FISHERY RESEARCH

CAPTIVE REARING PROGRAM FOR SALMON RIVER CHINOOK SALMON

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Captive Rearing Program for Salmon River Chinook Salmon

Project Progress Report

2003 Annual Report

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ABSTRACT

During 2003, the Idaho Department of Fish and Game continued to develop techniques to rear Chinook salmon *Oncorhynchus tshawytscha* to sexual maturity in captivity and to monitor their reproductive performance under natural conditions. Eyed-eggs were hydraulically collected from redds in the East Fork Salmon River (EFSR; N = 319 eggs) and the West Fork Yankee Fork Salmon River (WFYF; N = 338 eggs) to establish brood year 2003 culture cohorts. The eyed-eggs were incubated and reared at the Eagle Fish Hatchery, Eagle, Idaho (Eagle). Juveniles collected in 2001 were given a passive integrated transponder (PIT) tag, elastomer tagged, and vaccinated against *Vibrio* spp. and bacterial kidney disease (causative agent *Renibacterium salmoninarum*) prior to smoltification and transfer to the NOAA Fisheries, Manchester Research Station, Manchester, Washington (Manchester) for saltwater rearing through maturity. Smolt transfers included 257 individuals from the WFYF and 285 from the EFSR. Maturing fish transfers from Manchester to Eagle included 52 individuals from the Lemhi River (LEM), 89 from the WFYF, and 102 from the EFSR. This was the third year maturing adults were held on chilled water at Eagle to test if water temperature manipulations could advance spawn timing and improve egg quality. Adults from the EFSR and WFYF were divided into chilled (≈9°C) and ambient (≈13.5°C) temperature groups while at Eagle. Twenty-seven mature females from the EFSR (13 chilled, 14 ambient) were spawned at Eagle with 24 EFSR males in 2003. Mean spawn date for females in the chilled water group was statistically earlier (23.4 ± 4.68 d) than in the ambient group (32.7 ± 3.62 d). Egg survival to the eyed stage was also higher for test females (62.76%) than control females (34.91%), but this difference was not statistically different. Overall egg survival to the eyed stage averaged 46.59%. Personnel from the Shoshone-Bannock Tribes placed 16,154 eyed-eggs from these crosses in in-stream incubators in the EFSR. Mature adults were released into the WFYF (N = 88) to evaluate their reproductive performance, but by approximately one month after release all fish had died. This unprecedented level of prespawn mortality was not attributable to any specific cause. Diagnostic assays on tissue collected from a number of carcasses did not reveal any viral or bacterial pathogens except *Aeromonas* spp., which is generally considered a secondary infection or response to stress. Forty-one mature adults were released into the EFSR as well as 48 mature adults into the LEM. Almost all of these fish moved out of the areas shoreline observers had access to, so no spawning behavior was observed. Juvenile Chinook salmon were collected from the WFYF (N = 565) and Hayden Creek (tributary to the Lemhi River; N = 567) to assess production levels from volitional spawning program adults in the WFYF in 2002 or eyed-egg plants in Hayden Creek in 2002. Genetic material from these juveniles will be analyzed with samples from all program adults by the University of Idaho, and this information will be used in parental exclusion analyses.

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INTRODUCTION

Idaho Department of Fish and Game's (IDFG) long-term management objective for Chinook salmon *Oncorhynchus tshawytscha* is to maintain Snake River salmon populations at levels that will provide sustainable harvest (IDFG 2001). Restoring currently depressed populations to historic levels is a prerequisite to this condition. Artificial propagation of spring and summer Chinook salmon in the Salmon River basin, through Lower Snake River Compensation Plan (LSRCP) and Idaho Power Company hatcheries, was initiated to compensate for lost production and productivity caused by the construction and operation of private and federal hydroelectric facilities in the Snake River basin. The mitigation approach was to trap, spawn, and rear a portion of the historically productive local broodstock to produce a large number of smolts (Bowles 1993). When Chinook salmon trapping began in 1981 as part of the LSRCP, it was assumed that enough Chinook salmon adults would return to provide for harvest and continued hatchery production needs. It was also assumed that hatchery programs would not negatively affect the productivity or genetic viability of target or other populations of Chinook salmon and that natural populations would remain self-sustaining even with hydropower projects in place. In reality, smolt-to-adult survival in wild Snake River Chinook salmon declined abruptly with completion of the federal hydroelectric system by the mid-1970s (Petrosky and Schaller 1994; Petrosky et al. 1999), and numbers of naturally produced salmon declined at various rates throughout the Snake River basin (Petrosky et al. 1999). It now appears that the survival rate estimates used in the hatchery mitigation program models were substantially overestimated, which has led to hatchery programs that have not consistently mitigated for reductions in Chinook salmon production and productivity. Spring/summer Chinook salmon returns have been insufficient to meet artificial and natural smolt and adult production goals; much less provide a consistent harvestable surplus of adults (Hassemer 1998).

Development of the Snake River hydrosystem has substantially influenced the decline of local spring/summer Chinook salmon stocks by reducing productivity and survival (Raymond 1979; Schaller et al. 1999), and has contributed to the listing of Snake River Chinook salmon under the Endangered Species Act (ESA; NMFS 1992). A recovery strategy incorporating natural-river function is most likely to increase the smolt-to-adult return rate and provide for recovery of these populations (Marmorek et al. 1998). However, until smolt-to-adult survival increases, our challenge is to preserve the existing metapopulation structure (by preventing local or demographic extinctions) of these stocks to ensure they remain extant to benefit from future recovery actions. This project is developing technology that may be used in the recovery of the listed Snake River spring/summer Chinook salmon evolutionarily significant unit (ESU), which consists of 31 subpopulations (i.e., breeding units or stocks; McClure et al. 2003). Preserving the metapopulation structure of this ESU is consistent with the various Snake River Salmon Recovery Plans (NMFS 1995; Schmitten et al. 1997; McClure et al. 2003), and supports the Northwest Power and Conservation Council's goal of maintaining biological diversity while doubling salmon and steelhead runs (NPPC 1994).

Idaho and Oregon state, tribal, and federal fish managers met during 1993 and 1994 to discuss captive culture research and implementation in the Snake River basin. The outcome of those meetings was an agreement that the Oregon Department of Fish and Wildlife would initiate a captive broodstock program using selected Grande Ronde River Chinook salmon populations, and the IDFG would initiate captive rearing research using selected Salmon River Chinook salmon populations. Both captive culture techniques begin by bringing naturally produced eggs, parr, or smolts into captivity and rearing them in a hatchery to sexual maturity. At this point, the two techniques diverge. The F_1 generation in a captive rearing program is

returned to its natal stream and allowed to spawn naturally. The F_1 generation from a captive broodstock program is spawned in the hatchery, where the resulting F_2 progeny are held until smoltification. The F_2 smolts are then released to their natal streams to emigrate volitionally. The primary focus of these programs is to evaluate the effectiveness of the two forms of captive culture to meet population conservation objectives. Implicit within each research project is the objective to develop and test appropriate facilities and fish culture protocols specific to the captive culture of Chinook salmon for conservation management of depressed populations.

Little scientific information regarding captive culture techniques for Pacific salmonids was available at the inception of these programs, but a substantial amount of new literature has been published in the ensuing years. The Chinook Salmon Captive Propagation Technical Oversight Committee (CSCPTOC) was formed to convey this new information between the various state, federal, and tribal entities involved in the captive culture of Chinook salmon. The CSCPTOC meets approximately every two months, which allows an adaptive management approach to all phases of the program and provides a forum of peer review and discussion for all activities and culture protocols associated with this program. Flagg and Mahnken (1995) provided an initial literature review of captive rearing and captive broodstock technology, which provided the knowledge base upon which the program was designed. Using this work, the IDFG captive rearing program for Salmon River Chinook salmon was initiated to further the development of this technology by monitoring and evaluating captive-reared fish during rearing and post-release spawning phases. Berejikian et al. (2004) and Flagg et al. (2004) provide contemporary assessments of the benefits and risk of captive rearing and broodstocking, respectively. Since the program's inception, studies documenting the spawning behavior of captive-reared Chinook salmon (Berejikian et al. 2001b), coho salmon *O. kisutch* (Berejikian et al. 1997), and Atlantic salmon *Salmo salar* (Fleming et al. 1996) have been published. Other studies have also compared the competitive behavior of male captive-reared and wild coho salmon during spawning (Berejikian et al. 2001a) and the competitive differences between newly emerged fry produced by captive-reared and wild coho salmon (Berejikian et al. 1999). Finally, Hendry et al. (2000) report on the reproductive development of sockeye salmon *O. nerka* reared in captivity.

The IDFG captive rearing program was developed as a way to increase the number of naturally spawning adults and maintain metapopulation structure in selected populations at high risk of extinction while avoiding the impacts of multigenerational hatchery culture described in Reisenbichler and Rubin (1999). The strategy of captive rearing is to prevent cohort collapse in the target populations by returning captive-reared adults to natural spawning areas to augment depressed natural escapement or replace it in years when no natural escapement occurs. This maintains the continuum of generation-to-generation smolt production and provides the opportunity for population maintenance or increase should environmental conditions prove favorable for that cohort. However, the success of the captive rearing approach to produce adults with the desired morphological, physiological, and behavioral attributes to spawn successfully in the wild remains somewhat speculative (Fleming and Gross 1992, 1993; Joyce et al. 1993; Flagg and Mahnken 1995).

The IDFG captive rearing program was initiated in 1995 with the collection of brood year 1994 Chinook salmon parr from three study streams. Since then, naturally spawned Chinook salmon progeny from brood years 1995-2003 have been represented in captivity to continue the project. Hassemer et al. (1999, 2001) and Venditti et al. (2002, 2003a, 2003b) summarize project activities from inception through 2002. The streams selected for inclusion in the captive rearing program include the Lemhi River (LEM), the East Fork Salmon River (EFSR), and the West Fork Yankee Fork Salmon River (WFYF; Figure 1). Water temperatures are ideal for

juvenile Chinook salmon rearing in all three streams, while water quality ranges from sufficient to ideal. Habitat quality ranges from relatively pristine to areas of riparian degradation caused by sedimentation, grazing, mining, logging, road building, and irrigation diversion. The LEM drains productive basaltic parent material resulting in rapid fish growth. The lower section of this river flows through private land developed extensively for agriculture and grazing and typically reflects C channel conditions (Rosgen 1985). The EFSR drains a relatively sterile watershed of granitic parent material associated with the Idaho batholith. The lower 30 km of the EFSR runs through ranch and grazing property developed during the last century, but the upper reaches reflect near pristine conditions with little historical disturbance from logging, mining, or agriculture. Stream habitat in the EFSR typically reflects B and C conditions (Rosgen 1985). The WFYF, which drains a sterile watershed similar to the EFSR, remains primarily roadless and has remained nonimpacted by land use practices for nearly half a century. Stream habitat typically reflects B and C conditions (Rosgen 1985).

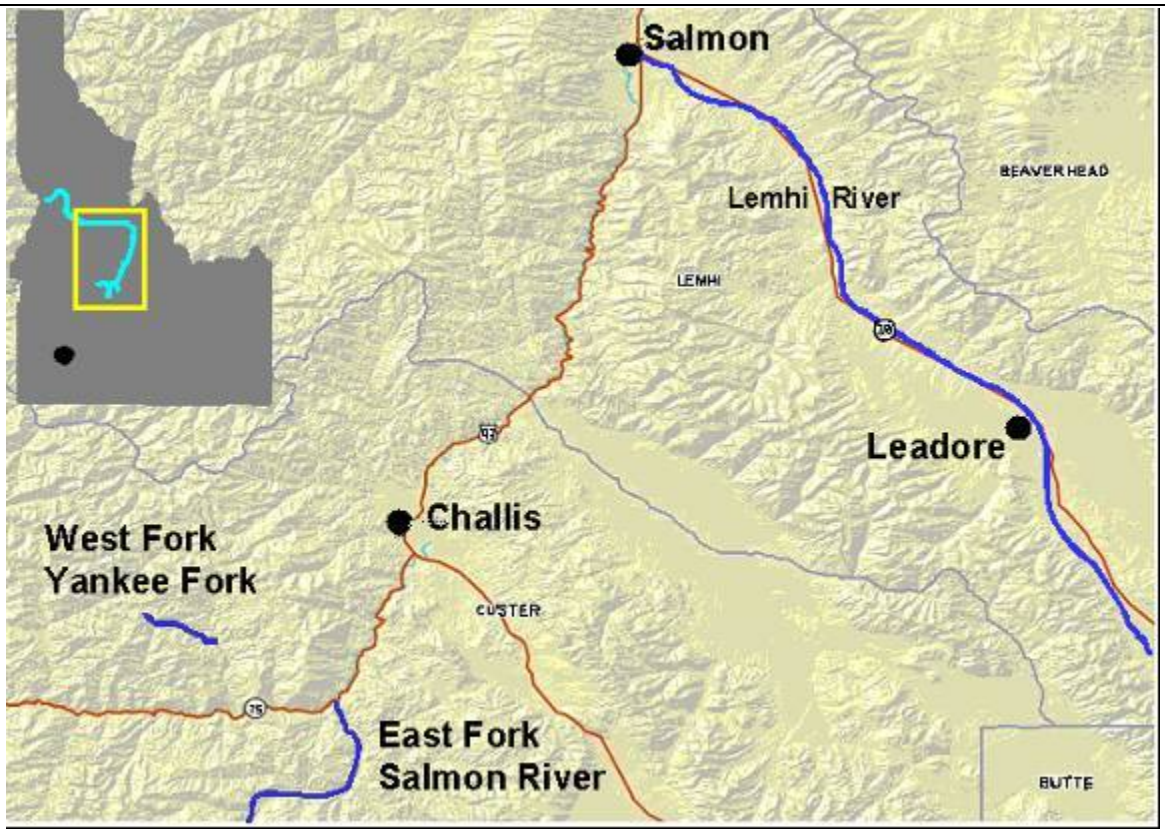

Figure 1. Location of study streams included in the Idaho Department of Fish and Game Captive Rearing Program for Salmon River Chinook Salmon.

The goal of the captive rearing program is to increase the number of naturally spawning adults and maintain metapopulation structure in selected Chinook salmon populations at high risk of extinction. We have identified two primary project objectives needed to realize this goal. These are to: 1) develop and implement culture practices and facility modifications necessary to rear Chinook salmon to adulthood in captivity having morphological, physiological, and behavioral characteristics similar to wild fish, and 2) evaluate the spawning behavior and

success of captive-reared individuals under hatchery and natural conditions. These objectives divide the program into two functional units including fish culture and field evaluations, but the success of the program is dependent on the synchronous development of both. This report documents activities performed in both aspects of the evaluation from January 1, 2003 through December 31, 2003. This project is coordinated with the Northwest Power and Conservation Council's Fish and Wildlife Program (NPPC 2000) and is identified as project 199700100. Funding is provided through the Bonneville Power Administration under contract 00004002.

METHODS

Culture Facilities

The IDFG Eagle Fish Hatchery, Eagle, Idaho (Eagle) was the primary Idaho site for the captive culture of program fish from collection through smoltification. Smolts were transferred to the NOAA Fisheries Manchester Research Station in Manchester, Washington (Manchester) for saltwater rearing through sexual maturity. Eagle was supplied with pathogen-free artesian water from three wells, and the artesian flow is augmented with three separate pump and motor systems. Ambient water temperature and total dissolved gas averaged 13.5°C and 100% after degassing, respectively. Water chilling capability was added in 1994 and expanded in 2001 for use during various stages of the captive rearing process. Water temperature was maintained between 7.0°C and 9.0°C during the egg incubation period of the rearing cycle. From ponding through transfer of smolts to saltwater, water temperature was maintained between 8.0°C and 10.0°C. Chilled water was also used in holding tanks of maturing, adult Chinook salmon prior to in-hatchery spawning or release for natural spawning. Backup and system redundancy was maintained for degassing, pumping, and power generation. Nine water level alarms were linked through an emergency service operator. Additional security was provided by limiting public access and by the presence of three onsite residences occupied by IDFG hatchery personnel.

Tanks of various sizes and configurations were maintained at Eagle to accommodate the various life stages and sizes of Chinook salmon maintained on station. Plastic incubators and fiberglass tanks ranging in size from 0.7–6.0 m in diameter were used to culture Chinook salmon from eggs to maturity. Fertilized eggs are held in incubators until swim-up, transferred to 0.7 m semisquare tanks (0.09 m^3), then transferred to 1.0 m diameter semisquare tanks (0.30 m^3), where they remained until they reached approximately 1 g. Fish were then moved to 2.0 m semisquare tanks (1.42 m^3), where they remained until transfer to saltwater at smoltification. At maturation, fish were transferred from saltwater back to freshwater at Eagle. Maturing fish were held in 3.0 m circular tanks (6.50 m^3) by stream origin until they were released into their natal waters or spawned in the hatchery to monitor reproductive success variables.

Flow to all tanks at Eagle was maintained at no less that 1.5 exchanges per hour, and shade covering (70%) and jump screens were used where appropriate. Tank discharge standpipes were assembled in two sections ("half-pipe" principle) to prevent tank dewatering when removed for tank cleaning.

Tanks and culture facilities utilized by the Chinook salmon captive rearing program were located in three general areas at Eagle. Spawning, incubation, and fry rearing took place in an enclosed building plumbed with chilled and ambient water, which allowed water temperature regulation through controlled mixing. The intermediate sized tanks were located adjacent to the

spawn building and received both chilled and ambient water. A roof covered tanks in this location, but the sides were not walled. The 3.0 m tanks used by this project were located approximately 100 m from the incubation building. Tanks in this area were exposed to both direct overhead and peripheral sunlight. A second water chiller was installed in 2001 to provide water temperature control to a portion of the 3.0 m tanks in this group; the other tanks received only ambient temperature water.

Saltwater rearing was provided for all study animals post smoltification at Manchester. This facility is located on Puget Sound near Seattle, Washington and was supplied with approximately 5,000 L/min of saltwater that ranged in temperature between 7°C and 14°C annually and averaged 29‰ salinity. Raw saltwater was passed through sand and cartridge filters to remove particles >5 μ, sanitized with ultraviolet light, and degassed prior to entering fish rearing tanks. Effluent from the rearing tanks was treated with ozone prior to being returned to Puget Sound (Frost et al. 2002).

Eyed-Egg Collection, Incubation, and Transport

Eyed-eggs to establish individual brood year captive cohorts were collected from redds spawned by wild Chinook salmon in study streams using hydraulic sampling methods described by McNeil (1964). The hydraulic sampling system consisted of two main components. The first was a gas-powered pump attached to a 3.8 cm diameter aluminum probe via flexible tubing (Figure 2A). Holes drilled near the top of the probe infused air into the water stream through venturi action. The second component was the collection net frame consisting of a "D" shaped aluminum frame with expanded plastic mesh along its curved portion and netting around the bottom and sides of its straight portion (Figure 2B). During operation, water was forced through the probe, which was worked into the substrate. The air/water stream then lifted eggs out of the substrate, where they were swept downstream into the net. The expanded plastic screen confined eggs lifted out near the periphery and channeled them into the net. In order to minimize disturbance to the redd, sampling generally began slightly downstream of estimated nest pocket locations and progressed upstream. This procedure prevented the fine materials lifted out of the substrate from settling back into the redd and possibly smothering the eggs. Care was also taken to keep personnel behind or to the side of the net frame to minimize redd trampling, which has been shown to kill eggs and pre-emergent fry in trout redds (Roberts and White 1992).

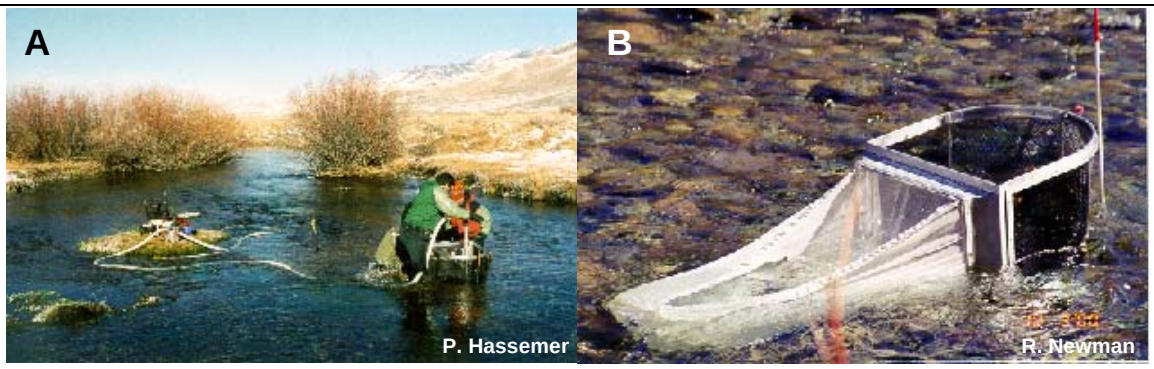

Figure 2. Hydraulic sampling gear including (A) the pump and probe, and (B) the collection net used to collect eyed-eggs from naturally spawned redds.

To facilitate eyed-egg collections, redd locations were marked, construction and completion dates determined, and stream temperatures monitored with recording thermographs. Program personnel walked portions of the study streams every 7–10 d to identify new redds and estimate completion dates of previously located redds. Redds were marked by placing flagging on shoreline vegetation near their location. Information on when the redd was first observed and the spawning state of fish seen associated with the redd (e.g., courting, digging, trenching, etc.) was recorded on the flagging. Thermographs deployed in the study streams recorded water temperature every 2 h, and the daily average water temperature was computed to track the number of Celsius temperature units (CTUs) received by the developing embryos in each stream. Eyed-eggs were collected after receiving 300-400 CTUs. During this period, eye pigmentation makes developing embryos readily identifiable, and egg structures are capable of withstanding collection.

Eyed-eggs were transferred from collection locations to Eagle using the following standardized protocols. Eyed-eggs from a single redd were placed in perforated shipping tubes, capped, and labeled to identify them to stream and redd. Tubes were wrapped in paper towels saturated with river water and packed in small, insulated coolers. Ice chips were added to maintain proper temperature and a moist environment during transport. Eggs were taken to Eagle as soon as possible after collection and were generally on site 4–6 h after extraction from the gravel.

Once at Eagle, familial groups of eyed-eggs were disinfected in 100 mg/L Iodophor for 30 min and transferred to separate incubators (14 cm diameter x 19 cm height, 2.5 L total operating volume), where they remained until the resulting fry were ready to begin feeding (Heindel et al. 2005). A constant flow (1.2 L/min) of chilled water (approximately 7-9°C) was maintained throughout incubation and was provided as upwelling from below the eggs (Figure 3A). Incubators were checked daily, and dead eggs were removed. After hatching, water flow was reversed to downwelling (Figure 3B).

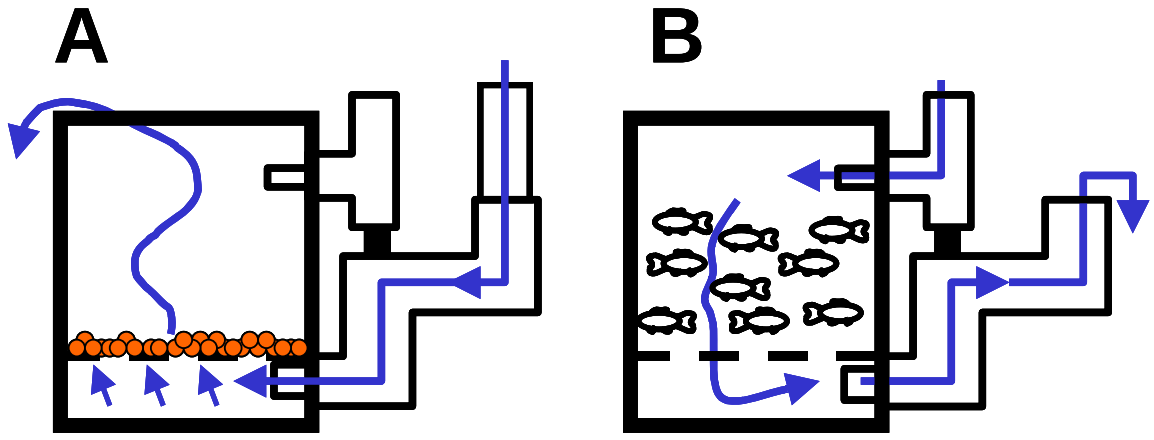

Figure 3. Schematic diagram of reversible flow incubators used to incubate eggs and rear newly emerged fry. A) Upwelling configuration for egg incubation, and B) downwelling configuration for fry rearing.

Juvenile Rearing, Marking, and Transportation

Fish husbandry practices employed at the Eagle facility ranged from traditional to experimental. Fish health issues were handled using only FDA and/or CVM (Center for Veterinary Medicine) approved therapeutants, and standard fish culture practices were employed whenever possible (for an overview of standard methods see Leitritz and Lewis 1976; Piper et al. 1982; Erdahl 1994; Bromage and Roberts 1995; McDaniel et al. 1994; Pennell and Barton 1996). However, due to the experimental nature of the work conducted at Eagle, some aspects of the incubation, rearing, and feeding protocols differed from those used at production hatcheries. Eggs were incubated in specially designed incubators (Heindel et al. 2005) that allowed siblings from individual spawn crosses or redds to be maintained separately until the juveniles are tagged with passive integrated transponder (PIT) tags (Prentice et al. 1990) to permit future familial identification. Rearing tank size, density, and food ration varied with fish age and were managed to promote optimum growth and the attainment of program objectives.

Ration and water temperature were manipulated to simulate the ration and temperature regimes that would be experienced in the natural environment to modulate growth and reduce precocial male development and has been developed collaboratively with NOAA Fisheries Project Number 199606700. Fish were fed standard commercial diets produced by Bio-Oregon, Inc. (Warrenton, Oregon) and Skretting (Vancouver, B.C.). Swim-up fry were fed for one week in their incubators prior to ponding to 0.7 m semisquare tanks. Fry were fed hourly during daylight hours, approximately eight times per day, until they reached approximately 1 g. Growth projections were developed at this time, and feeding rates were reduced to four times per day. As fish grew, ration and pellet size were adjusted accordingly. Juveniles were periodically anesthetized, weighed to the nearest 0.1 g, and measured to the nearest 1 mm fork length (FL) to track growth and to ensure that projected weights tracked closely with actual weights. Tanks received a mixture of ambient and chilled water that maintained a temperature of approximately 10°C and ensured approximately 1.5 turnovers/h.

Juvenile Chinook salmon were marked during two separate events at Eagle each year to aid in tracking fish in the program. The first involved injecting a PIT tag into the peritoneal cavity of age-1 juveniles. Fish were anesthetized in MS-222 (tricaine methanesulfonate; buffered to neutrality with sodium bicarbonate), weighed to the nearest 0.1 g, and measured to the nearest 1 mm FL. A modified 12-gauge hypodermic needle was then used to inject the PIT tag into the body cavity slightly anterior to the pelvic girdle and just off the ventral midline. The PIT tag gave each individual a unique identity within the program that can be used to track each fish through the remainder of its life. The second marking involved age-1 juveniles and was conducted shortly before they were transported to Manchester. Fish were again anesthetized in buffered MS-222, weighed to the nearest 0.1 g, and measured to the nearest 1 mm FL. A color-coded elastomer tag was injected into the clear tissue immediately posterior to the eye (Olsen and Vøllestad 2001; Close and Jones 2002) based on its stream of origin. Fish from the EFSR and WFYF received green and orange marks, respectively. The fish also received intraperitoneal injections of Renogen® (Aqua Health, Ltd., Charlottetown, PEI, Canada) *Arthrobacter* spp. to vaccinate against bacterial kidney disease (BKD) and Vibrogen® (Aqua Health, Ltd.) to vaccinate against *Vibrio* spp. After each marking event, fish were allowed to recover in coolers of freshwater before being returned to the general population.

All age-1 juvenile Chinook salmon were transported to Manchester as smolts for saltwater rearing. Smolts were transported between facilities in truck-mounted, insulated tanks (950 L capacity) with alarm and backup oxygen systems and "fresh flow" mechanical water

movement units on board. Loading volumes did not exceed 89 g/L (0.75 lb/gal). In addition, all vehicles had two-way radios and/or cellular telephones to provide routine or emergency communications. Prior to offloading, transport water was tempered to within 2.0°C of the receiving water, if necessary, and fish were moved, by stock, to 6.0 m circular tanks filled with full strength freshwater for saltwater acclimation. Once in the circular tanks, full strength saltwater flowed into the tanks until the freshwater was completely replaced (approximately 12 h, C. McAuley, NOAA Fisheries, personal communication).

Adult Rearing, Transportation, and Marking

Maturing Chinook salmon at Manchester were transported back to Eagle to complete the freshwater phase of their maturation and for spawning performance evaluation. Maturation state was determined for all individuals at Manchester by ultrasound examination using an Aloka SSD-500V ultrasound unit with an Aloka Electronic Linear Probe UST-556L-7.5. A second maturation sort was also conducted at Manchester several weeks after the initial sort to identify any maturing fish not detected in the earlier ultrasound examination. Adults were transported using similar equipment and techniques as described above, and loading volumes did not exceed 89 g/L. Maturing fish from multiple brood years were pooled by stock for transport to Eagle, although stocks that may have posed a health risk to other program fish were transported in separate vehicles. Tanks were loaded with ¼ saltwater and ¾ freshwater to begin freshwater acclimation during transport. Once at Eagle, fish were immediately placed in 3.0 m circular tanks filled with full strength freshwater.

Maturing Chinook salmon destined to be released for natural spawning were fitted with either disc tags or Floy tags prior to release. Disc tags were color-coded to identify the temperature treatment (see below) and brood year to which the fish belonged. Additionally, each disc tag had a unique number embossed upon it to identify the individual. Fish were anesthetized in buffered MS-222, weighed to the nearest 1.0 g, and measured to the nearest 1 mm FL. Water temperature in the anesthetic baths was determined by the temperature treatment the fish were being exposed to (see below). Disc tags were attached to the fish by passing a stainless steel pin through a hole in the center of the tag and passing the pin through the musculature of the dorsal surface just ventral to the midline of the dorsal fin. Then, a corresponding tag (same color code and number) was slipped onto the pin on the opposite side of the fish. The tag was secured by trimming the pin to length, and a loop was formed at the end with needle-nose pliers. When used, Floy tags were inserted into the left side of the dorsal musculature in a similar location as disc tags but did not protrude to the opposite side. Fish that received Floy tags were anesthetized using the same protocol as outlined for disc tagging procedures. After marking, all fish were allowed to recover in coolers containing the appropriate temperature water before being returned to the holding tanks.

Chilled Water Experiments

A common thread linking previous releases of captive-reared Chinook salmon has been that these fish have consistently spawned several weeks later than their naturally produced counterparts (Hassemer et al. 1999, 2001; Venditti et al. 2002, 2003a, b). In order to address this limitation, additional water chilling capacity was added at Eagle in 2001 to assess if water temperature manipulations between the time maturing adults were returned to freshwater and release could be used to advance their spawn timing. While we could find no instances where this has been tested on Chinook salmon, there is a substantial amount of literature describing

the effect of temperature on the timing of ovulation in other salmonid species. Elevated holding temperature prior to spawning has been shown to retard the onset of ovulation in rainbow trout *O. mykiss* (Pankhurst et al. 1996; Pankhurst and Thomas 1998; Davies and Bromage 2002), pink salmon *O. gorbuscha* (Beacham and Murray 1988), Atlantic salmon (Taranger and Hansen 1993), and Arctic charr *Salvelinus alpinus* (Gillet 1991; Jobling et al. 1995). However, Henderson (1963) did not observe this relationship in eastern brook trout *S. fontinalis*.

Maturing Chinook salmon stocks were separated into three groups for holding at two temperatures during their freshwater maturation at Eagle. Fish determined to be maturing during the first maturation sort at Eagle and Manchester were separated into control and test groups. Control fish were maintained on ambient well water that averaged approximately 13.5°C. In contrast with previous years when test fish were held on chilled water at one constant low temperature, test fish held on chilled water (averaging ≈9.0°C) now experienced water temperature changes designed to simulate those experienced by naturally migrating Chinook salmon passing up the Columbia and Snake rivers to spawning streams in the Salmon River drainage. Care was taken to ensure that the entire size range of fish present was represented in both treatment groups. Mean group weight in each treatment group was calculated for each stock and brood year. Means (within brood year) were compared by computing an estimate of the sample variance (adjusted with the finite population correction factor) and used to construct 95% confidence intervals (CI) around the means (Scheaffer et al. 1990). Fish were also assigned to size groups within brood years and treatment groups to determine if water temperature had a differential effect on spawn timing relative to body size. Fish weighing less than the group average (within brood year) were randomly assigned to either the test or the control group and were classified as “small.” Those weighing more than the group mean (within brood year) were also randomly divided between experimental groups and designated as “large.” The mean weight (and range) for each group was also reported. Mean weight differences between small and large classes of fish, in the various pairings, were not compared statistically because, by definition, the largest fish in the “small” group was smaller than the smallest fish in the “large” group. A third group of fish consisting of those determined to be maturing in the second maturation sort at Manchester (designated “late-arrivals”) were held on ambient temperature water but not included in the temperature experiment due to the different amount of time spent in freshwater compared to earlier groups.

Monitoring Programs

Hatchery Spawning and Gamete Evaluation

Typically, a small number of maturing fish from each treatment group (one stock) were retained annually at Eagle and spawned in the hatchery where eggs remain through the eyed stage of development. In addition to the date fish from each treatment group became ripe, hatchery spawning allows the comparison of egg quality (survival to the eyed stage) between the temperature groups. This is important since elevated water temperature prior to ovulation has been shown to reduce egg survival in salmonids (Pankhurst et al. 1996; Taranger and Hansen 1993; Gillet 1991). When one or more females were determined to be in spawning condition, milt was preharvested from males with the same treatment history. Ripe females were stripped of their eggs and total fecundity was estimated by calculating average egg weight from a subsample of approximately 50 eggs and dividing the total egg weight by average egg weight. Eggs from each female were divided into one to three sublots of approximately equal size depending on the number of eggs produced. Each sublot was fertilized with milt from a unique

male and placed in separate incubators (see Figure 3). Male use was subsequently equalized as each male spawns with approximately three females. The creation of multiple subfamilies increases the representation of parental genetic diversity in progeny groups (Flagg et al. 2004), and the factorial-mating design helps offset risks associated with individual sublot failure. Incubators were checked daily and opaque eggs or those with fungal growth were removed. When developing embryos had received approximately 325-350 CTUs, the eggs were shocked and those that become opaque were removed. Survival to the eyed stage was computed as the number of green eggs minus the number of dead or unfertilized eggs removed divided by the number of green eggs produced. Eyed-eggs produced from all hatchery crosses were provided to biologists with the Shoshone-Bannock Tribes for transfer to in-stream hatch boxes within the appropriate study system.

The effect of water temperature treatment on spawn timing and gamete quality was evaluated by comparing mean spawn date and mean embryo survival to the eyed stage of development in both treatment groups. The spawn date for each female was the number of days after the first female is spawned (day one). Based on these values the mean spawn date in both groups was computed, the variance of the means was estimated and adjusted with the finite population correction factor (Equation 1) for each group, and the bound on the error of estimation (Equation 2) was used to construct an approximate 95% CI around the means (Equation 3; Scheaffer et al. 1990). Statistical significance was assumed when the resulting intervals did not overlap. Embryo survival to eye-up was compared similarly. Survival was recorded for all egg-lots and a mean computed for females in both groups; the variance of the means was estimated (adjusted with the finite population correction factor) and used to construct approximate 95% CI around the means (Scheaffer et al. 1990). Significance was assumed when the intervals did not overlap.

$$\hat{V}(\bar{y}) = \frac{s^2}{n}\left(\frac{N-n}{N}\right)$$

Equation 1.

where

$$s^2 = \frac{\sum_{i=1}^{n}(y_i - \bar{y})^2}{n-1}$$

Equation 2.

$$B = 2\sqrt{\hat{V}(\bar{y})} = 2\sqrt{\frac{s^2}{n}\left(\frac{N-n}{N}\right)}$$

Equation 3.

$$\bar{y} \pm B$$

Fish Health Monitoring

The captive rearing program utilized disinfectants, antibiotics, vaccinations, and antifungal treatments to control pathogens. Dosage, purpose of use, and method of application for currently used drugs was as follows: 1) Antibiotic therapies: Erythromycin was administered orally, feeding medicated feed from Bio-Oregon to produce a dose of 100 mg/kg of body-weight. Fish were fed medicated feed for up to a 28 d period to either prevent or control BKD. When oral administration was not feasible, as with anadromous adults, an intraperitoneal injection of

erythromycin was given at a dose of 20 mg/kg of body weight. Fingerlings were fed Oxytetracycline or oxolinic acid medicated feed at a dose of 75 mg/kg of body weight for 10 d to control outbreaks of pathogenic aeromonads, pseudomonads, and myxobacteria, etc., as these cases arose. 2) Vaccinations: age-1 Chinook salmon were vaccinated prior to shipment to saltwater with intraperitoneal injections of Vibrogen (Aqua Health, Ltd., Charlottetown, PEI, Canada) to prevent *Vibrio* spp. and Renogen (Aqua Health Ltd.) to prevent BKD. 3) Egg disinfection: newly fertilized eggs were water hardened in 100 mg/L solution of Iodophor for 30 minutes to inactivate viral and/or bacterial pathogens on the egg surface and in the perivitelline space.

Fish health was checked daily by observing feeding response, external condition, and behavior of fish in each tank as initial indicators of developing problems. In particular, fish culturists looked for signs of lethargy, spiral swimming, side swimming, jumping, flashing, unusual respiratory activity, body surface abnormalities, and unusual coloration. Presence of any of these behaviors or conditions was immediately reported to the program fish pathologist. When a treatable pathogen was either detected or suspected, the program fish pathologist prescribed appropriate prophylactic and therapeutic drugs to control the problem. Dead fish were routinely analyzed for common bacterial and viral pathogens (e.g., BKD, infectious hematopoietic necrosis virus, etc.). Select carcasses may have been appropriately preserved for pathology, genetic, and other analyses. After necropsy, carcasses that were not vital to further analysis were disposed of as per language contained in the ESA Section 10 permit for the program.

Tissue samples were collected from dead program fish during necropsies to monitor for the presence of common bacterial and viral pathogens. American Fisheries Society “Bluebook” procedures were employed to isolate bacterial or viral pathogens and to identify parasite etiology (Thoesen 1994). All examinations were conducted under the direction of the program fish pathologist. Genetic samples were also collected from these fish in the event they may be needed in future mitochondrial DNA and/or nuclear DNA evaluations for Chinook salmon populations held in the program.

Spawning adults were analyzed for common bacterial and viral pathogens such as BKD, infectious hematopoietic necrosis virus, and viral hemorrhagic septicemia. Tissue samples were collected from the kidney and spleen of each fish, and ovarian fluid samples were collected from each female and analyzed at the Eagle Fish Health Laboratory. In addition, tissue from maturing Chinook salmon transferred to the State of Idaho from Manchester were screened for *Piscirickettsia salmonis*, and additional ovarian fluid was “blind passed” in a separate test for the North American strain of viral hemorrhagic septicemia. These pathogens are not currently present in Idaho but have recently been identified in fish reared at a seawater net pen location in close proximity to the Manchester site. Results of fish health analyses on spawned fish were used by IDFG and the CSCPTOC to determine the disposition of eggs and subsequent juveniles.

Growth and Survival of Completed Brood Years

Each program year, an individual brood cohort is terminated with respect to remaining live individuals of a certain age component (typically after year 5 or year 6 of culture). In order to track the contribution of individual cohorts through time, measures such as growth, sources and magnitudes of mortality, and maturation rates were evaluated for completed brood groups. Fish weights collected during routine sampling at both Eagle and Manchester were plotted over time,

and both individual fish weight and group means were presented graphically. Major sources of mortality were compiled including disease, tagging, mechanical (e.g., equipment failure), and maturation. Mortality at Eagle and Manchester were combined into a single analysis. Finally, we determined the total number of brood year program fish from each study stream that reached sexual maturity and computed the percentage that matured at age-2, -3, -4, and -5.

Volitional Spawning

Fish weirs were utilized in study streams receiving mature Chinook salmon from the captive-rearing program to assess spawning behavior and success in a natural environment. The components of a weir were transported to a construction site and assembled at the downstream end of a given section to ensure that project fish remained in the study area above the weir. Trap boxes built into the weir allowed wild Chinook salmon and other species to pass in either direction. Generally, study sections were divided into multiple reaches of varying lengths to permit systematic observations of Chinook salmon spawning above the weir. Thermographs were used to document the thermal histories of redds spawned by captive-reared individuals and provided a means to accurately determine when redds should be sampled to determine fertilization rates and survival to the eyed-egg stage of development.

Following weir construction, maturing captive-reared Chinook salmon were transported by truck from Eagle to a streamside site in preparation for release into the study section. Water temperature in the transport tank varied with respect to the stream temperature they were released into and represented a compromise temperature appropriate for the transport of both study groups. Fish were then released at various sites with the aid of a helicopter (WFYF) or transferred on foot (EFSR). Fish transported by helicopter were transferred to insulated coolers filled with water from the transport tank. The coolers were secured inside specially constructed steel frames (Figure 4) for transport under the helicopter during the approximately 2 km flight to the release site. Transport frames were secured to the helicopter with a 30.5 m steel cable (Figure 4). Fish transported and released on foot were transferred to either water-filled coolers or specially constructed, water-filled slings that were then carried to the release site.

Behavioral data collection began approximately 24 h after fish were released. Field observers were assigned stream reaches within the study section, and the entire study section was monitored daily. Observers walked slowly upstream watching for Chinook salmon. When one was encountered, the time was recorded, and the fish's habitat associations and behavior activities (Table 1) were observed and documented for 5 min. During this time, the observer also used binoculars and polarized sunglasses to determine if it was a wild or a study fish based on the presence or absence of a disc or Floy tag. If it was a study fish, the identification color combination and/or number of the tag was recorded. If the number could be determined (or the fish was wild), its location was recorded on a global positioning system (GPS) receiver. When multiple fish were observed simultaneously, their activity, habitat, and location information were recorded separately.

When spawning related behaviors were observed during the first 5 min of observation, additional time was spent recording the frequency of these behaviors to estimate how close the pair was to spawning. If, based on these frequencies, the observer believed spawning would occur within 1-2 h, the observer remained with that pair and recorded their behaviors until 30 min after spawning. Behavioral observations were recorded in 10 min blocks during this time to facilitate comparisons of courting, aggression, and digging frequencies as spawning approached.

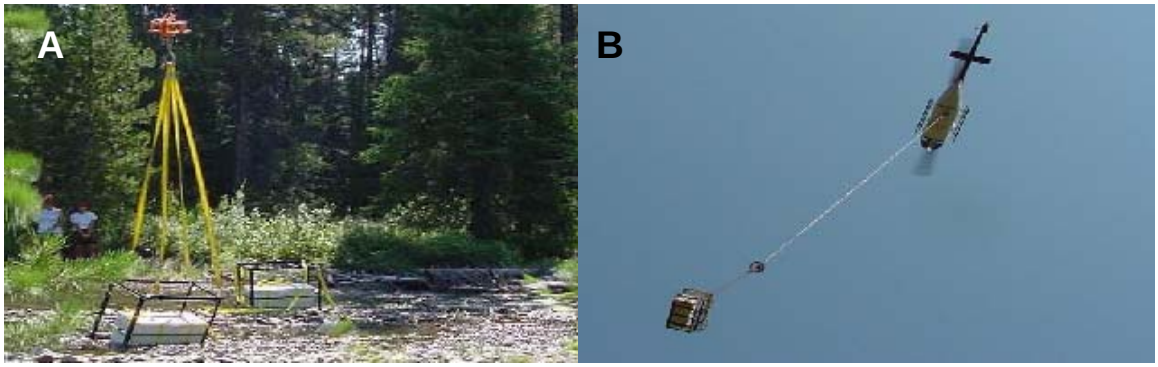

Figure 4. Equipment used to fly mature adult Chinook salmon into the West Fork Yankee Fork Salmon River for volitional spawning. A) Steel-frame cages with coolers securely fastened inside. B) Helicopter with synthetic cable carrying a steel-frame cage.

Table 1. Habitat and behavior variables recorded during observations of captive-reared Chinook salmon.

Habitat	**Definition**
Overhead vegetation	Associated with riparian vegetation overhanging the stream
Aquatic vegetation	Associated with aquatic vegetation
Cut bank	Under an overhanging bank
Pool	In a pool with no other structure
Riffle or run	In a riffle or run with no other structure
Riffle tail-out	In the tail-out section of a riffle with no other structure
Large woody debris	Within one body length of log(s)
General Behavior	**Definition**
Holding	Remaining in one position
Milling	Movement not resulting in displacement
Moving (A)	Movement in an upstream direction
Moving (B)	Movement in a downstream direction
Aggression	Aggression between Chinook of undetermined sex
Redd Holding	Maintaining position on or near a redd
Courting	Active male and receptive female
Spawn	Observed release of eggs and milt
Male Behavior	**Definition**
Quiver	Dart toward female ending with body vibrations
Crossover	Movement to opposite side, head passing over peduncle
Aggression (A)	Male on male aggression
Aggression (B)	Male on female aggression
Aggression (C)	Male on other species aggression
Following	Female present, no redd
Satellite	Holding away or downstream of a courting pair
Female Behavior	**Definition**
Aggression (A)	Female on female aggression
Aggression (B)	Female on male aggression
Aggression (C)	Female on other species aggression
Test dig	2–6 body flexures, not concentrated
Nest dig	5–8 body flexures in a concentrated area
Cover dig	8–12 body flexures along redd perimeter

Production Estimation

Chinook salmon parr were collected from streams that had previously received eyed-eggs or adults from the captive rearing program to obtain fin clips for genetic analysis to determine if the juveniles were the product of program parents. Parr were collected by snorkelers using aquarium dip-nets (Bonneau et al. 1995) throughout the study section, although particular emphasis was given to areas near known spawning locations of captive adults or egg-box incubators from the previous year. Once captured, the parr were transferred to tubs filled with fresh streamwater and lightly anesthetized with MS-222. A small portion of the anal fin was removed and preserved in 95% ethanol. Scissors used to remove fin tissues were swabbed with isopropyl alcohol between specimens to reduce the possibility of DNA cross-contamination. The fish were also measured to the nearest 1 mm FL before being placed into a tub of fresh stream water to recover. Parr were then released back into the stream near their point of collection. Microsatellite markers will be utilized to conduct parentage analysis (parental exclusion analysis; Colbourne et al. 1996; Talbot et al. 1996; Estoup et al. 1998; Bernatchez and Duchesne 2000; Eldridge et al. 2002) to determine the reproductive success of captive-reared adults (released for volitional spawning in the previous year) as well as in-stream incubator production (eggs produced from hatchery spawning and planted in the previous year) in terms of F_1 progeny (parr collections). Genetic analysis is currently ongoing and will be reported as results become available.

RESULTS AND DISCUSSION

Brood Year Report Outline

The following acronyms are used in the following section of the report to describe culture groups: NP refers to “natural parr” or fish collected from natal streams as wild parr, SN refers to “safety net” or fish generated from hatchery spawning events, and NE refers to “natural egg” or fish generated from the collection of eyed-eggs from redds constructed by wild adults.

Brood Year 1998

At the beginning of the reporting period, zero brood year 1998 Chinook salmon were in culture at Eagle. One (male) maturing EFSR-SN, 21 (16 females/4 males/1 unknown) maturing EFSR-NP, 17 (12 females/1 male/4 unknown) maturing LEM-NP and 14 (7 females/2 males/5 unknown) maturing WFYF-NP adults were transferred to Eagle from Manchester on May 6, May 7, and June 10, 2003 to complete their maturation in freshwater. Maturing LEM-NP (6 females/1 male/4 unknown) adults were released to the LEM on August 4, 2003, while maturing adults from the EFSR-SN (1 male), EFSR-NP (4 females/2 males/1 unknown) and WFYF-NP (5 females/2 males/5 unknown) were released for natural spawning and spawning observation studies on August 5 (EFSR) and August 6 (WFYF), 2003. Eleven maturing EFSR-NP (10 females/1 male) adults were used for hatchery spawning in 2003. At the end of the reporting period, zero BY98 captive Chinook (all stocks) remained in culture at Eagle (Tables 2, 3, 4).

Brood Year 1999

At the beginning of the reporting period, one EFSR-SN, six LEM-NE and four WFYF-SN brood year 1999 Chinook salmon were in culture at Eagle. Twenty-one (14 females/7 males) maturing EFSR-SN, 18 (16 females/2 males) maturing EFSR-NE, 35 (28 females/4 males/3 unknown) maturing LEM-NE, and 63 (47 females/16 males) maturing WFYF-SN adults were transferred to Eagle from Manchester on May 6, May 7 and June 10, 2003 to complete their maturation in freshwater. Maturing LEM-NP (29 females/4 males/4 unknown) adults were released to the LEM on August 4, 2003, while maturing adults from the EFSR-SN (7 females/5 males), EFSR-NE (7 females/1 male) and WFYF-NP (47 females/16 males/1 unknown) were released for natural spawning and spawning observation studies on August 5 (EFSR) and August 6 (WFYF), 2003. Nine maturing EFSR-SN (7 females/2 males) and nine maturing EFSR-NE (8 females/1 male) adults were used for hatchery spawning in 2003. At the end of the reporting period, zero BY99 captive Chinook (all stocks) remained in culture at Eagle (Tables 2, 3, 4).

Brood Year 2000

At the beginning of the reporting period, zero brood year 2000 Chinook salmon were in culture at Eagle. Forty-one (2 females/32 males/7 unknown) maturing EFSR-NE and 12 (8 females/4 males) maturing WFYF-NE adults were transferred to Eagle from Manchester on May 6, May 7 and June 10, 2003 to complete their maturation in freshwater. Captive Chinook cohorts were not collected in the Lemhi River system in calendar year 2000, with BY99 LEM cohorts (above) representing the final collection year for the Lemhi River stock. Maturing EFSR-NE (0 females/12 males/1 unknown) and WFYF-NE (8 females/4 males) adults were released for natural spawning and spawning observation studies on August 5 (EFSR) and August 6 (WFYF), 2003. Twenty-two maturing EFSR-NE (2 females/20 males) adults were used for hatchery spawning in 2003. At the end of the reporting period, zero BY00 captive Chinook (both stocks) remained in culture at Eagle (Tables 2, 4).

Brood Year 2001

At the beginning of the reporting period, 285 EFSR-NE and 257 WFYF-NE brood year 2001 presmolts were in culture at Eagle. On May 5, 2003, 285 EFSR-NE and 257 WFYF-NE BY01 Chinook smolts were transferred from Eagle to Manchester to complete rearing in saltwater (Tables 2, 4, 5). Brood year 2001 precocial males were not utilized in spawning or cryopreservation events in 2003. At the end of this reporting period, zero EFSR-NE and zero WFYF-NE captive Chinook remained in culture at Eagle (Tables 2, 4).

Brood Year 2002

At the beginning of the reporting period, 324 EFSR-NE and 277 WFYF-NE brood year 2002 fry were in culture at Eagle. At the end of the reporting period, 320 EFSR-NE and 272 WFYF-NE presmolts were on station at Eagle (Tables 2, 4).

Brood year 2003

Eyed-egg collections in 2003 resulted in an initial inventory of 319 EFSR-NE and 338 WFYF-NE eyed-eggs. At the end of the reporting period, 306 EFSR-NE and 306 WFYF-NE developing fry were in culture.

Table 2. Summary of losses and magnitude of mortality for eight East Fork Salmon River captive Chinook salmon culture groups reared at IDFG facilities in 2003. Culture groups are designated by brood year (BY) and by the method by which the group was sourced (NP = natural parr, SN = safety net, and NE = natural egg).

	Culture Groups							
	BY98 SN	**BY98 NP**	**BY99 SN**	**BY99 NE**	**BY00 NE**	**BY01 NE**	**BY02 NE**	**BY03 NE**
Starting Inventory (January 1, 2003)	0	0	1	0	0	285	324	319[a]
Eyed-Egg to Fry Undetermined[b]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	13
Mechanical Loss								
Handling	0	0	0	0	0	0	0	0
Jump-out	0	0	0	0	0	0	0	0
Transportation	0	0	0	0	0	0	0	0
Noninfectious								
Lymphosarcoma	0	0	0	0	0	0	0	0
Nephroblastoma	0	0	0	0	0	0	0	0
Other[c]	0	3	1	1	6	0	4	0
Infectious								
Bacterial	0	0	0	0	0	0	0	0
Viral	0	0	0	0	0	0	0	0
Other	0	0	0	0	0	0	0	0
Hatchery Spawning								
Male Spawners	0	1	2	1	20	0	0	0
Female Spawners	0	10	7	8	2	0	0	0
Cryopreservation	0	0	0	0	0	0	0	0
Relocation								
Transferred In	1	21	21	18	41	0	0	0
Transferred Out	0	0	0	0	0	285	0	0
Planted/Released	1	7	12	8	13	0	0	0
Ending Inventory (December 31, 2003)	0	0	0	0	0	0	320	306

[a] Fall 2003 inventory.
[b] Typical egg to fry mortality includes non-hatching eggs, abnormal fry, and swim-up loss.
[c] Includes mortality due to maturation; culling associated with cultural anomalies; and all undetermined, noninfectious mortality.

Table 3. Summary of losses and magnitude of mortality for two Lemhi River captive Chinook salmon culture groups reared at IDFG facilities in 2003. Culture groups are designated by brood year (BY) and by the method by which the group was sourced (NP = natural parr, and NE = natural egg).

	Culture groups	
	BY98-NP	**BY99-NE**
Starting Inventory (January 1, 2003)	0	6
Eyed-Egg to Fry		
Undetermined	n/a	n/a
Mechanical Loss		
Handling	0	0
Jump-out	0	0
Transportation	0	0
Noninfectious		
Lymphosarcoma	0	0
Nephroblastoma	0	0
Other[a]	6	4
Infectious		
Bacterial	0	0
Viral	0	0
Other	0	0
Hatchery Spawning		
Male Spawners	0	0
Female Spawners	0	0
Cryopreservation	0	0
Relocation		
Transferred In	17	35
Transferred Out	0	0
Planted/Released	11	37
Ending Inventory (December 31, 2003)	0	0

[a] Includes mortality due to maturation; culling associated with cultural anomalies; and all undetermined, noninfectious mortality.

Table 4. Summary of losses and magnitude of mortality for six West Fork Yankee Fork Salmon River captive Chinook salmon culture groups reared at IDFG facilities in 2003. Culture groups are designated by brood year (BY) and by the method by which the group was sourced (NP = natural parr, SN = safety net, and NE = natural egg).

	Culture Groups					
	BY98 NP	**BY99 SN**	**BY00 NE**	**BY01 NE**	**BY02 NE**	**BY03 NE**
Starting Inventory (January 1, 2003)	0	4	0	257	277	338[a]
Eyed-Egg to Fry						
Undetermined[b]	n/a	n/a	N/a	n/a	n/a	32
Mechanical Loss						
Handling	0	0	0	0	0	0
Jump-out	0	0	0	0	0	0
Transportation	0	0	0	0	0	0
Noninfectious						
Lymphosarcoma	0	0	0	0	0	0
Nephroblastoma	0	0	0	0	0	0
Other[c]	2	3	0	0	5	0
Infectious						
Bacterial	0	0	0	0	0	0
Viral	0	0	0	0	0	0
Other	0	0	0	0	0	0
Hatchery Spawning						
Male Spawners	0	0	0	0	0	0
Female Spawners	0	0	0	0	0	0
Cryopreservation	0	0	0	0	0	0
Relocation						
Transferred In	14	63	12	0	0	0
Transferred Out	0	0	0	257	0	0
Planted/Released	12	64	12	0	0	0
Ending Inventory (December 31, 2003)	0	0	0	0	272	306

[a] Fall 2003 inventory.
[b] Typical egg to fry mortality includes non-hatching eggs, abnormal fry, and swim-up loss.
[c] Includes mortality due to maturation; culling associated with cultural anomalies; and all undetermined, noninfectious mortality.

Eyed Egg Collection, Transport, and Incubation

Naturally spawned, eyed-eggs were collected from the EFSR and the WFYF to establish captive culture groups representing brood year 2003. Eyed-eggs were collected from five redds in the EFSR on September 25, 2003 and from six redds on the WFYF on September 27, 2003. Collections totaled 319 eyed-eggs from the EFSR and 338 from the WFYF (Table 5). The eyed-eggs were transported to Eagle as soon as possible after collection and were in incubators within 4-6 h of removal from the redds. Percent survival to ponding was 95.9% for the EFSR eggs and 90.5% for the WFYF eggs.

Eyed-eggs were produced at Eagle when maturing EFSR program fish were spawned to assess the effect of water temperature on maturation timing and gamete quality. Twenty-seven females (13 test and 14 control; 26 Manchester and 1 Eagle reared) and 24 males (23 Manchester and 1 Eagle reared) were used in these crosses (Appendix A). Eggs were incubated by subfamily at approximately 9.0°C. Incubators were checked daily, and dead eggs were removed and enumerated from each incubator. At approximately 280 CTUs, the eggs had developed a soft eye and were shocked. When eggs had accumulated approximately 325 CTUs, they were transferred to in-stream incubators operated by personnel from the Shoshone-Bannock Tribes.

Table 5. Summary of number of eyed-eggs collected and estimated Celsius temperature units (CTU) at collection in the East Fork Salmon River (EFSR) and the West Fork Yankee Fork Salmon River (WFYF) to establish brood year 2003 culture groups at the Eagle Fish Hatchery.

		Stream	Redd 1	Redd 2	Redd 3	Redd 4	Redd 5	Redd 6
Collection date	9/25/03	EFSR						
	Eyed-eggs		43	110	47	29	90	—
	CTU		374	331	385	374	292	
Collection date	9/20/02	WFYF						
	Eyed-eggs		62	22	23	58	127[a]	46
	CTU		380	347	339	349	362	292

[a] Eggs were collected twice from this redd.

Juvenile Rearing, Marking, and Transportation

On March 25, 2003, brood year 2001 juveniles from the WFYF and EFSR were marked with an elastomer tag and vaccinated against BKD (Renogen) and vibrio (Vibrogen) in preparation for transfer to Manchester. Juveniles from the WFYF averaged 155.6 mm FL and 47.2 g (N = 257, range 120–194 mm FL and 19–190 g), and those from the EFSR averaged 150.8 mm FL and 42.9 g (N = 285, range 118–190 mm FL and 21–87.5 g). Improved feed rationing and the availability of chilled water at Eagle during incubation and early rearing resulted in smolts that were closer in size to their wild counterparts than in 2002 (Venditti et al. 2003b).

Brood year 2001 juvenile Chinook salmon were transferred from Eagle to Manchester as smolts on May 5, 2003. Smolts transferred between facilities included 285 fish from the EFSR-NE group and 257 fish from the WFYF-NE group (Appendix B). All brood year 2001 program fish were transferred to Manchester, and precocially maturing males were culled at Manchester.

Two culture groups of juvenile Chinook salmon representing brood year 2002 (N = 592) were PIT tagged on December 15, 2003. A total of 320 EFSR fish and 272 WFYF fish were PIT tagged at that time. The length and weight of brood year 2002 juveniles were larger than in previous years (Venditti et al., 2003a, b). This larger size was to be expected as these juveniles were tagged approximately six months later than were previous cohorts. Fish from the WFYF averaged 116 mm FL and 19.0 g (range 825–144 mm FL, 6.8–36.6 g), while EFSR fish averaged 109 mm FL and 15.8 g (range 81–172 mm FL, 6.4–64.5 g).

Adult Rearing, Marking, and Transportation

Adult Chinook salmon from the WFYF, EFSR, and LEM stocks determined to be maturing at Manchester were transferred to Eagle on two occasions in 2003. The first groups were transported on May 6 and 7 and included fish from brood years 1998, 1999, and 2000. Two hundred thirty-one maturing adults were transferred at that time, including 52 fish from the LEM, 83 from the WFYF, and 96 from the EFSR (Appendix B). Adults that were determined to be maturing during a second sort were transferred on June 10 and contained six WFYF individuals (three brood year 1999 and three brood year 2000) and six brood year 2000 individuals from the EFSR (Appendix B).

Maturing fish were disc tagged at Eagle on July 22 and July 23, 2003 in preparation for release into their natal streams (Appendix C). A total of 129 fish were tagged during the two days. Eight brood year 1998 adults averaging 998 g (range 563–1,695 g), 20 brood year 1999 adults averaging 1,337 g (range 748–2,444 g), and 13 brood year 2000 adults averaging 1,199 g (range 414–1,653 g) were tagged from the EFSR. Twelve brood year 1998 adults averaging 3,357 g (range 985–5,037 g), 64 adults from brood year 1999 averaging 2,008 g (range 628–4,090 g), and 12 brood year 2000 adults averaging 1,555 g (range 634–2,446 g) were tagged from the WFYF stock.

Maturing LEM adults were Floy tagged on July 23, 2003 in preparation for release. Forty-eight adult Chinook salmon from this stock were tagged including 11 brood year 1998 individuals averaging 1,673 g (range 1,374–1,773 g) and 37 from brood year 1999 averaging 1,684 g (range 1,371–1,775 g).

Chilled Water Experiment

Experimental groups of fish exposed to the two temperature treatments experienced an average difference of 4.7°C during their freshwater maturation period at Eagle. Water temperature in the test tanks averaged 9.1°C (range 6.4°C–13.9°C), while water temperature in control tanks averaged 13.8°C (range 9.4°C–17.7°C; Figure 5).

Mean fish weight in the two temperature treatment groups were evenly divided for all brood years. Analysis of treatment classifications showed no size differences between experimental groups within brood years (Figure 6). However, mean weights and range are

presented for the various treatment and size groups within the temperature groups for illustrative purposes (Table 6).

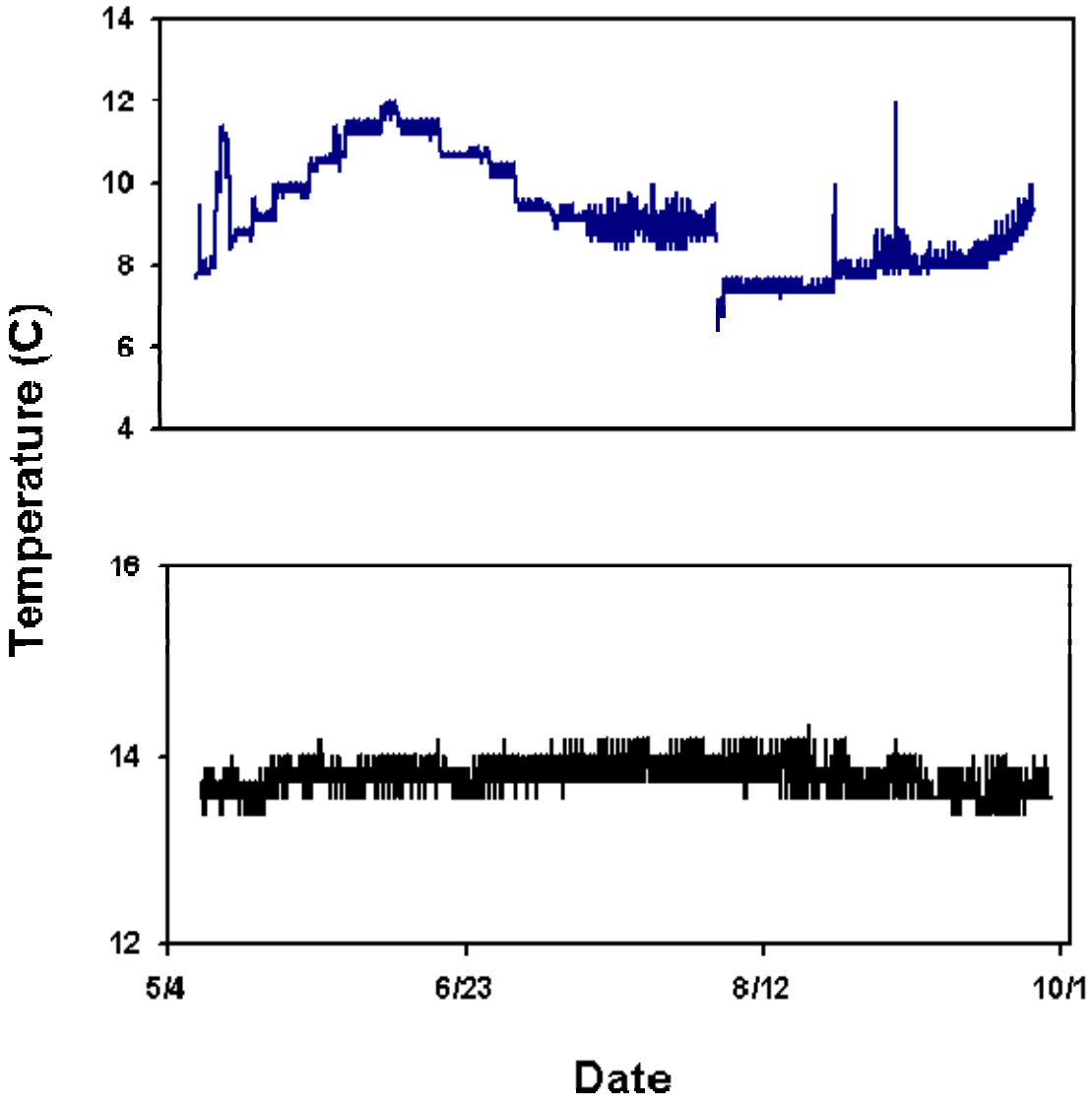

Figure 5. Chilled (upper pane) and ambient (lower pane) tank water temperatures experienced by maturing captive-reared Chinook salmon at the Eagle Fish Hatchery during their final freshwater maturation, May–October 2003.

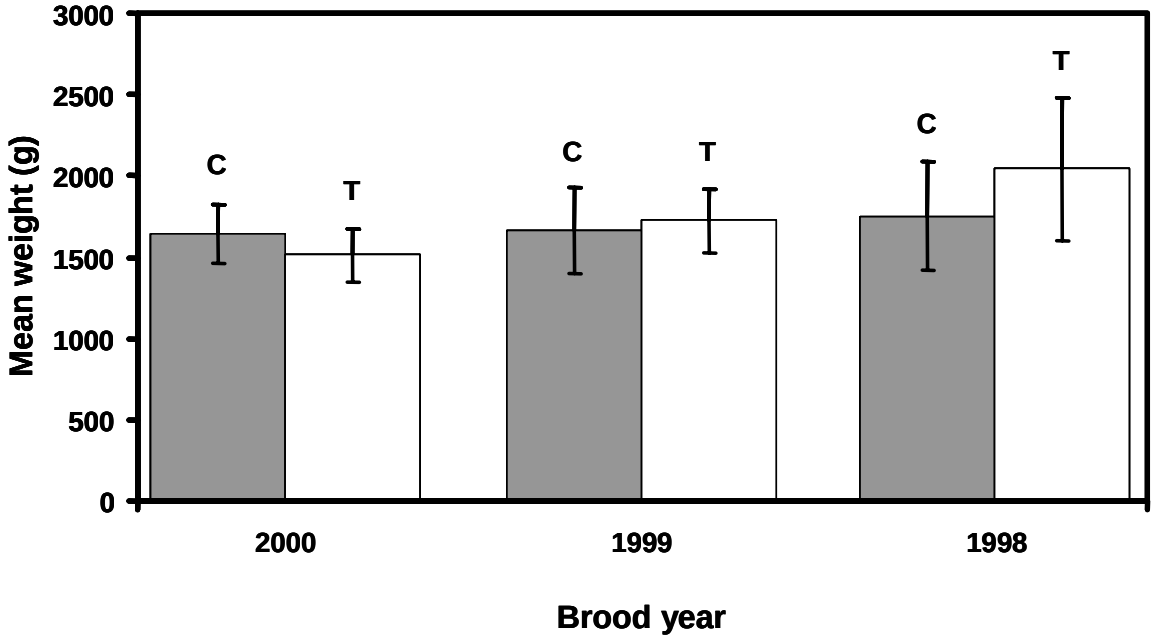

Figure 6. Mean weight (± 95% CI) of captive-reared Chinook salmon held on ambient (C) and chilled (T) water at the Eagle Fish Hatchery during 2003.

Hatchery Spawning and Gamete Evaluation

Maturing program fish from the EFSR stock were spawned at Eagle to assess the effect of water temperature on maturation timing and gamete quality. Twenty-seven females (26 Manchester and 1 Eagle reared) and 24 males (23 Manchester and 1 Eagle reared) were used in these crosses (Appendix A). The average spawn date (determined by the ordinal date based on the first female to ripen in either group) for females in the treatment temperature group (23.4 ± 4.68 d) was significantly earlier than for females in the control group (32.7 ± 3.62 d; Figure 7). Survival to the eyed stage was variable in both temperature treatment groups and averaged 46.6% overall (Appendix A) but did not differ statistically (Treatment: mean = 62.8 ± 13.0%, range 0.0%-95.2%; Control: mean = 34.9 ± 12.9%, range 1.1%-91.4%; Figure 8). When the eggs had reached the eyed stage of development, they were transferred from Eagle to in-stream incubators in the EFSR drainage on October 6 and 20 and November 10, 2003. Tribal cooperators received 1,539 eyed-eggs on the first date, 4,593 on the second, and 10,022 eggs on the third (16,154 total eyed). After distributing the eggs, Tribal biologists monitored the incubators to evaluate the hatch and emergence rates and dates.

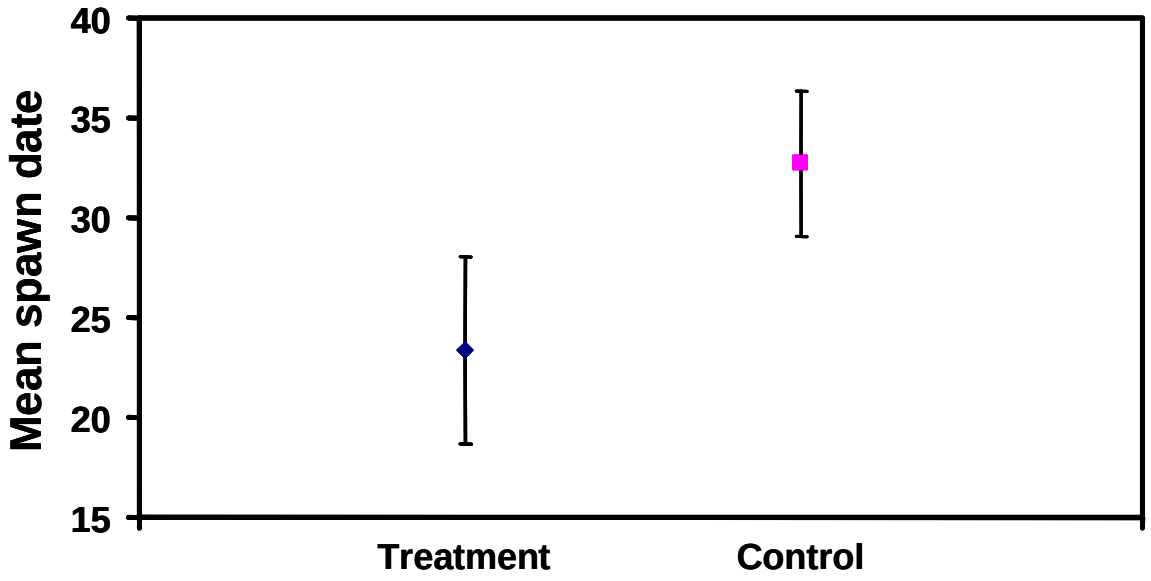

Figure 7. Mean spawn date (± 95% CI) in two groups of captive-reared Chinook salmon. Spawn date was determined by the ordinal date based on the first female to become ripe in either group. The treatment group was held on a chilled water regime that simulated natural in-river temperatures. The control group was held on a stable regime of ambient water at the Eagle Fish Hatchery (≈13.5°C).

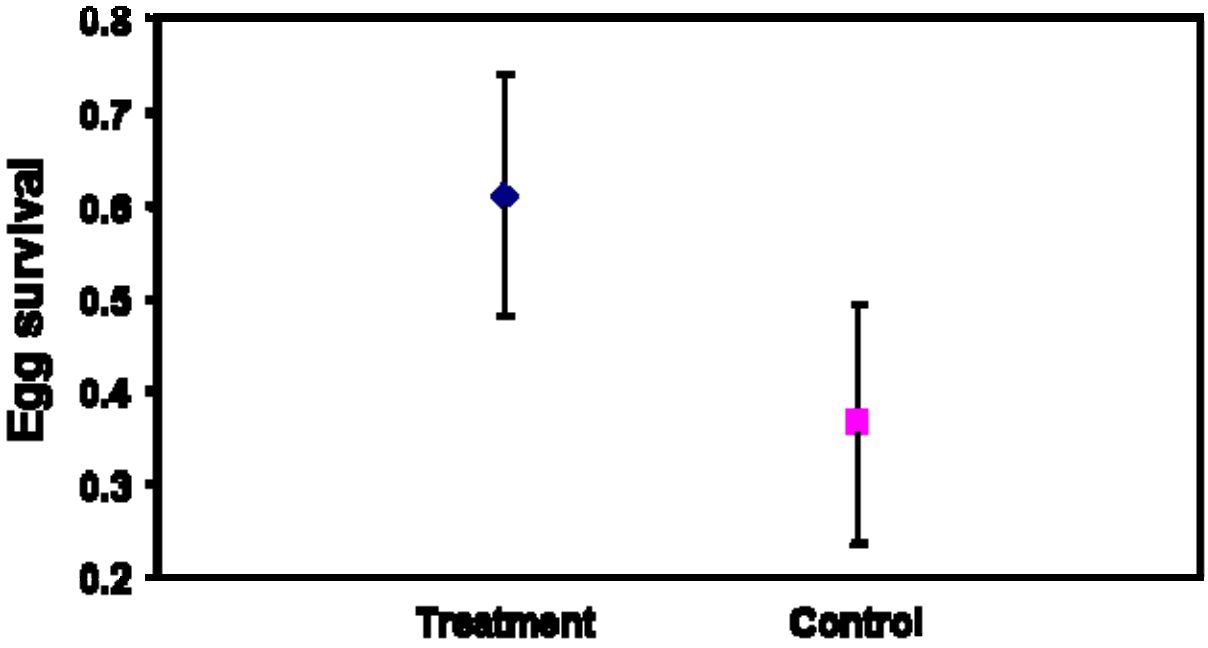

Figure 8. Mean egg survival (± 95% CI) to the eyed stage of development produced by two groups of captive-reared Chinook salmon. The treatment group was held on chilled water that simulated natural in-river temperatures. The control group was held on a stable regime of ambient water at the Eagle Fish Hatchery (≈13.5°C).

Table 6. Comparisons of mean and range of weights in East Fork Salmon River (EFSR) and West Fork Yankee Fork Salmon River (WFYF) captive-reared Chinook salmon from brood years (BY) 1998, 1999, and 2000. Fish were randomly assigned to either chilled (T) or ambient (C) water and designated either large (L) or small (S) depending on size relative to their overall group mean weight. Block one contains weights of treatment groups within brood years. Block two contains weights in size classes within brood years. Block three contains weights in treatment groups within brood year and size classes.

BY Stock	Group	Size	N	Mean wt. (g)	Range (g)
1998 EFSR	C		13	1,752.7	563–3,088
	T		9	2,042.7	1,029–3,737
1999 EFSR	C		19	1641.5	748–3,281
	T		22	1,768.4	666–3,255
2000 EFSR	C		16	1,642.6	695–2,732
	T		21	1,474.2	721–2,348
1998 WFYF	C		6	3,356.8	2,334–5,037
	T		7	3,256.0	985–5,023
1999 WFYF	C		29	2,159.7	1,045–3,650
	T		31	2,040.8	731–4,090
2000 WFYF	C		4	1,598.7	914–2,146
	T		5	1,566.4	634–2,446
1998 EFSR		L	10	2,694.7	1,880–3,737
		S	12	1,185.2	563–1,723
1999 EFSR		L	18	2,357.6	1,746–3,281
		S	23	1,202.5	666–1,680
2000 EFSR		L	17	1,991.6	1,607–2,732
		S	20	1,169.2	695–1,538
1998 WFYF		L	6	4,665.3	3,622–5,037
		S	8	2,514.0	985–3,380
1999 WFYF		L	34	2,554.7	2,113–4,090
		S	29	1,437.8	731–2,064
2000 WFYF		L	5	1,999.8	1,582–2,446
		S	4	1,057.0	634–1,480
1998 EFSR	C	L	6	2,633.2	1,880–3,088
	T	L	4	2,787.0	2,223–3,737
1998 EFSR	C	S	7	998.0	563–1,723
	T	S	5	1,447.2	1,029–1,710
1999 EFSR	C	L	7	2,392.9	2,009–3,281
	T	L	11	2,335.1	1,746–3,255
1999 EFSR	C	S	12	1,203.3	748–1,585
	T	S	11	1,201.6	666–1,680
2000 EFSR	C	L	8	2,019.4	1,634–2,732
	T	L	9	1,966.9	1,607–2,348
2000 EFSR	C	S	8	1,265.9	695–1,498
	T	S	12	1,104.7	721–1,538
1998 WFYF	C	L	2	4,841.0	4,645–5,037
	T	L	4	4,577.5	3,622–5,023
1998 WFYF	C	S	4	2,614.8	2,334–3,010
	T	S	4	2,413.3	985–3,380
1999 WFYF	C	L	17	2,523.2	2,150–3,650
	T	L	17	2,586.2	2,113–4,090
1999 WFYF	C	S	15	1,493.0	1,045–2,060
	T	S	14	1,378.6	731–2,064
2000 WFYF	C	L	3	1,827.0	1,582–2,136
	T	L	2	2,259.0	2,072–2,446
2000 WFYF	C	S	1	914.0	—
	T	S	3	1,104.7	634–1,480

Fish Health Monitoring

Juvenile Chinook salmon from brood year 2001 culture groups destined for transfer to Manchester for saltwater rearing received intraperitoneal injections to provide a measure of protection from BKD and Vibrio spp. A total of 285 fish from the EFSR and 258 WFYF juveniles were vaccinated on March 25, 2003.

In 2003, 43 laboratory accessions (representing 67 fish) were generated at the Eagle Fish Health Laboratory for captive-reared Chinook salmon. Cause of mortality and magnitude of loss for Chinook salmon maintained at Eagle during this reporting period are presented in Tables 2 through 4.

Maturing Chinook salmon transferred to the State of Idaho from Manchester were screened for the North American strain of viral hemorrhagic septicemia (NA VHS). There was no evidence of the virus demonstrated from the routine or "blind passed" procedures described above.

Monitoring for BKD in captive-reared Chinook salmon has been routinely conducted since the inception of the program in 1995. In 2003, 65 of 67 juveniles that died at Eagle were tested for this disease using an enzyme-linked immunosorbent assay (ELISA). None of these produced ELISA values reflecting clinical levels of BKD. This is the second year of not detecting BKD in juvenile Chinook salmon at Eagle and reflects the transition to sourcing brood groups by safety net or eyed-eggs in lieu of natural parr. Erythromycin medicated feed for a 21-day duration was administered once as an additional prophylactic treatment to BY01 groups prior to saltwater transfer.

In 2003, brood year 1998 LEM Chinook salmon juveniles were not found to be infested with the gill parasite *Salmincola*, indicating that the gastric intubation treatment with the parasiticide Ivermectin and the shift from juvenile to eyed-egg collections was successful in eliminating this fish health concern. In years prior to 2000, this infestation debilitated rearing groups of LEM Chinook salmon.

Naturally produced juvenile Chinook salmon from the LEM (and to a lesser extent the WFYF & EFSR) are known to be exposed to *M. cerebralis*. The prevalence of *M. cerebralis* infection in LEM captive-rearing groups was 17% in 2003 (1/6 tested positive), which is lower than previously observed and reflects the benefits of originating broodstocks from eyed-eggs. All WFYF and EFSR Chinook adults tested for *M. cerebralis* in 2003 tested negative for this parasite. In past years, mortality has not been attributed to the parasite, but occasional deformities have been observed.

No captive-reared females constructed redds in the WFYF in 2003 year due to what appears to have been complete prespawn mortality. Nearly 80% of all fish released into the WFYF were recovered as carcasses by September 6, 2003, and no live fish were observed during stream observations on or after that date. There were no distinguishing irregularities between brood year or treatment group mortalities that would identify one group as being predominately affected. However, observers noted that in contrast to previous years, program fish began to display fungal infections (*Saprolegnia* spp.) on the head and peduncle within days of release. Kidney and spleen samples were collected from five carcasses (N = 3 BY1999, control; N = 1 BY2000, control; and N = 1 BY1998, treatment) and sent to the IDFG Eagle Fish Health Laboratory. Results indicated clinical levels of infection of *Aeromonas* spp. in all

samples. *Aeromonas* infections are ubiquitous throughout freshwater fish species (Piper et al. 1982), and fungus outbreaks are a common secondary response to an underlying stressor. Unfortunately, this underlying stressor has not been positively identified.

Growth and Survival of Brood Year 1998

Growth rate comparisons of brood year 1998 captive-reared Chinook salmon indicated that those from Manchester attained a larger size than those reared at Eagle. Sample weights collected from fish at Eagle in August of 2000, 2001, and 2002 show that program fish averaged 127.5 g, 970.9 g, 2,037.4 g, respectively (Figure 9). There were no brood year 1998 fish in culture at Eagle during 2003. Sample weights collected at Manchester at approximately the same times indicated that fish there were consistently larger than those at Eagle. Average weights of program fish at Manchester were 145.5 g, 1,502.6 g, 2,524.7 g, and 2,415.6 g in August of 2000, 2001, 2002, and 2003, respectively (Figure 10). Chinook salmon reared at Manchester exhibited little growth during their fifth year of life, which is consistent with previous observations (Venditti et al. 2002, 2003a, b), and were similar in size to those released at age-4 (Figure 10).

General sources of mortality in this cohort were similar to those observed previously (Hassemer et al. 2001; Venditti et al. 2002, 2003a, b), although losses to BKD were much lower than for some previous brood years (Venditti et al. 2003b). Primary sources of mortality in this group included unexplained tank deaths, maturation, and handling (Figure 11). Mortality due to handling (including transportation) increased substantially in brood year 1998 compared to earlier brood years. Following smolt transfers from Eagle to Manchester in 2000, 97 immature Chinook salmon died within one week after transport. Although myxobacteria and/or *Ichthyobodo* (*Costia*) were isolated from some carcasses, these infections are believed to be a secondary infection in response to an underlying stressor. Mortality in the EFSR-SN continued until December 2000, with only 19.2% survival in this group after transfer to saltwater. Despite intense pathological examinations, no infectious agents were isolated as the cause of death (C. McAuley, NOAA Fisheries Manchester Research Station, personal communication). Immediately after a maturation sort at Eagle on June 14, 2001, 23 juvenile and one mature Chinook salmon died, apparently from the stresses associated with this activity. After inspection by pathologists at the IDFG Eagle Fish Health Laboratory, these fish were identified as being infected with a motile aeromonad septicemia (MAS) bacteria, although they did not display any clinical signs of MAS. Activities associated with the maturation sort (i.e., crowding, handling, and tank movements) likely exacerbated the situation to the point where the fish were unable to handle the additional stress.

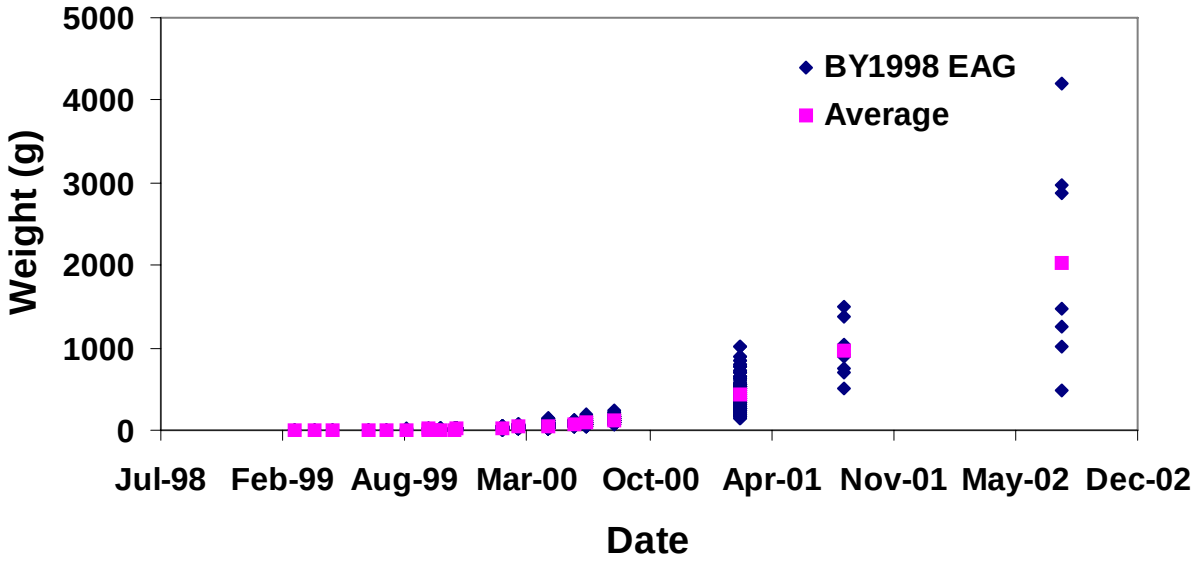

Figure 9. Growth data for brood year 1998 fish reared in freshwater at the Eagle Fish Hatchery during their duration in the captive rearing program.

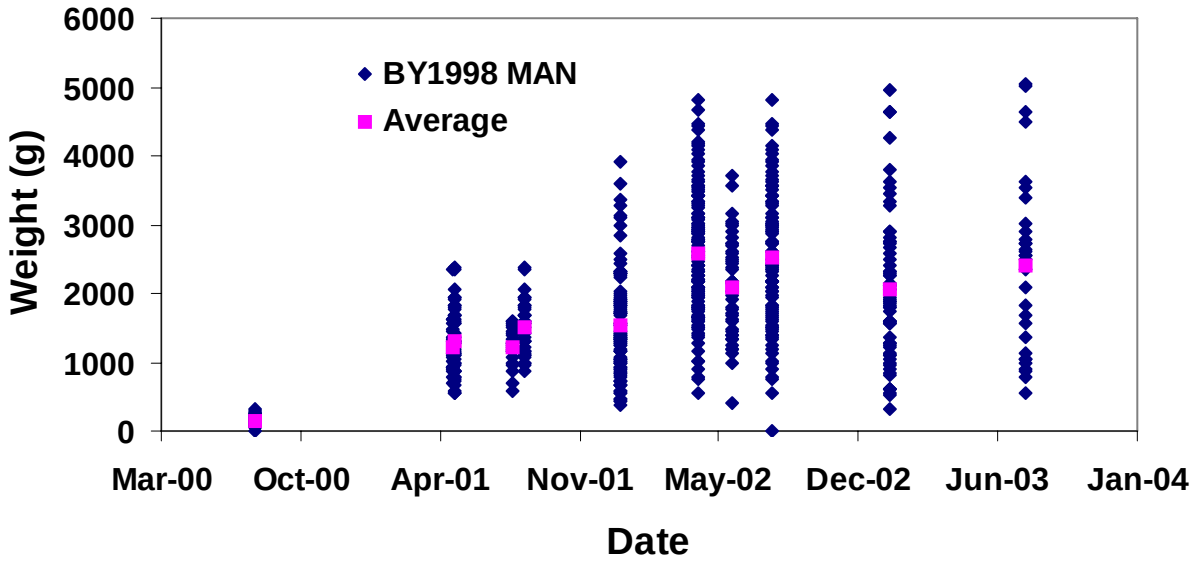

Figure 10. Growth data for brood year 1998 fish reared in saltwater at the Manchester Research Station during their duration in the captive rearing program.

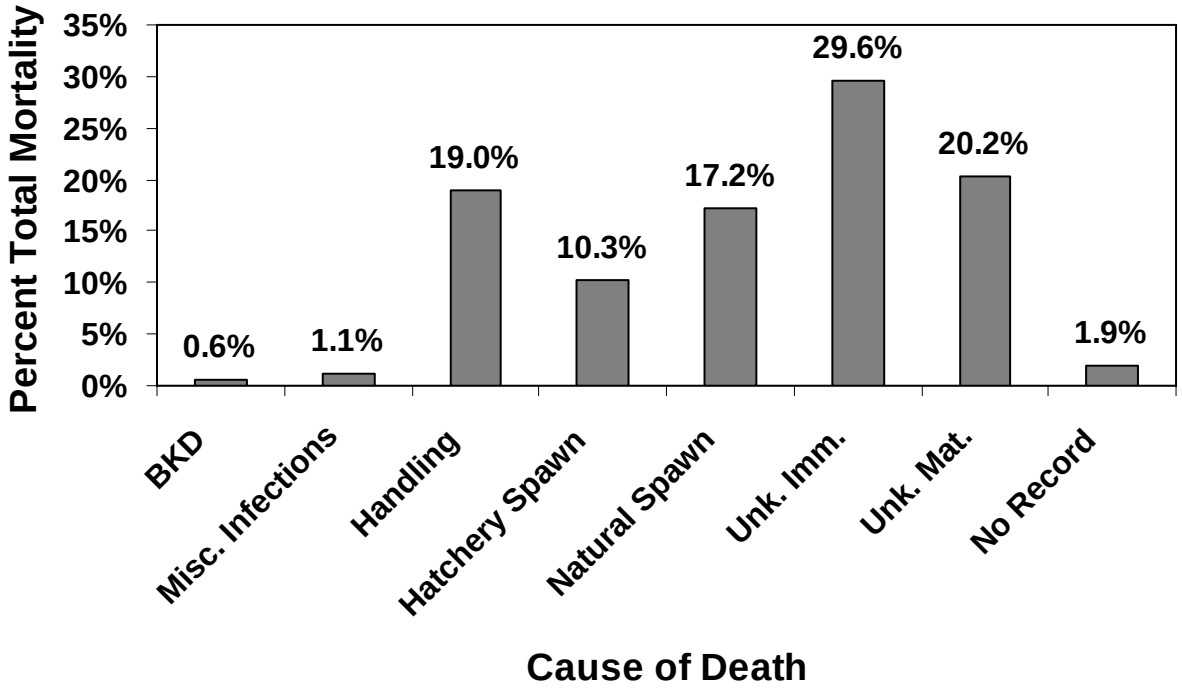

Figure 11. Primary sources of mortality in brood year 1998 captive-reared Chinook salmon at the Eagle Fish Hatchery and Manchester Research Station. Abbreviations: BKD = bacterial kidney disease, Unk. = Unknown. Hatchery spawns include cryopreservation.

Brood year 1998 captive-reared Chinook salmon from the WFYF and LEM matured at a similar overall rate as previous cohorts, but maturation in the EFSR group was lower than usual. Overall, 150 of 219 fish (68.5%) from the WFYF brought into the program matured; of these, 27 males (18.0%) matured at age-2, two males (1.3%) and 43 (28.7%) unknown sex at release matured at age-3, 42 females (28.0%), 17 males (11.3%), and six (4.0%) unknown sex at release matured at age-4, while nine females (6.0%), three males (2.0%), and one (0.7%) unknown sex at release matured at age-5. Precocity was higher than observed in earlier cohorts from the WFYF (Hassemer et al. 2001; Venditti et al. 2002), but similar to results observed in brood years 1996 and 1997 (Venditti et al. 2003a, b). In the LEM stock, 116 of 186 (62.4%) brood year 1997 program fish matured. Precocial maturation in this group was 11.2% (13 fish), while 24 (20.7%) males, four females (3.4%), and one (0.9%) unknown sex at release matured at age-3, seven males (6.0%), 50 females (43.1%) matured at age-4, while one male (0.9%), 12 females (10.3%), and four (3.4%) unknown sex at release matured at age-5. Maturation in this group was slightly lower than that observed in the previous cohorts (Venditti et al., 2003b), and males still typically matured at a younger age than females. In the EFSR, 164 out of 430 (38.1%) brood year 1998 fish matured. Of these, 47 males (28.7%) and one female (0.6%) matured as precocials. Forty-four males (26.8%) matured at age-3, seven males (4.3%), 38 females (23.2%), and six unknown sex at release (3.7%) matured at age-4, while four males (2.4%), 16 females (9.8%), and one unknown sex at release (0.6%) matured at age-5. Maturation in this stock was significantly lower than that experienced in previous cohorts (Venditti et al. 2002, 2003a, b). Such a low level of maturation is reflective of increased mortality

experienced in this cohort. A greater percentage of brood year 1998 EFSR-NP (53.2%) matured overall in comparison to the EFSR-SN (28.0%) from the same brood year. This difference was surely influenced by the extensive unexplained mortality experienced by the EFSR-SN group after transfer to Manchester.

Volitional Spawning

After being disc tagged, 88 WFYF, 41 EFSR, and 48 LEM adults were released back into their natal streams for volitional spawning. Releases into the LEM and EFSR occurred on August 4 and August 5, 2003, respectively, and adult Chinook salmon were flown into the WFYF and released on August 6, 2003 (Appendix B). Release sites in all streams were widely spaced in order to reduce the density of fish at any one particular location.

Behavior and habitat associations of captive-reared Chinook salmon observed in the WFYF were similar to observations recorded in previous years directly following release. Initially, study fish were generally observed to be associated with pools, large woody debris, or runs (Figure 12). Holding was the dominant behavior observed during August and September 2003, accounting for approximately 90% of all observations, whereas milling was the second most dominant with 8% (Figure 13). These behaviors were also the dominant associations observed from August to September 2002, averaging 86% and 6%, respectively (Venditti et al. 2003b). Such behavior and habitat associations are in accord with prespawn Atlantic salmon reported by Bardonnet and Baglinière (2000). This behavioral adaptation of selecting habitats with low water velocity and complex structures may benefit them by helping to conserve depleted energy reserves for future spawning activities (Torgersen et al. 1999) or by providing refuge from predators.

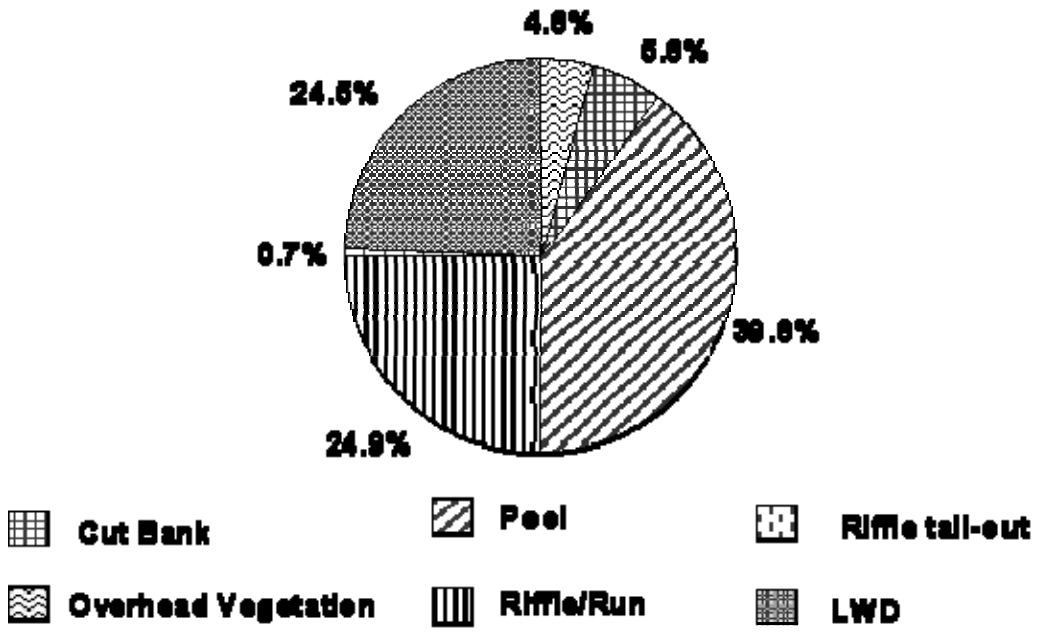

Figure 12. Habitat associations of captive-reared Chinook salmon released into the West Fork Yankee Fork Salmon River in the summer of 2003. Data were collected during standardized observation intervals of 5 min.

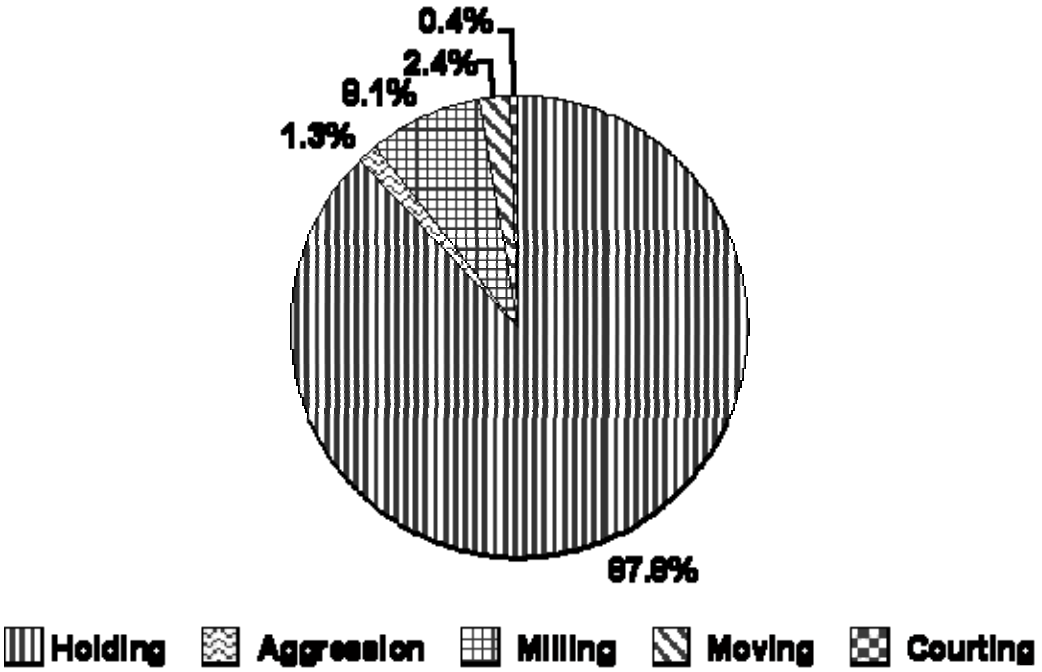

Figure 13. General behaviors of captive-reared Chinook salmon released into the West Fork Yankee fork Salmon River in the summer of 2003. Data were collected during standardized observation intervals of 5 min.

No captive-reared females constructed redds in the WFYF in 2003 year due to what appears to have been complete prespawn mortality. Carcasses from nearly 80% of all fish released into the WFYF were recovered by September 6, 2003 (Figure 14), and no live fish were observed during stream observations on or after that date. There are no distinguishing patterns in the mortality between brood year and treatment groups that would identify one group as being predominately affected (Figures 15 and 16). However, observers noted that in contrast to previous years, program fish began to display fungal infections on the head and peduncle within 2–3 d of release. Kidney and spleen samples were collected from five carcasses (N = 3 BY1999, control; N = 1 BY2000, control; and N = 1 BY1998, treatment) and sent to the IDFG Eagle Fish Health Laboratory. Results indicated clinical levels of infection of *Aeromonas* spp. *Aeromonas* infections are ubiquitous throughout freshwater fish species (Piper et al. 1982), and fungus outbreaks are a common secondary response to an underlying stressor.

No compelling evidence has been identified to suggest what conditions in culture or in the WFYF were responsible for the unprecedented level of prespawn mortality. Transport and prerelease mortality at Eagle were the lowest on record and remained extremely low for adults held there for hatchery spawning. Except for the change in the chilled water temperature regime (which was also experienced by the adults that remained at Eagle), no other protocols such as rearing, handling, marking, and transporting were altered in 2003. Several unspawned, wild carcasses were recovered in the WFYF in 2003, and prespawn mortality in the South Fork Salmon River and several hatcheries throughout Idaho was higher than usual in 2003. This suggests that an environmental factor may be responsible, and elevated water temperature and adverse migration conditions have been commonly identified as the prime suspects (K. Johnson, IDFG, personal communication). However, thermograph data from the WFYF

indicate stream temperatures (daily maximum, average, and minimum) in 2003 were similar or slightly lower than those recorded in 2001 but several degrees higher than in 2002 (Figure 17). The spawning performance of captive-reared Chinook salmon (based on the proportion of females that constructed redds) was higher in 2002 than in 2001 (Venditti et al. 2003a, b), which lends a measure of support to the supposition that elevated water temperature may have affected survival and therefore spawning success. However, in 2001 (the year with the highest maximum and mean daily temperatures), most fish survived at least to the date program fish began initiating spawning activity, and many were still alive well into September.

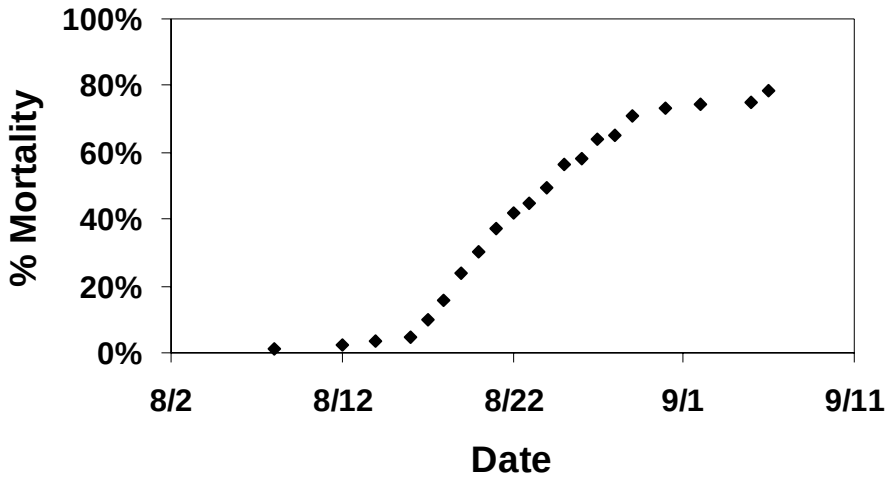

Figure 14. Overall percent mortality of captive Chinook carcasses recovered on the West Fork Yankee Fork Salmon River during the 2003 field season.

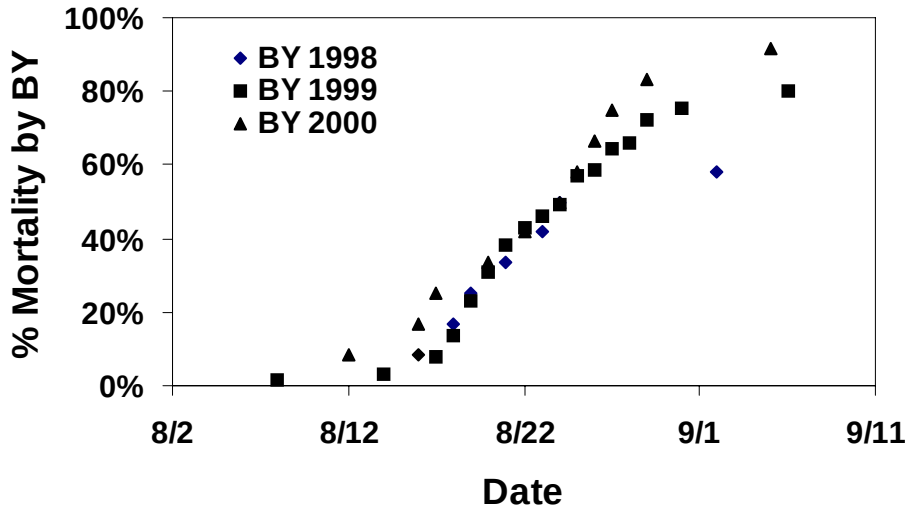

Figure 15. Running mortality record for brood year carcasses recovered on the West Fork Yankee Fork Salmon River during 2003.

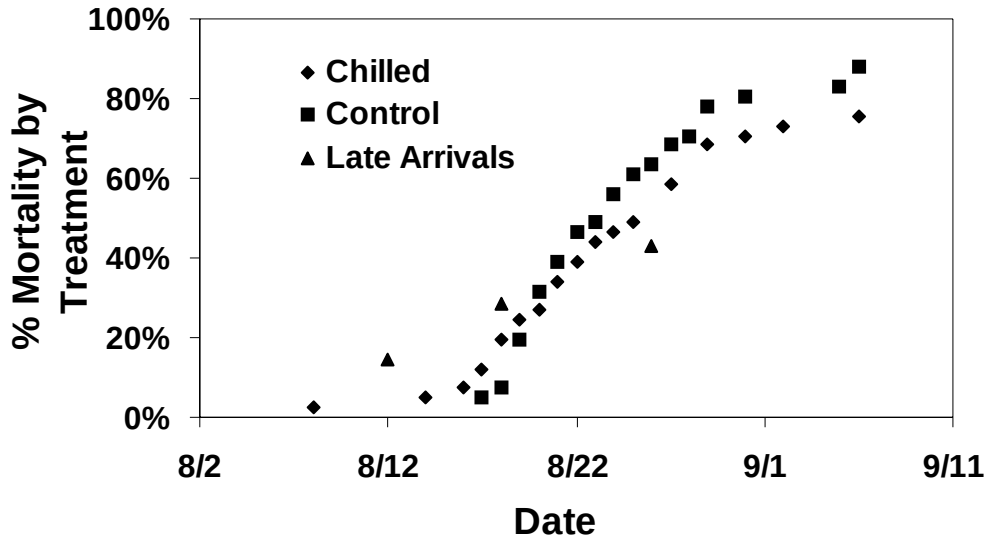

Figure 16. Running mortality record for treatment group carcasses recovered on the West Fork Yankee Fork Salmon River during 2003.

Production Estimation

During August and September 2003, we collected DNA samples from naturally produced Chinook salmon parr in the WFYF and Hayden Creek (HYDN) that were potentially produced by volitionally spawning program fish or eyed-eggs produced from program parents. Five hundred sixty-five parr were collected from 93 different sites in the WFYF near areas of known spawning in 2002. Parr from the WFYF were collected between August 10 and September 3, 2003. Five hundred sixty-seven parr were collected from 58 sites on HYDN below egg-box locations in 2002. Parr were collected from HYDN between September 14 and 22, 2003. Samples collected from the WFYF were sent to the University of Idaho's Aquaculture Research Institute, along with tissue samples from spawn year 2002 mature captive- and ocean-reared adult samples, for parental exclusion analysis on December 3, 2003. Fork-length from parr averaged 60.7 mm (range, 23–111 mm) in the WFYF and 74.0 mm (range, 37–94 mm) in HYDN. No parr mortality resulted from the collection of these samples.

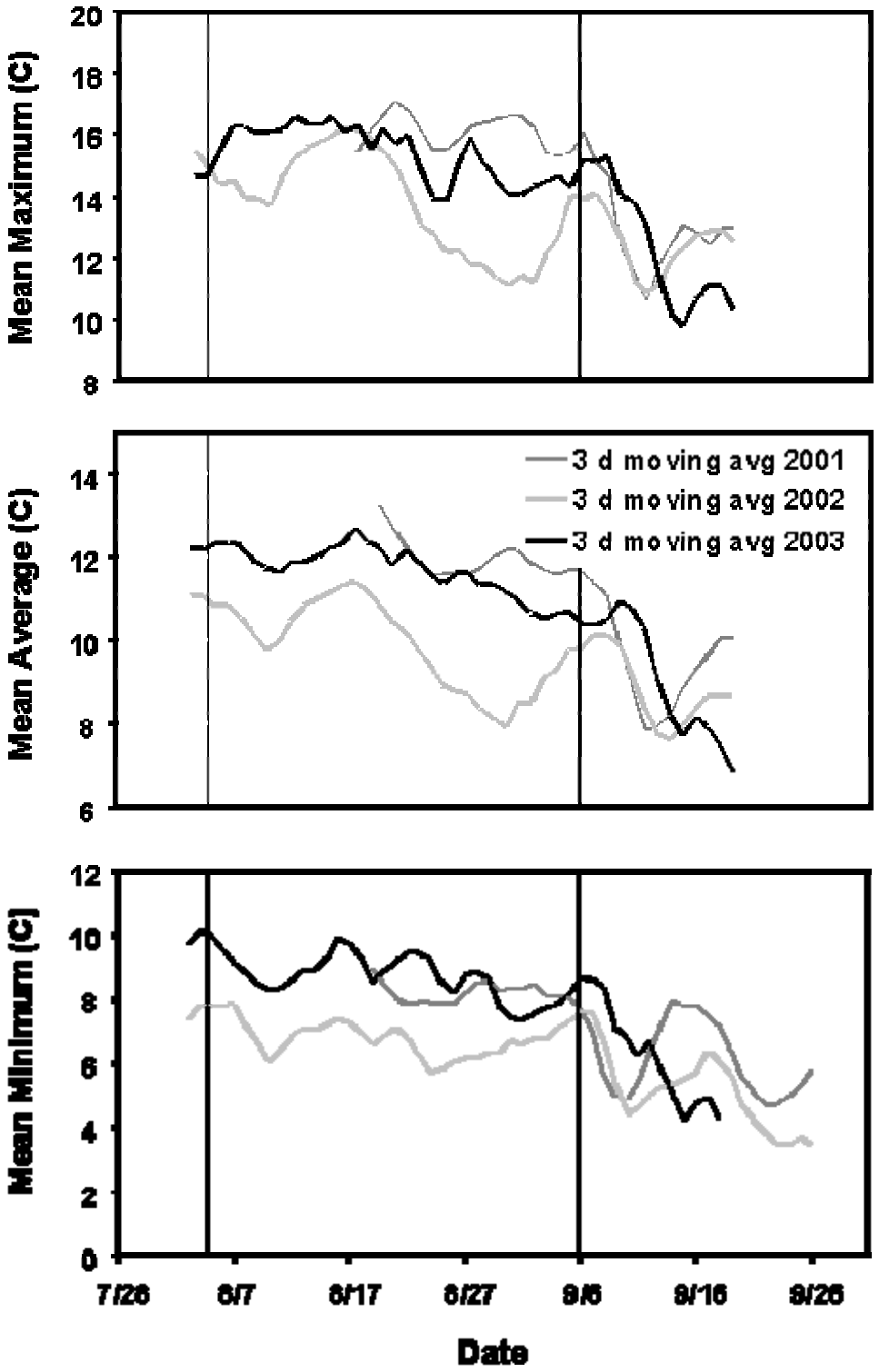

Figure 17. Comparison of three measures of water temperatures in the West Fork Yankee Fork Salmon River for the years 2001–2003 calculated from thermograph collections at 2 h intervals. Three-day moving averages are used to smooth the data. Vertical lines represent the date captive-reared Chinook salmon were released in 2003 and the date by which all program fish were presumed dead.

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APPENDICES

Appendix A. Summary of spawning activities involving captive-reared Chinook salmon from the East Fork Salmon River at the Eagle Fish Hatchery in 2003. Fish were separated into two groups: one held on chilled water and one on ambient temperature water to determine the effect of temperature on maturation timing and egg survival to the eyed stage of development. Males identified by ***bold/italic*** type were "chaser" males whose milt was introduced approximately 2 min after the milt from the primary male. Facility limitations precluded the incubation of individual sublots after the second spawn date. After that time, sublots were combined and incubated together.

Spawn Date	Female ID	Treatment	Female BY	Female Lineage	Male BY	Male Lineage	Green Eggs	Eyed Eggs	Survival
8/26/2003	3D9.1BF0ECD6AE	Chilled	1998	NP	1999	SN	343	272	79.3%
8/26/2003	3D9.1BF0ECD6AE	Chilled	1998	NP	2000	NE	346	288	83.2%
8/26/2003	3D9.1BF0ECD6AE	Chilled	1998	NP	2000	NE	324	255	78.7%
9/3/2003	3D9.1BF0DF58F9	Ambient	1998	NP	2000	NE	309	267	86.4%
9/3/2003	3D9.1BF0DF58F9	Ambient	1998	NP	2000	NE	304	289	95.1%
9/3/2003	3D9.1BF0DF58F9	Ambient	1998	NP	1999	SN	300	268	89.3%
9/9/2003	3D9.1BF0EDA220	Ambient	1998	NP	1999	SN			
9/9/2003	3D9.1BF0EDA220	Ambient	1998	NP	2000	NE			
					2000	***NE***	1,073	12	1.1%
9/9/2003	3D9.1BF0ED3955	Chilled	1999	SN	2000	NE			
9/9/2003	3D9.1BF0ED3955	Chilled	1999	SN	2000	NE			
					2000	***NE***	1,430	1,142	79.9%
9/9/2003	3D9.1BF0EC59D7	Chilled	1998	NP	1999	SN			
9/9/2003	3D9.1BF0EC59D7	Chilled	1998	NP	2000	NE			
					2000	***NE***	467	218	46.7%
9/9/2003	3D9.1BF0EC5D90	Chilled	1999	SN	2000	NE			
9/9/2003	3D9.1BF0EC5D90	Chilled	1999	SN	2000	NE			
					2000	***NE***	1,925	1,759	91.4%
9/9/2003	3D9.1BF0ED3224	Chilled	1998	NP	2000	NE			
9/9/2003	3D9.1BF0ED3224	Chilled	1998	NP	2000	NE			
					2000	***NE***	177	139	78.5%
9/9/2003	3D9.1BF0DFADB4	Chilled	1998	NP	2000	NE			
9/9/2003	3D9.1BF0DFADB4	Chilled	1998	NP	2000	NE			
					2000	***NE***	198	41	20.7%
9/16/2003	3D9.1BF0EC470B	Chilled	1998	NP	2000	NE			
9/16/2003	3D9.1BF0EC470B	Chilled	1998	NP	2000	NE			
9/16/2003	3D9.1BF0EC470B	Chilled	1998	NP	2000	NE	1,648	1,282	77.8%
9/22/2003	3D9.1BF0EC44A1	Ambient	1999	NE	1998	NP			
9/22/2003	3D9.1BF0EC44A1	Ambient	1999	NE	2000	NE			
	3D9.1BF0EC44A1			NE	2000	***NE***	557	463	83.1%
9/22/2003	3D9.1BF0ECEA68	Chilled	1999	NE	1998	NP			
9/22/2003	3D9.1BF0ECEA68	Chilled	1999	NE	2000	NE			
	3D9.1BF0ECEA68			NE	2000	***NE***	669	267	39.9%
9/26/2003	3D9.1BF0EC4ABA	Ambient	1998	NP	2000	NE			
9/26/2003	3D9.1BF0EC4ABA	Ambient	1998	NP	2000	NE			
	3D9.1BF0EC4ABA			NP	2000	***NE***	1,512	130	8.6%
9/26/2003	3D9.1BF0EE2D33	Chilled	1999	SN	2000	NE			
9/26/2003	3D9.1BF0EE2D33	Chilled	1999	SN	2000	NE			
	3D9.1BF0EE2D33			SN	2000	***NE***	1,864	595	31.9%
9/29/2003	3D9.1BF0ED379B	Chilled	1998	NP	2000	NE			
9/29/2003	3D9.1BF0ED379B	Chilled	1998	NP	2000	NE			
	3D9.1BF0ED379B			NP	2000	***NE***	632	72	11.4%
9/29/2003	3D9.1BF0ED4727	Chilled	1999	NE	2000	NE			
9/29/2003	3D9.1BF0ED4727	Chilled	1999	NE	2000	NE			
	3D9.1BF0ED4727			NE	2000	***NE***	2,269	2,049	90.3%
9/29/2003	3D9.1BF11A9D82	Ambient	2000	NE	1999	SN			

Appendix A. Continued.

Spawn Date	Female ID	Treatment	Female BY	Female Lineage	Male BY	Male Lineage	Green Eggs	Eyed Eggs	Survival
9/29/2003	3D9.1BF11A9D82	Ambient	2000	NE	2000	NE			
	3D9.1BF11A9D82			NE	2000	***NE***	1,121	492	43.9%
9/29/2003	3D9.1BF0EC3200	Ambient	1999	NE	2000	NE			
9/29/2003	3D9.1BF0EC3200	Ambient	1999	NE	1999	SN			
	3D9.1BF0EC3200			NE	2000	***NE***	769	158	20.5%
9/29/2003	3D9.1BF0EE2F82	Ambient	1999	NE	2000	NE			
9/29/2003	3D9.1BF0EE2F82	Ambient	1999	NE	2000	NE			
	3D9.1BF0EE2F82			NE	2000	***NE***	2,464	90	3.7%
9/29/2003	3D9.1BF0ED3464	Ambient	1999	SN	2000	NE			
9/29/2003	3D9.1BF0ED3464	Ambient	1999	SN	2000	NE			
	3D9.1BF0ED3464			SN	2000	***NE***	2,544	2,422	95.2%
9/29/2003	3D9.1BF0EE100A	Ambient	1999	NE	2000	NE			
9/29/2003	3D9.1BF0EE100A	Ambient	1999	NE	2000	NE			
	3D9.1BF0EE100A			NE	2000	***NE***	895	548	61.2%
9/29/2003	3D9.1BF0EE6864	Ambient	1999	SN	1999	SN			
9/29/2003	3D9.1BF0EE6864	Ambient	1999	SN	2000	NE			
	3D9.1BF0EE6864			SN	2000	***NE***	1,908	7	0.4%
10/2/2003	3D9.1BF0ED278F	Chilled	1999	NE	2000	NE			
10/2/2003	3D9.1BF0ED278F	Chilled	1999	NE	2000	NE			
	3D9.1BF0ED278F			NE	2000	***NE***	954	837	87.7%
10/2/2003	3D9.1BF0EE1404	Chilled	1999	NE	2000	NE			
10/2/2003	3D9.1BF0EE1404	Chilled	1999	NE	2000	NE			
	3D9.1BF0EE1404			NE	2000	***NE***	1,302	14	1.1%
10/2/2003	3D9.1BF0ED5667	Ambient	1999	NE	2000	NE			
10/2/2003	3D9.1BF0ED5667	Ambient	1999	NE	2000	NE			
	3D9.1BF0ED5667			NE	2000	***NE***	1,270	694	54.6%
10/2/2003	3D9.1BF11AE8F0	Ambient	2000	NE	2000	NE			
10/2/2003	3D9.1BF11AE8F0	Ambient	2000	NE	2000	NE			
	3D9.1BF11AE8F0			NE	2000	***NE***	918	434	47.3%
10/8/2003	3D9.1BF0EE7065	Ambient	1998	NP	2000	NE			
10/8/2003	3D9.1BF0EE7065	Ambient	1998	NE	2000	NE			
	3D9.1BF0EE7065			NE	2000	***NE***	1,610	0	0.0%
10/8/2003	3D9.1BF0ED4C08	Ambient	1999	SN	2000	NE			
10/8/2003	3D9.1BF0ED4C08	Ambient	1999	SN	1999	NE			
	3D9.1BF0ED4C08			SN	2000	***NE***	2,569	750	29.2%

Appendix B. Summary of fish transfers conducted by the Chinook salmon captive rearing project during 2003. LEM–Lemhi River, WFYF–West Fork Yankee Fork Salmon River, EFSR–East Fork Salmon River, MAN–Manchester Research Station, EAG–Eagle Fish Hatchery. NP, NE, and SN refer to natural parr, natural egg, and safety net groups, respectively.

Source Stream	BY	EAG to MAN	Transfer Date	MAN to EAG	Transfer Date	EAG to WFYF	Transfer Date	EAG to EFSR	Transfer Date	EAG to LEM	Transfer Date
LEM-NP	98			17	05/6-05/7					11	08/04
LEM-NE	99			35	05/6-05/7					37	08/04
WFYF-NP	98			14	05/6-05/7	12	08/06				
WFYF-NE	99			60	05/6-05/7	59	08/06				
WFYF-SN	99			3	06/10	5	08/06				
WFYF-NE	00			9	05/6-05/7	12	08/06				
WFYF-NE	00			3	06/10						
WFYF-NE	01	257	05/05								
EFSR-NP	98			21	05/6-05/7			7	08/05		
EFSR-SN	98			1	05/6-05/7			1	08/05		
EFSR-NE	99			21	05/6-05/7			11	08/05		
EFSR-SN	99			18	05/6-05/7			9	08/05		
EFSR-NE	00			35	05/6-05/7			13	08/05		
EFSR-NE	00			6	06/10						
EFSR-NE	01	285	05/05								

Appendix C. Tag and identification summary for captive-reared Chinook salmon released for volitional spawning in the West Fork Yankee Fork Salmon River (WFYF), the East Fork Salmon River (EFSR), and the Lemhi River (LEM) in 2003. Fish from the EFSR and WFYF received disc tags, while those from the LEM received Floy tags. We used Ultrasound at the Manchester Research Station (MAN) to determine sex, U–undetermined, F–female, M–male. Disc tag colors included combinations of W–white, B–blue, Y–yellow, O–orange, and P–pink. Floy tags were dark green (Dk. Green). Treatment group refers to the temperature experienced during freshwater maturation at EAG. Test fish (T) were held on chilled water (≈9.0°C); control fish (C) were held on ambient water (≈13.5°C); late arrivals (LA)—those fish transferred to freshwater about six weeks later than the others were held on ambient water but not included in the temperature study. Fish heavier than the group mean for their stock and brood year (BY) were classified as large (L), while those lighter were classed as small (S).

PIT Code	BY	Sex	Color	Number	Stock	Size	Treatment	Rearing
3D9.1BF0DF8159	1998	M	OW	12	WFYF	L	T	MAN
3D9.1BF0ED36A1	1998	F	OW	17	WFYF	L	T	MAN
3D9.1BF0EC4104	1998	U	OW	42	WFYF	S	T	MAN
3D9.1BF0ED4D5A	1998	F	OW	46	WFYF	S	T	MAN
3D9.1BF0EC50FA	1998	U	OW	47	WFYF	S	T	MAN
3D9.1BF0ECE492	1998	F	OW	49	WFYF	L	T	MAN
3D9.1BF0ED46B0	1998	F	RW	52	WFYF	S	C	MAN
3D9.1BF0EC6005	1998	U	RW	53	WFYF	S	C	MAN
3D9.1BF0EC46C3	1998	U	RW	74	WFYF	S	C	MAN
3D9.1BF0EC4866	1998	U	RW	77	WFYF	L	C	MAN
3D9.1BF0ED2C49	1998	F	RW	79	WFYF	L	C	MAN
3D9.1BF0ED38E1	1998	M	RW	88	WFYF	S	C	MAN
3D9.1BF0ED4E66	1999	F	BW	50	WFYF	S	C	MAN
3D9.1BF0DF089D	1999	F	BW	51	WFYF	L	C	MAN
3D9.1BF0ED40AE	1999	M	BW	52	WFYF	S	C	MAN
3D9.1BF0DF9B74	1999	F	BW	53	WFYF	L	C	MAN
3D9.1BF0EC48C1	1999	F	BW	54	WFYF	S	C	MAN
3D9.1BF0DF14F5	1999	F	BW	55	WFYF	L	C	MAN
3D9.1BF0DF9F55	1999	M	BW	57	WFYF	S	C	MAN
3D9.1BF0E028F3	1999	F	BW	59	WFYF	L	C	MAN
3D9.1BF0EC45D3	1999	F	BW	61	WFYF	L	C	EAG
3D9.1BF0EE6682	1999	F	BW	62	WFYF	L	C	MAN
3D9.1BF0DF147E	1999	U	BW	63	WFYF	S	C	EAG
3D9.1BF0EC402B	1999	M	BW	64	WFYF	S	C	MAN
3D9.1BF0ED43F9	1999	F	BW	65	WFYF	L	C	MAN
3D9.1BF0DFA1F8	1999	M	BW	67	WFYF	L	C	MAN
3D9.1BF0EC4EE6	1999	M	BW	70	WFYF	L	C	MAN
3D9.1BF0EC41E0	1999	F	BW	72	WFYF	S	C	MAN
3D9.1BF0EE2032	1999	F	BW	73	WFYF	S	C	MAN
3D9.1BF0EC2D28	1999	F	BW	74	WFYF	L	C	MAN
3D9.1BF0ECD256	1999	M	BW	75	WFYF	S	C	MAN
3D9.1BF0DF19AC	1999	F	BW	77	WFYF	L	C	MAN
3D9.1BF0EC5410	1999	F	BW	78	WFYF	L	C	MAN
3D9.1BF0DF1F37	1999	F	BW	82	WFYF	S	C	MAN
3D9.1BF0DF2155	1999	F	BW	84	WFYF	L	C	MAN
3D9.1BF0E0244D	1999	F	BW	85	WFYF	L	C	EAG
3D9.1BF0EE697F	1999	F	BW	91	WFYF	S	C	MAN
3D9.1BF0DFA9E8	1999	F	BW	92	WFYF	S	C	MAN

Appendix C. Continued.

PIT Code	BY	Sex	Color	Number	Stock	Size	Treatment	Rearing
3D9.1BF0EC3CDC	1999	F	BW	94	WFYF	L	C	MAN
3D9.1BF0EC5753	1999	M	BW	95	WFYF	S	C	MAN
3D9.1BF0EC3BED	1999	F	BW	96	WFYF	L	C	MAN
3D9.1BF0ED31EE	1999	F	BW	97	WFYF	L	C	MAN
3D9.1BF0EC4021	1999	F	OO	14	WFYF		LA	MAN
3D9.1BF0DF2200	1999	F	OO	28	WFYF		LA	MAN
3D9.1BF0EC44A0	1999	F	OO	34	WFYF		LA	MAN
3D9.1BF0EC3205	1999	M	WW	92	WFYF	S	C	MAN
3D9.1BF0DF1304	1999	F	YW	2	WFYF	S	T	MAN
3D9.1BF0EC52A1	1999	F	YW	4	WFYF	L	T	MAN
3D9.1BF0ED3642	1999	F	YW	5	WFYF	L	T	MAN
3D9.1BF0EE2F7F	1999	F	YW	7	WFYF	L	T	MAN
3D9.1BF0DF22F4	1999	M	YW	11	WFYF	S	T	MAN
3D9.1BF0EC3D30	1999	F	YW	14	WFYF	S	T	MAN
3D9.1BF0EC365D	1999	F	YW	15	WFYF	L	T	MAN
3D9.1BF0ED3081	1999	M	YW	17	WFYF	S	T	MAN
3D9.1BF0DF9522	1999	F	YW	20	WFYF	L	T	MAN
3D9.1BF0EC5839	1999	F	YW	21	WFYF	S	T	MAN
3D9.1BF0DF9CD3	1999	F	YW	23	WFYF	L	T	MAN
3D9.1BF0E021DE	1999	F	YW	25	WFYF	L	T	MAN
3D9.1BF0EE6525	1999	F	YW	26	WFYF	S	T	MAN
3D9.1BF0DF1FDE	1999	F	YW	27	WFYF	L	T	MAN
3D9.1BF0E02A7D	1999	F	YW	28	WFYF	S	T	MAN
3D9.1BF0EC410F	1999	F	YW	29	WFYF	S	T	MAN
3D9.1BF0EC533E	1999	F	YW	32	WFYF	S	T	MAN
3D9.1BF0DF9EDE	1999	M	YW	33	WFYF	S	T	MAN
3D9.1BF0DFA9A3	1999	M	YW	34	WFYF	S	T	MAN
3D9.1BF0E02C0D	1999	M	YW	35	WFYF	L	T	MAN
3D9.1BF0DF079F	1999	U	YW	37	WFYF	L	T	MAN
3D9.1BF0EC452A	1999	F	YW	40	WFYF	L	T	MAN
3D9.1BF0ED54A4	1999	M	YW	41	WFYF	S	T	MAN
3D9.1BF0DF0EFA	1999	M	YW	42	WFYF	L	T	MAN
3D9.1BF0ED4BCD	1999	M	YW	43	WFYF	S	T	MAN
3D9.1BF0E02884	1999	F	YW	45	WFYF	L	T	MAN
3D9.1BF0EC3C6B	1999	F	YW	46	WFYF	L	T	MAN
3D9.1BF0DF164F	1999	F	YW	47	WFYF	L	T	MAN
3D9.1BF0EC55DC	1999	F	YW	48	WFYF	L	T	MAN
3D9.1BF0EC45EA	1999	F	YW	49	WFYF	S	T	MAN
3D9.1BF11AB648	2000	M	GW	57	WFYF	S	T	MAN
3D9.1BF11AF258	2000	F	GW	65	WFYF	S	T	MAN
3D9.1BF11ADFF4	2000	M	GW	71	WFYF	S	T	MAN
3D9.1BF11AEE39	2000	F	GW	74	WFYF	L	T	MAN
3D9.1BF11AAC9E	2000	F	GW	96	WFYF	L	T	MAN
3D9.1BF11AE57B	2000	F	OO	0	WFYF		LA	MAN
3D9.1BF11B074E	2000	M	OO	12	WFYF		LA	MAN
3D9.1BF11AE476	2000	F	OO	25	WFYF		LA	MAN
3D9.1BF11EA5C2	2000	F	WW	51	WFYF	L	C	MAN
3D9.1BF11AE95D	2000	F	WW	69	WFYF	L	C	MAN
3D9.1BF11AD5E2	2000	M	WW	77	WFYF	S	C	MAN
3D9.1BF11AE443	2000	F	WW	88	WFYF	L	C	MAN
No Tag Detected	2000	M	OO	44	EFSR		LA	MAN
3D9.1BF11AB3D5	2000	M	WW	62	EFSR	S	C	MAN
3D9.1BF11ABAAA	2000	M	YW	24	EFSR	S	T	MAN
3D9.1BF11AC0C0	2000	U	YW	0	EFSR	S	T	MAN

Appendix C. Continued.

PIT Code	BY	Sex	Color	Number	Stock	Size	Treatment	Rearing
3D9.1BF11AC312	2000	M	OO	43	EFSR		LA	MAN
3D9.1BF11ADDD2	2000	M	YW	39	EFSR	S	T	MAN
3D9.1BF11ADDD8	2000	M	OO	40	EFSR		LA	MAN
3D9.1BF11AE26E	2000	M	YW	36	EFSR	S	T	MAN
3D9.1BF11AE584	2000	M	YW	44	EFSR	L	T	MAN
3D9.1BF11AE5B6	2000	M	OO	29	EFSR		LA	MAN
3D9.1BF11AF0D6	2000	M	YW	31	EFSR	S	T	MAN
3D9.1BF11BA6C8	2000	M	WW	97	EFSR	S	C	MAN
3D9.1BF11EA364	2000	M	OO	4	EFSR		LA	MAN
3D9.1BF0EC2DFB	1999	F	GW	88	EFSR	L	T	MAN
3D9.1BF0EC3EE9	1999	M	BW	58	EFSR	S	C	MAN
3D9.1BF0EC401D	1999	M	GW	64	EFSR	S	T	MAN
3D9.1BF0EC428E	1999	F	BW	83	EFSR	L	C	MAN
3D9.1BF0EC443B	1999	M	BW	93	EFSR	S	C	MAN
3D9.1BF0EC521E	1999	F	GW	78	EFSR	S	T	MAN
3D9.1BF0EC56D6	1999	M	GW	87	EFSR	L	T	MAN
3D9.1BF0EC5E30	1999	F	BW	87	EFSR	S	C	MAN
3D9.1BF0EC5EDC	1999	M	BW	81	EFSR	S	C	MAN
3D9.1BF0ECF16A	1999	F	BW	90	EFSR	S	C	MAN
3D9.1BF0ED19BD	1999	F	GW	94	EFSR	L	T	MAN
3D9.1BF0ED2573	1999	F	GW	53	EFSR	S	T	MAN
3D9.1BF0ED3E81	1999	F	BW	88	EFSR	S	C	MAN
3D9.1BF0ED4711	1999	F	BW	98	EFSR	L	C	EAG
3D9.1BF0ED4A1D	1999	F	GW	59	EFSR	S	T	MAN
3D9.1BF0ED4D4F	1999	F	GW	72	EFSR	S	T	MAN
3D9.1BF0EE358D	1999	F	BW	56	EFSR	S	C	MAN
3D9.1BF0EE387F	1999	M	GW	95	EFSR	S	T	MAN
3D9.1BF0EE6918	1999	F	GW	84	EFSR	S	T	MAN
3D9.1BF0EE6CD6	1999	F	BW	71	EFSR	S	C	MAN
3D9.1BF0EC4511	1998	F	OW	40	EFSR	S	T	MAN
3D9.1BF0ED567F	1998	F	OW	4	EFSR	S	T	MAN
3D9.1BF0EC5A37	1998	F	RW	51	EFSR	S	C	MAN
3D9.1BF0ED464A	1998	F	RW	62	EFSR	S	C	MAN
3D9.1BF0DFF403	1998	M	RW	58	EFSR	S	C	MAN
3D9.1BF0DFB90C	1998	M	RW	92	EFSR	S	C	MAN
3D9.1BF0EC4555	1998	U	RW	87	EFSR	S	C	MAN
3D9.1BF0ED57FE	1998	M	RW	78	EFSR	S	C	MAN
3D9.1BF0DF3EBB	1998	F	Dk. Green	1653	LEM			MAN
3D9.1BF0DF4807	1998	M	Dk. Green	1675	LEM			MAN
3D9.1BF0DFC7A7	1998	F	Dk. Green	1664	LEM			MAN
3D9.1BF0DFDC2D	1998	U	Dk. Green	1742	LEM			MAN
3D9.1BF0DFEBF9	1998	U	Dk. Green	1374	LEM			MAN
3D9.1BF0DFEF12	1998	F	Dk. Green	1763	LEM			MAN
3D9.1BF0E001BA	1998	F	Dk. Green	1768	LEM			MAN
3D9.1BF0E00722	1998	F	Dk. Green	1773	LEM			MAN
3D9.1BF0E00E21	1998	F	Dk. Green	1669	LEM			MAN
3D9.1BF0E01AB6	1998	U	Dk. Green	1654	LEM			MAN
3D9.1BF0E01C26	1998	U	Dk. Green	1667	LEM			MAN
3D9.1BF0DEFE4A	1999	F	Dk. Green	1662	LEM			MAN
3D9.1BF0DF1E36	1999	M	Dk. Green	1769	LEM			MAN
3D9.1BF0DF260B	1999	F	Dk. Green	1759	LEM			MAN
3D9.1BF0DF99E4	1999	F	Dk. Green	1671	LEM			MAN
3D9.1BF0EC348C	1999	F	Dk. Green	1766	LEM			MAN
3D9.1BF0EC38FC	1999	F	Dk. Green	1674	LEM			MAN

Appendix C. Continued.

PIT Code	BY	Sex	Color	Number	Stock	Size	Treatment	Rearing
3D9.1BF0EC4010	1999	F	Dk. Green	1744	LEM			MAN
3D9.1BF0EC4294	1999	F	Dk. Green	1661	LEM			MAN
3D9.1BF0EC436D	1999	F	Dk. Green	1670	LEM			MAN
3D9.1BF0EC4AAD	1999	F	Dk. Green	1668	LEM			MAN
3D9.1BF0EC4CF5	1999	F	Dk. Green	1373	LEM			MAN
3D9.1BF0EC4DB7	1999	F	Dk. Green	1738	LEM			MAN
3D9.1BF0EC4FF9	1999	F	Dk. Green	1660	LEM			MAN
3D9.1BF0EC5015	1999	F	Dk. Green	1651	LEM			MAN
3D9.1BF0EC5268	1999	F	Dk. Green	1743	LEM			EAG
3D9.1BF0EC5898	1999	F	Dk. Green	1770	LEM			MAN
3D9.1BF0EC58D4	1999	F	Dk. Green	1657	LEM			MAN
3D9.1BF0EC5934	1999	M	Dk. Green	1767	LEM			MAN
3D9.1BF0EC5966	1999	F	Dk. Green	1658	LEM			MAN
3D9.1BF0EC5F10	1999	U	Dk. Green	1762	LEM			MAN
3D9.1BF0EC5F16	1999	M	Dk. Green	1672	LEM			MAN
3D9.1BF0EC5F66	1999	F	Dk. Green	1656	LEM			MAN
3D9.1BF0ECCECF	1999	M	Dk. Green	1764	LEM			MAN
3D9.1BF0ED3679	1999	F	Dk. Green	1673	LEM			MAN
3D9.1BF0ED394F	1999	F	Dk. Green	1772	LEM			MAN
3D9.1BF0ED3C76	1999	F	Dk. Green	1771	LEM			MAN
3D9.1BF0ED3FFD	1999	U	Dk. Green	1739	LEM			MAN
3D9.1BF0ED4B37	1999	U	Dk. Green	???	LEM			EAG
3D9.1BF0ED4B45	1999	F	Dk. Green	1666	LEM			MAN
3D9.1BF0ED4EFE	1999	F	Dk. Green	1775	LEM			MAN
3D9.1BF0ED5348	1999	F	Dk. Green	1741	LEM			MAN
3D9.1BF0ED5556	1999	F	Dk. Green	1774	LEM			MAN
3D9.1BF0ED6394	1999	U	Dk. Green	1659	LEM			MAN
3D9.1BF0EE3C52	1999	F	Dk. Green	1371	LEM			MAN
3D9.1BF0EE3E79	1999	F	Dk. Green	1372	LEM			MAN
3D9.1BF0EE6661	1999	F	Dk. Green	1740	LEM			MAN
3D9.1BF0EE7064	1999	F	Dk. Green	1665	LEM			MAN

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