

TASK 6. MONITORING REPRODUCTIVE DEVELOPMENT IN CAPTIVE  
BROODSTOCK AND ANADROMOUS HATCHERY STOCKS OF SNAKE RIVER  
SPRING CHINOOK SALMON DURING THE FRESHWATER PHASE OF ADULT  
MIGRATION.

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by

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## INTRODUCTION

In a captive rearing strategy, such as the one employed by the Idaho Fish and Game Department (IDFG) Spring Chinook Salmon Captive Broodstock Program, adult fish are released into their native streams to spawn with wild counterparts. This strategy is presently limited because the seasonal timing of ovulation and spermiation in some of the captive populations has been 3-5 weeks later than wild counterparts (P. Kline, IDFG, personal communication), making spawning of captively reared adults with wild fish improbable. The delay in spawning time in captively reared fish relative to wild fish of the same stock is not uncommon. Other captive broodstock programs for Oregon stocks of Snake River spring chinook salmon, Redfish Lake sockeye salmon and Sacramento River winter run chinook salmon have observed varying degrees of delayed spawning. The degree of the spawning delay varies with the stock and even year to year within the same stock of fish. Captively reared females can also display a high rate of egg retention and, in some cases, abnormal ovarian development leading to reduced fertility, egg size and number, and atretic eggs. In concert with the delay in spawning time, a progressive decline in egg quality has been observed; female Redfish Lake sockeye salmon captive broodstock spawned early in the season produce offspring with higher survival to the eyed stage than females spawned late in the season (C. McAuley, NMFS, personal communication).

The factors causing delayed maturation are not known, but are likely due to rearing environment since overt genetic selection for spawn time has not occurred in the captive broodstock programs. The seasonal delay in ovulation and asynchronous maturation of oocytes within the ovary may be a manifestation of abnormalities in the rate of oocyte development due to inappropriate environmental cues, such as temperature. Abnormal ovarian development may be due to rearing conditions early in the life cycle, during the final year prior to spawning when secondary oocyte growth is occurring, and/or during the final stages of oocyte maturation prior to ovulation. In order to develop rearing conditions that allow for proper seasonal timing of oocyte growth and maturation, a better understanding of the phase of reproduction during which the delay first occurs is needed. In other words, is the delayed development of the egg occurring during yolk incorporation (seawater rearing) or just during the final phase of oocyte maturation and ovulation (freshwater rearing prior to spawning)? By comparing reproductive development of captive broodstock with that of a closely related stock of migrating hatchery fish it may be possible to determine at what stage the captively reared fish are delayed, and to develop a rational approach to solving this problem.

Therefore, in the present study, reproductive development was compared in two stocks of Snake River spring chinook salmon: adults returning to the IDFG Rapid River Hatchery and Lemhi River captive broodstock. The goals of the study were to compare the progress of sexual maturation by histological analyses of gonad samples and measurement of reproductive hormones, to evaluate the bioenergetics of migration in the migrating hatchery fish, and to determine the morphological changes that occur during

sexual maturation in spring chinook salmon. Concurrent with the hormonal changes that occur in sexually maturing salmon as they ascend the river is a depletion of body stores of protein and lipid. This depletion occurs due to cessation of feeding after entering fresh water, energy expenditure during upstream migration and transfer of somatic nutrients to the gonads. The relationships among hormonal changes, body composition and gonad development have not been well documented in migrating adult chinook salmon. Not surprisingly, anecdotal information suggests that the compositional changes that occur in captive broodstock are far less than that of wild fish that undergo an upriver migration. The large returns of adult chinook during 2001 provided a unique opportunity to obtain important physiological information from migrating adult spring chinook salmon. The data will provide a template by which to compare captively-reared adult chinook salmon, and important information to aid in understanding the underlying causes of the delayed maturation in the captive broodstock.

## **Work Completed**

## **Materials and Methods**

**Sampling protocol-** Samples of gonads, blood, pituitary glands and carcasses were collected from adults that returned to the IDFG Rapid River Fish Hatchery (Riggins, Idaho) at monthly intervals from May through spawning in September 2001. A total of 10 males and 10 females were sacrificed each month. Photographs using a digital camera were taken of whole bodies and gonads. Similar samples were collected during 2001 from female (BY97) Lemhi River spring chinook salmon captive broodstock at three time points: May (just prior to transfer from the saltwater rearing tanks to fresh water), August (prior to the normal time of release into the spawning stream) and September/October (when fish were spawned in the hatchery). Six to nine females at each time point were sampled. Lemhi River captive broodstock were reared in filtered and UV-treated saltwater at the NMFS Manchester Research Station (MRS) from May 1999 through early June 2001. On June 8, 2001 fish were transferred to fresh water and reared through spawning at the IDFG Eagle Hatchery, Eagle, ID. In June 2001, an additional treatment group was added to determine if temperature of fresh water influenced spawn timing. Lemhi River captive broodstock were divided into two groups: chilled (9 °C) and ambient (14 °C). Samples were collected from females in both treatments during August and at spawning.

During the sampling of Lemhi River captive broodstock in May, substantial gastric distention and ovarian atresia, particularly of the left ovary was observed. To determine whether this was specific to the Lemhi River stock, or due to the rearing in saltwater at the MRS, 10 females from the Catherine Creek spring chinook salmon captive broodstock maintained in fresh water at the Bonneville Hatchery, and in saltwater at the MRS were sampled during May.

**Histology-** Testicular tissue was preserved in Bouin's fixative for 48 hours at 4 °C and transferred to 70% ethanol for storage. Tissue was processed and embedded in

paraffin. Sections were cut at 4 microns on a standard rotary microtome and stained with routine hematoxylin and eosin. Ovarian tissue was preserved in Karnovsky's fixative for 48 hours at 4 °C and transferred to 70% ethanol for storage. Tissue was processed and embedded in glycol methacrylate resin using the Technovit 7100® Kit. Sections were cut at 4 microns on an automated microtome (Leica RM 2165) and stained with a modified hematoxylin and eosin procedure.

**Plasma and pituitary hormone analyses-** Blood was collected from caudal vein using 18 gauge needles and heparinized syringes. Blood was immediately transferred to 15 ml polypropylene tubes containing aprotinin and trypsin inhibitor and stored on ice prior to centrifugation. Plasma was stored on dry ice until transferred to -70 °C freezer for long term storage. Pituitary glands were frozen immediately on dry ice and stored at -70 °C until analyzed for gonadotropin content. Plasma and pituitaries were analyzed for follicle stimulating hormone (FSH) and luteinizing hormone (LH) content by radioimmunoassays (RIAs) (Swanson et al. 1989). Levels of testosterone (T), 11-ketotestosterone (11-KT), and estradiol 17 $\beta$  (E) were determined in plasma samples by either RIAs or enzyme immunoassays (EIA) (Cuisset et al. 1994, Rodriguez et al. 2001, Sower and Schreck 1982). Plasma insulin-like growth factor (IGF-I) was measured using GroPep components as described by Shimizu et al. (1999).

**Body composition-** Carcasses, gonads and visceral were collected separately and stored at -20 °C until analyzed for fat and protein content. Gonads, viscera or carcasses were partially thawed, ground in a food processor and a subsample of 100 g of wet material was dried, then reground in a coffee grinder, and a subsample was taken for analysis (0.5 g for protein and 2 g for fat). Moisture was determined by drying to constant weight at 105 °C. Fat was determined using a Soxhlet device (Buchi 810, Brinkman Instruments, Westbury, NY) with dichloromethane as the solvent. Protein was calculated by multiplying percent nitrogen determined using a nitrogen analyzer (Leco FP2000, Leco Corp., Henderson, NV) by a factor of 6.25. Proximate composition values are expressed on a wet weight basis.

**Morphometrics-** Fish were euthanized and photographed with a digital camera on their left side along side a ruler. Cartesian coordinate information for 19 landmarks was collected from each image with the digitizing program *tpsDig* (Rohlf 1998a). Thirty-two distance measurements were calculated between 15 landmarks in a truss network pattern after Winans (1984). Landmarks at the anterior tip of the dorsal and anal fins, and at the insertion and distal point of the left pectoral fin were digitized for dorsal and anal fin height, and pectoral fin length, respectively. Distances were calculated using the Pythagorean theorem. Two analyses, principal component (PC) and relative warp (RW) analyses (Cadrin 2000) were conducted to assess multivariate shape variation. Morphometric distance data were analyzed in a PC analysis of the variance-covariance matrix. RW analyses, representing geometric-landmark methods (Bookstein 1991), were conducted using *TPSRELW* (Rohlf 1998b) to assess the geometry of each fish relative to an average or consensus body shape. Individual PC and RW scores were analyzed and plotted for the first major components, respectfully. Bivariate confidence ellipses were calculated from standard deviations and sample covariation.

### Work to be Completed

As of May 2002, analyses of hormone levels in plasma and pituitary samples were completed with the exception of plasma vitellogenin (females) and 17 $\beta$ , 20 $\alpha$ -dihydroxy-4-pregnen-3-one (both sexes). It is anticipated that these will be completed by December 2002. Analyses of carcass, viscera, and gonad composition are completed and a preliminary analysis of the data has been completed. Initial analyses of morphometric data are completed. Histological analyses of testis development in male Rapid River spring chinook salmon are completed, but ovarian histology has not yet been completed. Samples have been processed, but quantification of oocyte stages by image analysis will be done during the FY02 work period. Data on spawning time have been collected and analyzed. All data collection and analyses will be completed during the FY02 work period and will be reported in the final report for FY02.

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