the junction of the arms and main channel to monitor fish movement in and out of each arm.

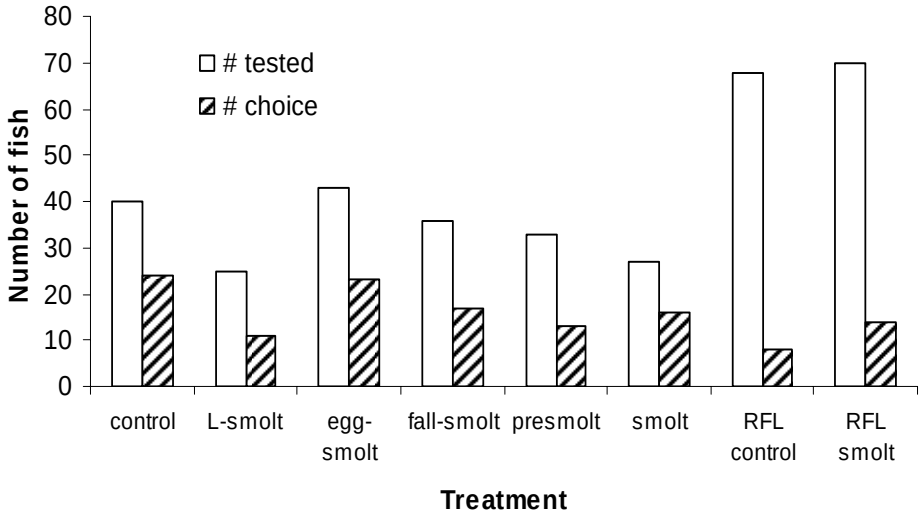

Figure 3. The number of fish from each treatment group tested in the Y-maze and the number of those making a choice by swimming into one or both arms during the trial.

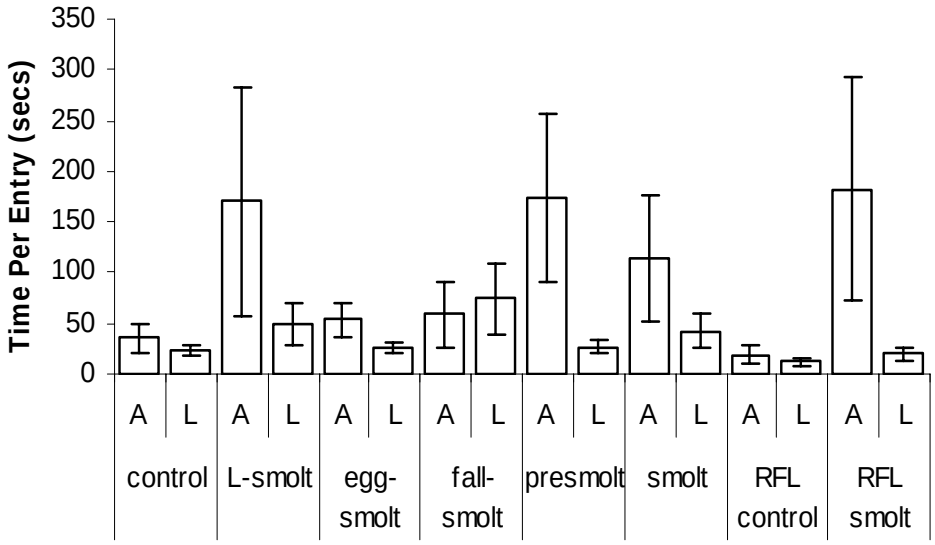

Figure 4. Average time per entry, in seconds, for each fish. Values for both the L-Arginine (A) and L-Leucine (L) arms are given for each group as the mean time per entry ± S.E.M.

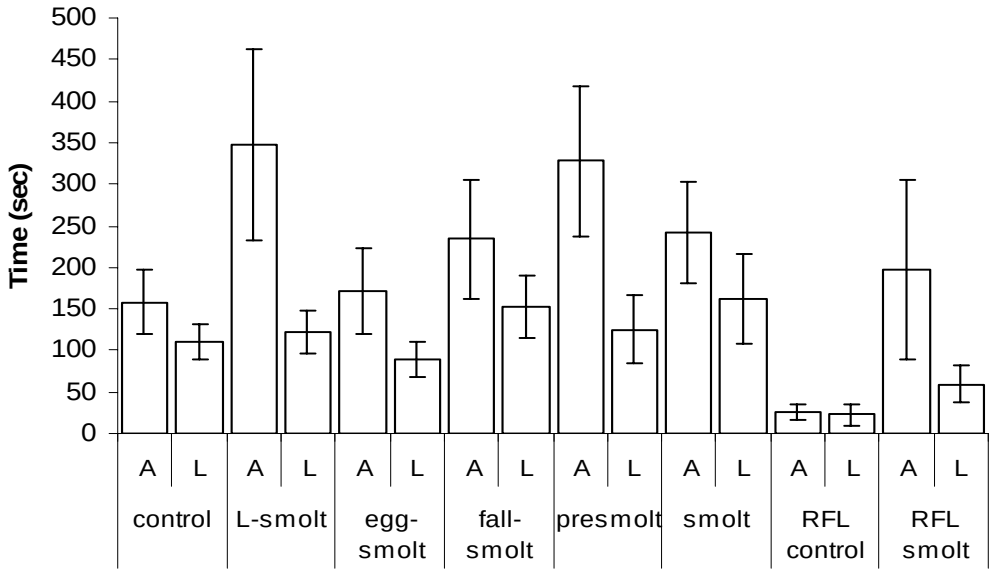

Figure 5. Average time spent in each odor arm/trial. Odorants tested were L-Arginine (A) vs. L-Leucine (L). Bars represent the mean in seconds ± the S.E.M.

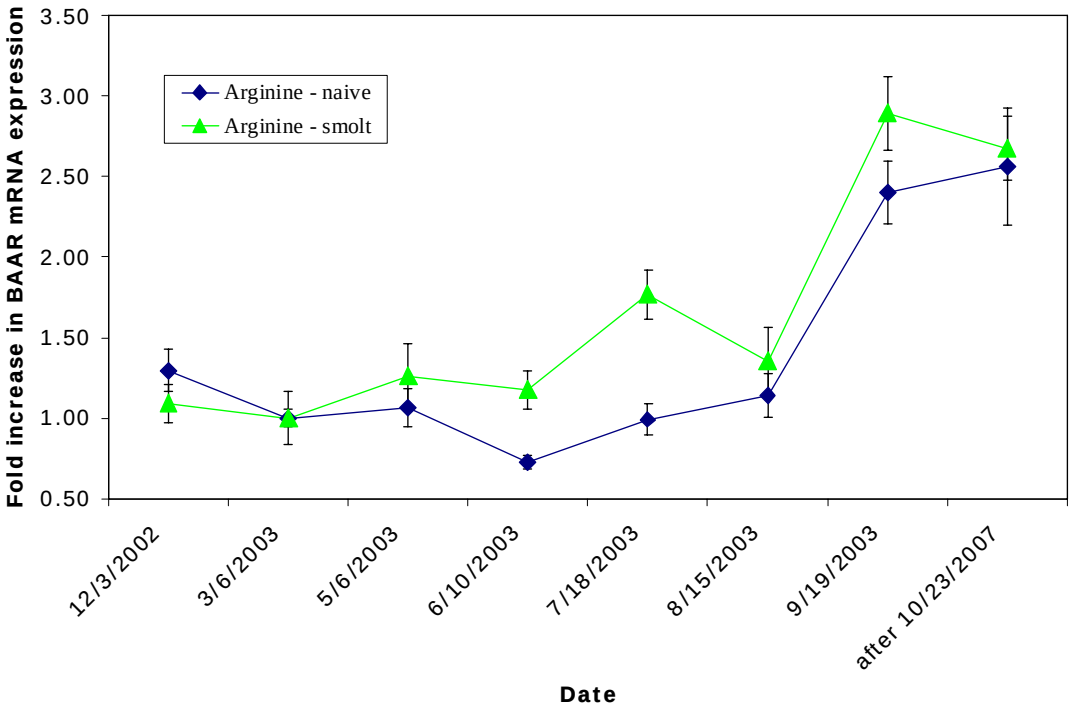

Figure 6. Changes in expression levels of BAAR mRNA during maturation in Stanley Basin sockeye salmon.

OBJECTIVE 3 - USE ENVIRONMENTAL FACTORS TO MATCH WILD PHENOTYPES IN CHINOOK AND SOCKEYE SALMON REARED IN HATCHERY SUPPLEMENTATION PROGRAMS - EFFECTS OF PHOTOPERIOD AND GROWTH AFTER EMERGENCE ON AGE OF MALE MTURATION IN SPRING CHINOOK SALMON: PROGRESS REPORT

by

Brian Beckman, Paul Parkins, Dina Spangenberg, Deborah Harsted, Kathleen Cooper, Donald Larsen, and Penny Swanson

Introduction

A major concern with captive rearing and supplementation programs for depleted or ESA-listed Pacific salmon is that artificial selection may lead to altered genotypes and phenotypes, thus diminishing fitness when these fish breed in natural environments. Therefore, one of the goals of supplementation programs (conservation hatcheries or captive broodstock programs) is to release fish with similar characteristics to that of wild fish, i.e. those that are ecologically equivalent to wild fish with similar patterns of mortality and survival. The intent is to maintain genetic and phenotypic similarities between natural and artificially propagated fish populations.

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. In both the hatchery and natural environment, early life histories of these species are variable; smolting occurs at various sizes, seasons and ages, and the proportions of males maturing at age 1 and 2 are variable. Both smolting and age of male maturity depend on interactions between environmental factors and genetic composition of the stock (c.f. Hankin et al. 1993, Heath et al. 1994), particularly those that influence body growth. 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.

While selection might be low in the hatchery (high egg to smolt survival), selection post-release can be severe (low smolt-to-adult survival) and focused on attributes of the released fish that are the result of hatchery rearing environment (size, smolting characters, early male maturation). Thus, the genetic composition of artificially propagated salmon populations may shift away from that of natural populations through survival and mortality patterns that take place outside of the hatchery. Accordingly, the focus of research in Objective 3 is to assess impacts of hatchery rearing conditions on life history characters at release, particularly seasonal timing of smoltification of juveniles and age of maturity in males. One aim 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 at release. The effects of two environmental factors,

photoperiod (at emergence) and food availability (growth during early rearing), on smolting and age of male maturity are being examined in spring Chinook salmon.

Effects of growth on age of maturity

Numerous studies in both Atlantic and Pacific salmon have shown that both body growth and fat levels during critical periods of the life cycle affect 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; Swanson et al. 2004; Shearer et al. 2006). Indeed, exceptionally high rates of early (precocious) male maturation have been observed in fish reared in hatcheries or captive broodstock programs (e.g. Larsen et al. 2004), in part due to the high early growth afforded by the captive environment. To address this problem and develop rearing regimes to reduce early male maturation, a series of studies were conducted during previous funding periods to; a) evaluate the relative effects of growth rate and body fat levels on rates of age-2 male maturation, b) determine critical periods when high growth influenced maturation in males and c) determine effects of growth on ovarian growth. Results indicated that in spring Chinook salmon, growth rate/body size was the main factor affecting age-2 maturation, and fat levels influenced maturation only in small fish (Shearer et al. 2006). Furthermore, the critical period wherein growth affected onset of maturation in spring Chinook salmon was the fall through late winter (Campbell et al. 2003; Swanson et al. 2004, 2005). This suggests that a growth regime that maintains low growth during this critical period (September-February) of the first year of rearing would be effective in reducing rates of age-2 maturation.

Consequences of variation in emergence timing in the hatchery environment

Fry emergence from gravel incubation beds is a critical developmental event when fish initiate feeding and are exposed to natural photoperiod and predators. Emergence timing varies between individuals, families, populations and species. Current theory holds that the trait of emergence timing is under strong stabilizing selection with winter floods selecting for emergence late in the season and spring productivity selecting for emergence early in the season (Vøllestad et al. 2004). In addition, it has been shown that fry that emerge relatively early in development are potentially subjected to elevated predation rates as the incompletely absorbed yolk sac is a major hydrodynamic obstacle to evading predators. Thus, the seasonal timing of emergence and the physiological completion of the alevin-to-fry transition are both important traits that influence survival and subsequent growth.

Emergence timing is also related to later life history events. For example, in Atlantic salmon, individual fish with elevated metabolic rates tend to emerge earlier, grow faster, and display heightened tendencies to either smolt at age 1 (instead of 2) or mature precociously if male (Metcalfe and Thorpe 1992). Within most artificial rearing programs salmon fry are not allowed to volitionally emerge; rather, fry are directly transferred from egg and alevin incubation trays into early rearing troughs or even directly into hatchery raceways (commonly referred to as ponding). In the hatchery, incubation temperatures have been altered to synchronize or advance ponding. In many cases, natural variation in emergence timing is limited as eggs originating from earlier or later parts of the spawning season are incubated at differing temperatures to synchronize pond timing and thus

increase the efficiency of hatchery operations. Routinely, elevated incubation temperature is used to advance emergence timing of Chinook salmon by four to five months. This may occur unintentionally because eggs are generally incubated in pathogen free groundwater, which is routinely warmer than surface of hyporheic water. Intentional acceleration of early development of Chinook salmon may also allow hatchery managers to free egg-incubation facilities for steelhead eggs (hatchery steelhead populations may start spawning in December) and to increase feeding and growth opportunities.

The potential effects of hatchery-induced modification of emergence timing are almost entirely unexplored in Pacific salmon. However, recent data from a study on Sacramento River Winter Run Chinook salmon (Beckman et al. 2007) suggests that the timing of emergence (ponding) had profound effects on subsequent life history pathways.

In that study, altering the photoperiod at ponding changed smolt timing from spring (yearling) to the autumn and altered the rate of early male maturation. In this study, replicate groups of first emergent fry from the same parents (genetically identical) and of the same age were placed into different photoperiod regimes using controlled lighting systems. A second variable was growth pattern post-ponding, which was manipulated using ration (either high or low). Feeding regimes were controlled so that fish within one ration treatment, irrespective of photoperiod were of the same size (no differences in growth rate due to photoperiod). Results of this study demonstrated that altering the timing of emergence so that fish emerge under different photoperiods profoundly alters life history phenotype by increasing the rate of males maturing at age 1.

Although winter-run Chinook salmon from the Sacramento River are unique in terms of seasonal timing of life history events (Healey 1994), this may be a general phenomenon among Pacific salmon in the Columbia Basin. Anecdotal evidence from the Yakima River Spring Chinook Salmon Supplementation Program suggests that emergence timing also affects life history pathways in this species. Early male maturation at age 1 (one year after the parents spawned) is common in both wild and hatchery spring Chinook populations (Mullan et al. 1992), including wild-reared Yakima spring Chinook salmon (Pearsons et al. 2004). However, over a number of years of study, only one age-1 mature male has been found in groups of fish reared at the Cle Elum Hatchery (> 5,000 fish assessed, Larsen and Beckman unpublished).

The Yakima Program intentionally chills water during incubation of eggs and alevins to delay ponding of fry until April, thus avoiding problems associated with high silt loads in March runoff that clogs hatchery raceways and makes feeding and cleaning problematic. Fish are subsequently reared on high growth to attain sufficient size at tagging in October. Based on the results of the Sacramento River winter run Chinook salmon study, the lack of age-1 male maturation in the spring Chinook salmon at the Cle Elum hatchery may be due to late ponding (effectively later emergence timing than wild fish). At the same time, maturation at age 2 increased in this hatchery population. Current estimates suggest that production releases contain 20 – 50 % early maturing males (age 2, Larsen et al. 2004), which have very poor reproductive success on the spawning grounds (Pearsons et al. 2004). The net result could be elimination of a specific component of the juvenile population (i.e. fast-growing males) since age-2 maturation is linked to high early growth.

This phenomenon is not unique to the Yakima Program. Male spring Chinook salmon maturing at age 2 from several upper Columbia or Snake River hatchery programs were relatively abundant at Columbia River dams (Beckman and Larsen 2005). Genetic studies have not been done to evaluate the potential effect of this selection pressure on salmon populations. However, selective elimination of fast-growing males and age-1 mature males from the hatchery supplementation populations is highly unlikely to match the selective pressures found in a naturally rearing population.

Emergence time is also altered in captive broodstock programs, but in some cases growth after ponding is controlled to match size of wild fry. For example, captive Redfish Lake sockeye salmon are ponded in January. Subsequently, fry are placed in chilled water and reduced ration since high growth rates are associated early smolting (under-yearling) and high rates of early male maturation (William Fairgrieve, personal communication). There has been no direct observation of fry emergence timing in Redfish Lake, but coupling thermograph data from the lake to development -temperature relationships established for sockeye salmon embryos suggests that fry in Redfish Lake emerge later than January. The Redfish Lake Captive Broodstock Program has been relatively proactive in addressing life history shifts induced by hatchery rearing by altering rearing temperatures and feeding rates to keep fry below a 1g threshold before 1 May. However, the Redfish Lake Program has the advantage of rearing fish through out their life cycle. Thus it relatively easy to monitor any shifts in proportions of life history phenotypes (early male maturation). Most supplementation programs release fish (presumed smolts) before early male maturation can be easily assessed. Moreover, most hatchery supplementation programs do not have the facilities or the budget to allow for chilling water temperatures to drastically reduce growth of fry and parr to compensate for earlier emergence. Thus, there is little opportunity to modify potentially altered life histories induced by early emergence timing in many programs.

It would be more cost-effective to control emergence timing to match wild life history phenotypes, if this phenomenon is universal to Pacific salmon. Therefore, research under Objective 3 is examining the effects of photoperiod at emergence and growth after ponding on timing of smolting and age of male maturation in Yakima River spring Chinook salmon.

Materials and Methods, and Description of Project Area

Fish rearing and sampling

This experiment tests the following hypotheses:

H_{01}: Photoperiod at emergence does not alter seasonal timing of smoltification or age of male maturation in Yakima River spring Chinook salmon.

H_{02}: Growth (ration) after ponding does not alter seasonal timing of smoltification or age of male maturation in Yakima River spring Chinook .

Emergence timing of hatchery salmonids is often earlier than found in wild fish because of elevated water temperatures used for early incubation in hatchery programs. These practices may inadvertently affect later timing of smolting and manifestation of the

precocious male phenotype in hatchery-produced fish relative to wild fish. Delayed or advanced emergence exposes fry to a seasonally advanced or delayed photoperiod, respectively, and alters the time allowed for growth prior to release. In Sacramento River winter-run Chinook to salmon, both of these factors influence life history phenotype in terms of smolt timing and occurrence of early male maturation (Beckman et al. 2007). Under Objective 3, an experiment is being conducted to examine the independent and interactive effects of photoperiod at emergence (3 different regimes) and growth (3 rations) post-ponding on age of maturity in males. Buttoned up fry 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 (~ 10ºC) and late emergence represents conditions found in very cold, high elevation Snake River tributaries. This range of photoperiods should provoke the entire range of phenotypic responses found for Chinook salmon under current environmental conditions.

Ponding occurred on day # 45 (mid-February). Because of this design fish in all treatments are the same age throughout the experiment even though they are exposed to different photoperiods. Within each photoperiod treatment fish are being fed under three feeding regimes: Satiation, High Feed and Low Feed. Target sizes after first year will be 10 and 25g on day # 270 (1 October) for Low Feed and High 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 Low Feed and High Feed treatments, respectively. Feeding rate is being adjusted so that fish from a given feeding treatment (High Feed or Low Feed) will be the same size regardless of photoperiod treatment.

Sampling and Laboratory Methods

After hatching (~400 degree days post fertilization), alevins (N=12 fish) were sampled at 2-week intervals through 1000 degree days posthatch to define the schedule of sex differentiation and gonadal development. Once yolk sacs were completely adsorbed (button-up; ~360 degree days, posthatch), fry were placed into 1.3 m circular tanks. Based on other salmonids, sex differentiation should occur at ~710-1050 degree days post fertilization or ~300-400 degree days posthatch (Foyle 1993).

At approximately 4-week intervals after ponding, size is being assessed (3 batch weights of 50 fish) and sub-samples of fish (8 fish/tank, 16 fish/treatment) are being sacrificed to obtain gill tissue, blood plasma and gonad tissue. Gill Na-K ATPase activity will be measured as an index of smolting (McCormick et al. 1993). Plasma insulin-like growth factor I (IGF-I) levels will be measured as an index of growth rate (Shimizu et al. 2000) and plasma 11-ketotestosterone (11-KT) will be measured as an early index of male maturation (Cuisset et al. 1994, Larsen et al. 2004). Gonads are being weighed from all sacrificed males to assess degree of male maturation. Seawater challenges are being conducted monthly (72 hours in 37.5 ppt seawater) as another assessment of smolting. On approximately day 225 of the experiment (1 August) 100 fish from each tank were sacrificed to assess age-1 male maturation (testis weight and plasma 11-KT levels).

Beginning at approximately day # 285 (1 October), all the remaining individual fish were examined for exterior appearance indicative of male maturation at age 1 (bronzy-yellowish color, enlarged yellowish anal fin, reappearance of parr marks) and for the ability to extrude milt (gentle pressure on abdomen). No fish were sacrificed during this examination and fish exhibiting signs of early make maturation will be adipose fin clipped in order to examine future survival, smolting, or re-maturation. All remaining fish will be sacrificed and testis will be examined to determine male maturation at age 2 at approximately day # 590 (30 August of year 2). Maturing males will be readily identified by gonado-somatic indices (GSIs), however the stage of testis development of immature males will aid in determining whether some males are maturing in the subsequent year (age 3). Therefore, at the end of the experiment, testes of immature males will be examined histologically.

Results and Discussion

The experiment described in this objective is still ongoing therefore, only data on growth and age one maturation are shown. Final samples will be collected in August 2008 and final results of all sample and data analyses will be reported in subsequent reports. Data on growth, smoltification and rates of maturation in yearling males are shown in Figures 1-3. The growth in length of fish during the first year of the experiment is shown in Figure 1. By the end of December 2007, fish that were on the early emergence treatment and fed to satiation were the largest while fish reared under late emergence and low ration were the smallest of all treatments. Both the ration and photoperiod treatment appeared to affect growth in the experimental animals.

In the fall of 2007, yearling fish were subjected to seawater challenge tests to assess the progress of smoltification (Figure 2). Again both photoperiod and ration treatments appeared to affect the degree of smoltification as indicated by survival after 24-hour seawater challenges. Nearly 50% of fish on the early emergence and satiation treatment survived the seawater challenge indicating that a large proportion of fish in this group had smolted. Fish on the low or high ration and early emergence photoperiod has substantially greater mortality indicating only about 10% of the fish in these groups had smolted. Similarly, only 5% of the fish on middle emergence and either high ration or fed to satiation survived the seawater challenge test. Fish exposed to the late emergence photoperiod had 100% mortality after the seawater challenge irrespective of ration treatment.

Throughout the first year of rearing, gonad development was monitored in all sampled fish and the data were compiled to determine the percentage of males were maturing at age one (Figure 3). Both photoperiod at emergence and ration post-ponding affected the number of males maturing at age one. Nearly 70% of the males in the early emergence and satiation fed group matured after the first year of rearing, while none of the fish reared on late emergence photoperiod (equivalent to emergence on May 1) matured during this time irrespective of ration treatment. Within the early emergence groups, reducing growth using ration (low or high) appeared to reduce the number of males maturing at age one from 70% to 40-50%. Maturation rates of fish that emerged in a photoperiod equivalent to mid-February (middle emergence) ranged from 10-25%.

Together these data indicate that the seasonal timing of fry emergence and growth after ponding can alter life history patterns in spring Chinook salmon. These results are similar to those found for Sacramento River winter run Chinook salmon (Beckman et al. 2007), with early emerging fish having the highest rates of precocious male maturation. The results imply that hatchery rearing practices that alter seasonal timing of fry emergence can have drastic effects on life history patterns in juvenile Chinook salmon.

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. Data analyses that are incomplete will be included in the subsequent annual report(s).

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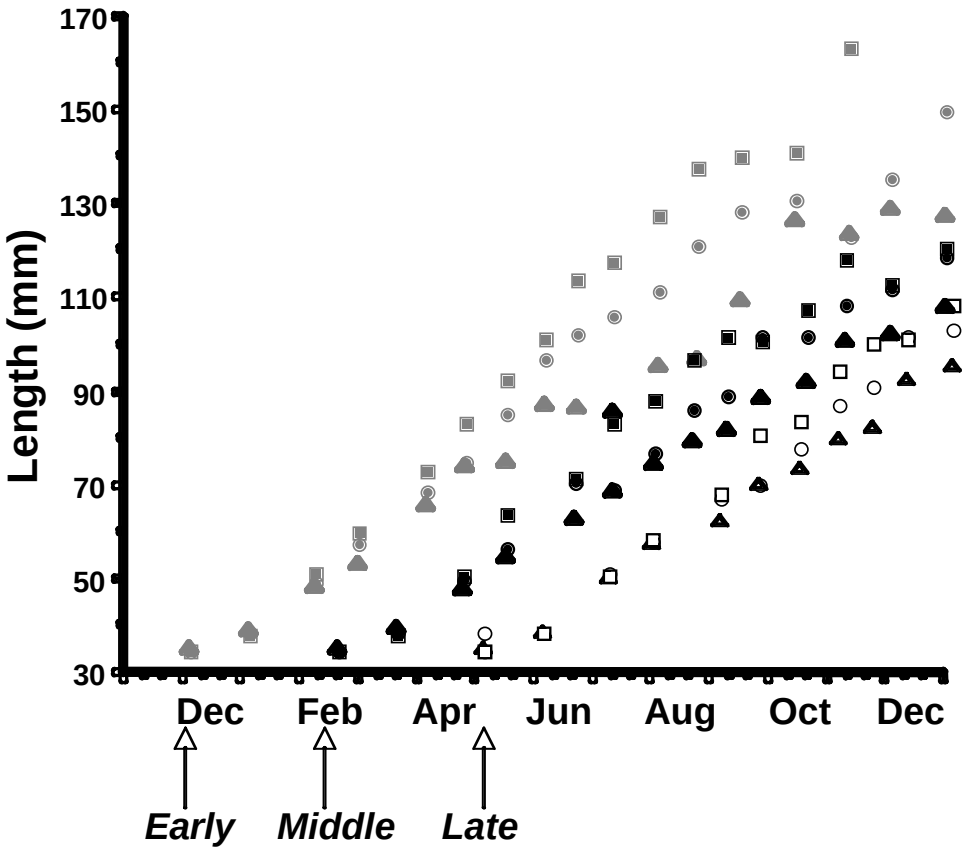

Figure 1. Length of Yakima River spring Chinook salmon ponded at three different points along a seasonal photoperiod cycle: Early, 1 December (gray); Middle, 15 February (black); and Late, 1 May (white). Within each photoperiod treatment fish were divided into three groups and fed three different rations: Satiation (squares), High Feed (circles) and Low Feed (triangles).

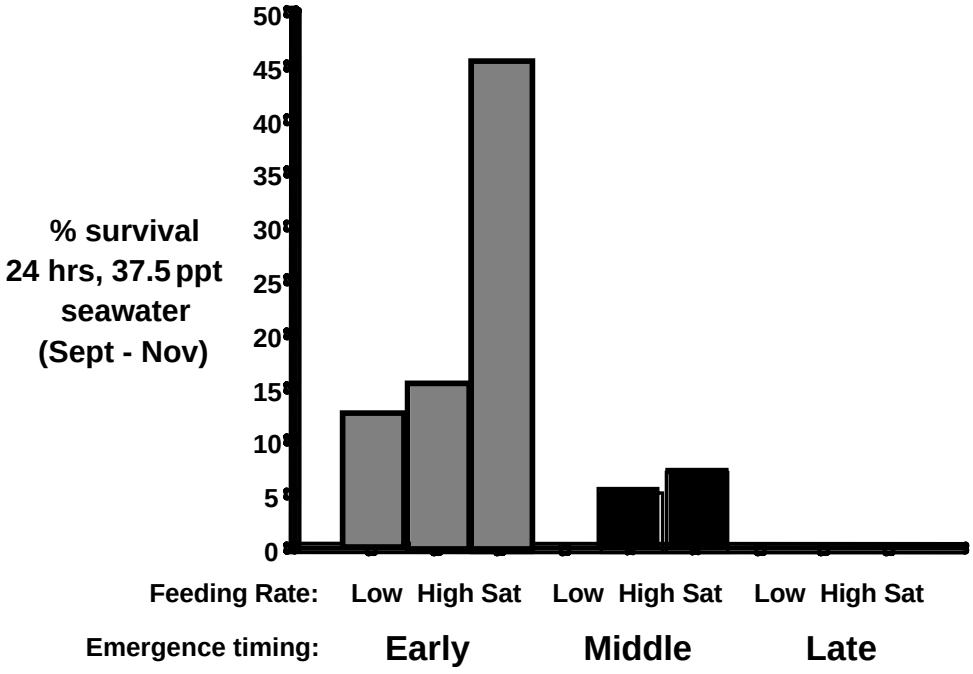

Figure 2. Twenty four-hour seawater survival of Yakima River spring Chinook salmon ponded at three different points along a seasonal photoperiod cycle: Early, 1 December (gray); Middle, 15 February (black); and Late, 1 May (white). Within each photoperiod treatment fish were divided into three groups and fed three different rations: Satiation (Sat), High Feed (High) and Low Feed (Low).

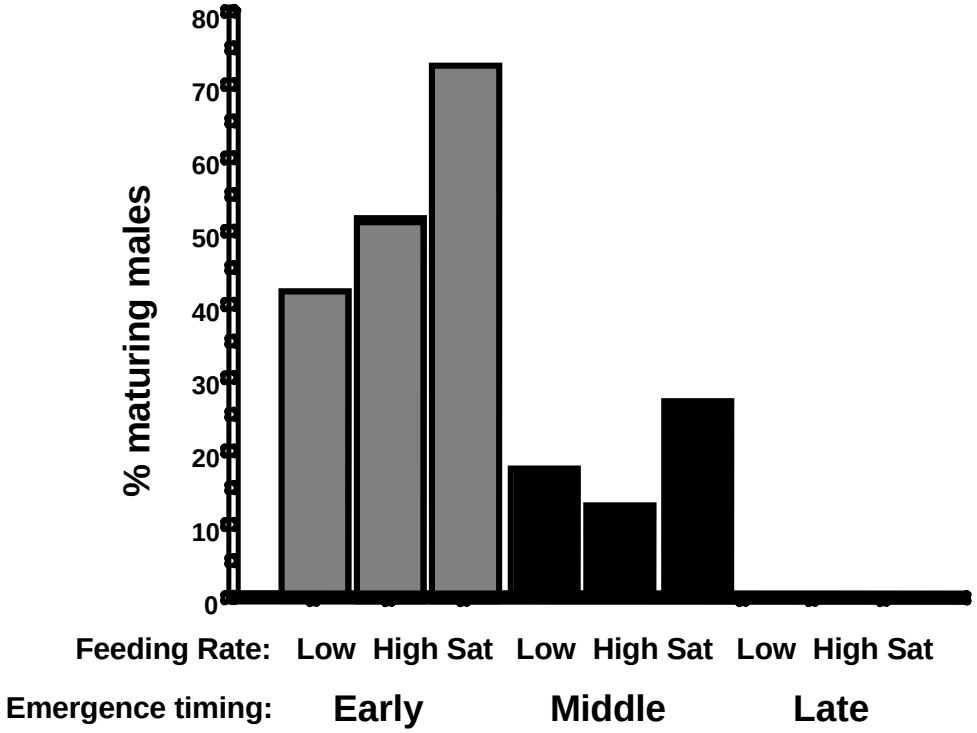

Figure 3. Percentage of male Yakima River spring Chinook that matured at age 1 from experimental groups of fry ponded at three different points along a seasonal photoperiod cycle: Early, 1 December (gray); Middle, 15 February (black); and Late, 1 May (white). Within each photoperiod treatment fish were divided into three groups and fed three different rations: Satiation (Sat), High Feed (High) and Low Feed (Low). Result is a composite from all fish sampled in the summer and fall (June - November).