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Fish Culture Technology and Practices for Captive Broodstock Rearing of ESA-listed Salmon Stocks

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Table of Contents

List of Figures v
List of Tables vii
Executive Summary ix
Introduction 1
Broodstock Histories 3
ESA-listed Endangered Snake River Sockeye Salmon 3
ESA-listed Threatened Snake River Spring/summer Chinook Salmon 4
Broodstock Coordination 6
Fish Culture Facilities 7
Facilities Selection 7
Seawater Facilities 9
Freshwater Facilities 11
Fish Culture Practices 15
Biosecurity Protocols 15
Rearing Densities 17
Loading Densities 17
Turnover Rates 19
Tank and Raceway Cover 19
Feed 19
Spawning Procedures 20
Egg Incubation 22
Egg and Fish Transportation 24
Snake River Sockeye Salmon 24
Snake River Chinook Salmon 26
Program Production 27
Snake River Sockeye Salmon 27
Snake River Spring/summer Chinook Salmon 27
Biological Metrics of NMFS Captive Reared Fish 33
Snake River Sockeye Salmon Culture 33

Chinook Salmon Culture 39
Adult Fish Transportation 47
Insights and Recommendations 50
References 53
Appendix A: Transport Details for Intersite Fish Transfers 59

List of Figures

Figure 1. Diagram of the path that fish follow in NMFS's Snake River sockeye salmon captive broodstock and rearing operations 4

Figure 2. Flow diagram illustrating ODFW's approach of coupling captive broodstock technology with conventional hatchery production to restore Grande Ronde spring Chinook salmon 5

Figure 3. IDFG's captive rearing approach to Salmon River spring/summer Chinook salmon rebuilding 5

Figure 4. Smolt-to-adult rearing tanks in main ESA seawater rearing building at MRS 10

Figure 5. Fry-to-presmolt rearing room at BCH 12

Figure 6. Smolt-to-adult rearing tanks at BCH 12

Figure 7. Raceways inside greenhouse type building used for final freshwater maturation of sockeye salmon 13

Figure 8. Incubation buckets in aluminum troughs at BCH 13

Figure 9. Illustrations of isolation egg incubator system and its individual components 14

Figure 10. Growth rate projection for Redfish Lake sockeye salmon reared in 10°C freshwater at BCH 20

Figure 11. NMFS annual production of prespawning sockeye salmon adults for release in Stanley Basin lakes 28

Figure 12. NMFS sockeye salmon eyed egg production available for recovery actions 28

Figure 13. Number of eggs NMFS retained each year for incorporation into its captive broodstock program 29

Figure 14. Number of eggs NMFS retained for incorporation into adult release production 29

Figure 15. Number of eggs and juvenile fish IDFG supplied each broodyear for incorporation into NMFS captive broodstock and rearing programs 30

Figure 16. Number of Salmon River spring/summer Chinook salmon adults produced from each broodyear for stock rebuilding activities in Idaho 30

Figure 17. Number of Grande Ronde River spring/summer Chinook salmon produced from each broodyear for stock rebuilding activities in Oregon 31

Figure 18. Number of Salmon River spring/summer Chinook salmon smolts transferred to MRS from Idaho 31

Figure 19. Number of Grande Ronde River spring/summer Chinook salmon smolts transferred to MRS from Oregon 32

Figure 20. Average survival from green egg to eyed egg stage of Redfish Lake sockeye salmon by spawn year at NWFSC facilities, 1994–2008 33

Figure 21. Average survival by broodyear from eyed egg stage to mature adult for Redfish Lake sockeye salmon captive broodstock reared at NWFSC facilities 34

Figure 22. Percent by broodyear of Redfish Lake sockeye salmon males in the captive broodstock program maturing as 2-, 3-, and 4-year-old adults 35

Figure 23. Percent of broodyear 1991–2004 female Redfish Lake sockeye salmon reared in freshwater at NWFSC facilities maturing as 2-, 3-, and 4-year-old fish 36

Figure 24. Average fork length for mature 3- and 4-year-old female Redfish Lake sockeye salmon spawned at NWFSC freshwater facilities, 2003–2007 36

Figure 25. Average fecundity of 3-year-old female Redfish Lake sockeye salmon captive broodstock females spawned at NWFSC facilities, 1994–2008 37

Figure 26. Average fecundity of 4-year-old female Redfish Lake sockeye salmon captive broodstock females spawned at NWFSC facilities, 1994–2008 38

Figure 27. Average number of eggs produced per gram of 3-year-old spawning Redfish Lake sockeye salmon captive broodstock, 1996–2008 38

Figure 28. Average green egg weight by spawn year for 3-year-old spawning Redfish Lake sockeye salmon captive broodstock, 2003–2008 39

Figure 29. Average eyed egg weight for 3-year-old spawning Redfish Lake sockeye salmon captive broodstock, broodyear 1991–2008 40

Figure 30. Average eyed egg weight for 4-year-old spawning Redfish Lake sockeye salmon captive broodstock, 1993–2008 40

Figure 31. Percent survival by broodyear for West Fork Yankee Fork stock of Salmon River spring/summer Chinook salmon at MRS 41

Figure 32. Percent survival by broodyear for Lemhi River stock of Salmon River spring/summer Chinook salmon at MRS 42

Figure 33. Percent survival by broodyear for East Fork stock Salmon River spring/summer Chinook salmon at MRS 42

Figure 34. Percent survival by broodyear for Lostine River stock of Grande Ronde River spring/summer Chinook salmon at MRS 43

Figure 35. Percent survival by broodyear for Catherine Creek stock of Grande Ronde River spring/summer Chinook salmon at MRS 43

Figure 36. Percent survival by broodyear for Grande Ronde River stock of Grande Ronde River spring/summer Chinook salmon at MRS 44

Figure 37. Average fork length of prespawning 3- and 4-year-old adult Salmon River and Grande Ronde River spring/summer Chinook salmon at MRS 45

Figure 38. Average weight of prespawning 3- and 4-year-old adult Salmon River and Grande Ronde River spring/summer Chinook salmon at MRS 45

Figure 39. Percent of Salmon River stocks of Snake River spring/summer Chinook salmon reared at MRS maturing as 2-, 3-, 4-, 5-, and 6-year-old fish 46

Figure 40. Percent of Grande Ronde River stocks of Snake River spring/summer Chinook salmon reared at MRS maturing as 2-, 3-, 4-, 5-, and 6-year-old fish 46

List of Tables

Table 1. NMFS biosecurity protocols for facilities operation 16
Table 2. Summary of adult fish transfer success from MRS facilities to Oregon and Idaho 48
Table A-1. Transport details for intersite fish transfers 60
Table A-2. Transport details for intersite fish transfers, Salmon River spring Chinook salmon 63
Table A-3. Transport details for intersite fish transfers, Snake River (Redfish Lake) sockeye salmon 65

Executive Summary

The goals of captive broodstock programs for Pacific salmon listed under the U.S. Endangered Species Act (ESA) include 1) rescuing small extant populations on the verge of extinction and 2) increasing population size in captivity so that sufficient numbers of individuals can be returned to their native habitat in attempts to rebuild natural populations to self-sustaining levels. Captive broodstock programs are a form of artificial propagation where salmon are reared through all or most of their life protected from predation and other forms of natural mortality. Theoretically, captive broodstock programs could increase the number of fish surviving to maturation by more than two orders of magnitude over survival in nature. However, at the start of these programs, full term survival to spawning adults was problematic and research and technological development was needed to reach the programs' potential. The National Marine Fisheries Service's (NMFS) Northwest Fisheries Science Center (NWFSC) captive broodstock programs for Redfish Lake sockeye salmon (*Oncorhynchus nerka*) and spring/summer Chinook salmon (*O. tshawytscha*) have been in operation for almost 20 years with notable success and a great deal learned. This report describes the facilities, fish culture practices, production, and biological metrics of these programs.

In 1991 Snake River sockeye salmon were listed as endangered under the ESA. That same year, NMFS and the Idaho Department of Fish and Game (IDFG) initiated a Bonneville Power Administration–funded cooperative captive broodstock for those sockeye salmon. After listing, the majority of the remaining anadromous population was taken into captivity for a gene rescue program. NMFS and IDFG maintained parallel captive broodstocks for this critically endangered evolutionarily significant unit. The dual broodstocks in separate federal and state facilities comprise a hedge against catastrophic loss of important components of the gene pool. This technical memorandum describes only the NMFS portion of the total broodstock effort. NWFSC provides full life cycle culture of Snake River sockeye salmon that includes freshwater and marine rearing, spawning, and egg incubation. Our cooperative program has successfully maintained fish in safety net captive broodstocks for more than five generations and is now providing a substantial number of juveniles to Idaho for reintroduction into their natural environment. The successful reintroduction effort has resulted in the return of several thousand sea-run adult sockeye salmon to Idaho's Stanley Basin.

Snake River spring/summer Chinook salmon were listed as threatened under the ESA in 1992. Subsequently in the mid-1990s, captive broodstock safety nets were initiated for three Idaho Salmon River and three Oregon Grande Ronde River populations. Oregon cooperators choose a traditional captive breeding approach for their Grand Ronde River stocks in which fish are reared to maturity at the NWFSC Manchester Research Station (MRS), Manchester, Washington, then transferred to the Oregon Department of Fish and Wildlife (ODFW) for spawning and rearing of offspring in conventional hatcheries for release as smolts that migrate to sea and return as sea-run adults. IDFG adopted a captive rearing approach for the Salmon River stocks in which fish are reared to adult in captivity, then returned to Idaho for release into their

natal streams for natural spawning. The NWFSC role for both captive broodstock programs was to provide seawater rearing during the marine phase of their life cycle.

NWFSC has freshwater and seawater facilities, enabling it to accommodate a range of environmental needs of anadromous fish over the course of their life cycle. NWFSC's Burley Creek Hatchery (BCH) is used for freshwater final maturation of prespawning adults, spawning, incubation, and fry-to-smolt rearing. Marine rearing during the smolt-to-prespawning-adult stages of the life cycle is conducted at MRS. BCH has pathogen-free ground water to minimize development of fish disease and facilitate fish transfer between fish health management zones. Seawater at MRS is filtered to five microns and disinfected with ultraviolet (UV) radiation. At both facilities, effluent water is treated with either ozone or UV radiation to eliminate potential pathogen transfer. Primary and secondary backup generators are employed at both facilities to ensure a constant delivery of power to pumps and tertiary backup oxygen is available to maintain fish during electrical maintenance and in the rare event of complete electrical failure. The fish are protected from predation and vandalism at both facilities by rearing in secure buildings. Artificial lighting in fish rearing areas follows a natural photoperiod for proper entrainment of natural circadian and seasonal rhythms, and slowly ramps up at dawn and down at dusk to avoid startle responses. Marine rearing at MRS is done in circular tanks ranging from 4.1 to 6.1 m diameter and freshwater rearing and final maturation occur in circular tanks ranging from 1.5 to 3.7 m and in 5.8 m long raceways at BCH.

NWFSC has adopted a suite of fish culture practices for its captive broodstocks that ensures excellent fish health, maximizes survival, minimizes the risk of inbreeding, and maximizes safety net population genetic diversity. These practices begin with stringent biosecurity protocols designed to prevent the spread of disease from local fish to the ESA stocks and among various ESA stocks. Stress is minimized by handling fish with extreme care such as minimizing de-watering of fish during transport and processing. Fish health is monitored daily and mortalities are examined for cause of death and to control any pathogen outbreaks early in their development. Additional proactive fish health measures include vaccination against *Vibrio anguillarum* before transfer to seawater and giving prophylactic and therapeutic erythromycin and azithromycin treatments as deemed prudent.

Stress is minimized by holding rearing densities below 8 kg/m^3 (0.5 lbs/ft^3) for immature fish and below 15 kg/m^3 (1.0 lbs/ft^3) when fish near maturity. Except for nonfeeding adults held in oxygen-supplemented water, loading densities are kept below 0.84 kg/L/min (7 lbs/gal/min) to ensure that the rearing environment does not induce stress that may lead to disease outbreaks.

Maturing and immature fish are identified and sorted each spring using ultrasound imaging to assess gonadal development. Maturing spring/summer Chinook salmon are transferred to their state of origin, where they are spawned artificially or released into the wild for natural spawning. Maturing sockeye salmon are transferred from seawater at MRS to freshwater at BCH in June and July. In September several hundred of the maturing sockeye salmon in fresh water are transported to Redfish Lake Idaho to spawn naturally. NWFSC retains approximately 300 maturing sockeye salmon each year for spawning at BCH.

For the Redfish Lake sockeye salmon program, standard spawning protocols are used to identify ripe females, strip gametes, and fertilize eggs. Gonadotropin-releasing hormone analog

implanting of almost all males is required to synchronize final maturation with females. All males and females are sampled for bacterial and viral pathogens at spawning. Fertilization crosses are conducted following an inbreeding-avoidance matrix using allele sharing coefficients developed by IDFG. Each female is mated with two males and when possible every male is mated with two females to reduce the loss of unique genes held by individuals of either sex. Fertilized egg incubation follows standard salmon culture protocols. Each full sibling lot is held in an individual down-well bucket incubator to minimize potential pathogen transfer.

NWFSC has transferred eyed eggs, smolts, and prespawning adults to facilities in Washington, Oregon, and Idaho. Through 2008, NWFSC has supplied 2,210 maturing adults and 1,922,700 eyed eggs to Idaho's Redfish Lake sockeye salmon program. As of July 2008, NMFS has reared 3,523 prespawning Chinook salmon that were delivered to Idaho for reintroduction to the Salmon River watershed and provided 4,031 adult Chinook salmon to ODFW. Eyed eggs are shipped in plastic mesh tubes wrapped in moist towels held in insulated containers. Eyed egg shipments are via air to Idaho and truck to Oregon. Prespawning Chinook salmon are transferred by truck from seawater at MRS to freshwater in Idaho and Oregon. Transport water consists of 25% seawater and 75% freshwater with fish density less than 0.06 kg/L (0.5 lbs/gal). Transport survival of captive broodstock fish has been excellent. There has been no mortality during 12 years of adult sockeye salmon transfer from NMFS facilities to Redfish Lake and of adult Chinook salmon transfer to ODFW's Bonneville Fish Hatchery. There was no mortality for 5 years of transfer of adult Chinook salmon to IDFG's Eagle Fish Hatchery.

Survival of sockeye salmon and Chinook salmon in the captive programs was substantially higher than expected in nature. Survival of sockeye salmon from the green-to-eyed stage at NMFS facilities averaged 62.2% between years 1994 and 2008. The average survival of sockeye salmon captive broodstock during the longer eyed-egg-to-mature-adult period was 61.7%. Overall green-egg-to-adult survival was 38.4%, which is at least two orders of magnitude higher than the estimated 0.3% or less survival expected for a relatively stable population in nature. The overall average survival of Chinook salmon (broodyears 1994–2003) was 58.8% for Salmon River stocks and 67.9% for the Grand Ronde River stocks.

The fish culture technologies described in this report have successfully maintained ESA-listed sockeye salmon and Chinook salmon stocks in safety net programs. The programs have maintained significant genetic diversity of the founding populations and generated large numbers of fish for use in restoration. Key factors leading to the success of safety net programs include pathogen-free water, onshore tanks, fully enclosed buildings, and rigorous biosecurity protocols. Rearing sockeye and Chinook salmon at low densities in large tanks is critical for high fish survival. Use of mating strategies designed to reduce loss of unique genetic traits and minimize inbreeding helps maintain genetic diversity. Maintenance of duplicate genetic groups in geographically separate facilities greatly reduces the potential for catastrophic loss of stocks. Rigorous fish transportation measures have led to excellent fish survival in moving fish among facilities. The technologies and standards developed for full life cycle captive salmon rearing have provided a valuable tool for rebuilding ESA-listed populations of Pacific salmon.

Introduction

This technical memorandum documents the current fish culture practices, technologies, and results that the National Marine Fisheries Service's (NMFS) Northwest Fisheries Science Center (NWFSC) utilizes for captive broodstock efforts for selected stocks of Pacific salmon (*Oncorhynchus* spp.) of the Columbia River basin listed under the U.S. Endangered Species Act (ESA). Since the early 1990s, NWFSC has been involved with three major captive broodstock efforts for Columbia River basin stocks: 1) ESA-listed endangered Snake River sockeye salmon (*O. nerka*), 2) three stocks of ESA-listed threatened Snake River spring/summer Chinook salmon (*O. tshawytscha*) from the Salmon River basin in Idaho, and 3) three stocks of ESA-listed threatened Snake River spring/summer Chinook salmon from the Grande Ronde basin in Oregon. Each of these efforts is partnered with the local, state, and tribal agencies involved with recovery planning and implementation actions for the individual stocks.

Captive broodstock programs are a form of artificial propagation. However, they differ from traditional hatchery programs in one important respect: fish are cultured in captivity for their entire life cycle (Flagg and Mahnken 1995). The high fecundity of Pacific salmon, coupled with their potentially high survival in protective culture, affords an opportunity for captive broodstocks to aid restoration efforts (Flagg et al. 1995a, Schiewe et al. 1997). The high egg-to-smolt survival offered by the protective culture environment is used to rapidly increase the number of spawning adults above that which would occur if they had been released to the environment and gone to sea. Most captive broodstock programs follow a breeding approach where captive maturing fish are spawned, offspring incubated, and egg-to-smolt stages released into native watersheds. A captive rearing approach is also sometimes utilized where maturing adults are released back into their natal streams to spawn naturally. All forms of captive broodstock programs can be viewed as gene rescue efforts to preserve and maintain the genetics of populations at risk of extinction or, if adequate source stock is available, at levels to supplement natural salmon populations.

At the initiation of the NWFSC programs, captive broodstock rearing of Pacific salmon was considered an evolving technology, without well-defined standards (Flagg and Mahnken 1995). Full-term rearing to adult was considered problematic: high mortality rates and low egg viability were common in previously attempted programs. Egg-to-adult survival rates generally ranged well below 30%, viability of eggs to the eyed stage from captive-reared brood was commonly only 30–60% compared to viabilities of more than 80% for the eggs from wild cohorts, and the size of captive-reared adults was generally smaller than that of wild fish (Flagg et al. 1995a).

During the evolution of NWFSC participation in the current captive broodstock programs, a number of engineering advances have occurred in available equipment and considerable research and development have been conducted on captive broodstock technologies (Swanson et al. 1996, Berejikian 2000, 2002, 2004, Berejikian and Nash 2001, 2003). Specialized captive broodstock rearing facilities were developed and refined. Additionally,

guidelines have been proposed covering when to implement and how to conduct a captive broodstock action (Pollard and Flagg 2004). The overall success of captive broodstock rearing has improved dramatically; egg-to-adult survival now routinely ranges 50–80%, egg viability has improved greatly to more than 75%, and size and body conformation of fish is often equivalent to the wild progenitors.

In this document, we describe the development and use of these captive broodstock technologies at NWFSC facilities and provide our current set of facility and culture guidelines on the health and well-being of captive broodstock fish. Although the information presented is specific to fish reared at NWFSC facilities, we believe the results can be generalized to expectations for other facilities following similar guidelines. Additional details on NMFS culture protocols and facilities are available in a series of annual reports to the Bonneville Power Administration (BPA) (Flagg 1993, Flagg and McAuley 1994, Flagg et al. 1996, 2001, Frost et al. 2003a, 2003b, 2005b, 2006, 2008a, 2008b, Maynard et al. 2003a, 2003b, 2005, 2006, 2007, 2009a, 2009b, 2009c).

Broodstock Histories

ESA-listed Endangered Snake River Sockeye Salmon

Snake River sockeye salmon were listed as endangered under the ESA in 1991 (Waples et al. 1991). The last known remnants of the stock return to Redfish Lake in the Sawtooth Valley of Idaho. Only minimal numbers of fish (0–8 per year) had returned to Redfish Lake in the years preceding the listing and the probability of extinction was high. On the basis of critically low population numbers, aggressive gene rescue actions were initiated during the year of ESA listing to stem extinction risks for Snake River sockeye salmon. These measures included taking a majority of the remaining population into captivity and initiating actions in the freshwater rearing habitat to promote survival of reintroduced fish (Flagg et al. 1995a, 2004). Participants included NMFS, Idaho Department of Fish and Game (IDFG), Shoshone-Bannock Tribes of Idaho, University of Idaho, and BPA.

At project initiation, it was recognized that the effective population size for establishment of the captive broodstock was likely to be extremely small. During the 1990s, a total of 16 wild fish (0–8 per year) returned to Redfish Lake. IDFG captured all returning fish and placed them in a captive broodstock program as a gene rescue effort (Flagg et al. 1995b). In addition, approximately 900 smolts and 25 residual sockeye salmon were captured for captive broodstock rearing in 1991–1993. To reduce the risk of catastrophic loss, the fish have been maintained at NWFSC and IDFG facilities. The program currently has fish that have been in captive broodstock culture for multiple generations. Core lineages are tracked by IDFG and mated so as to maintain genetic diversity (Kozfkay et al. 2008). On an annual basis, IDFG ships groups of approximately 450–1,000 eyed eggs to NWFSC for its portion of the captive broodstock program. The program has had success during the gene rescue phase and is currently transitioning to include a large supplementation effort (see Flagg et al. 2004, Hebdon et al. 2004, Peterson et al. 2008, Kozfkay et al. 2008).

The NMFS portion of the work includes captive broodstock and captive rearing as illustrated in Figure 1. The rearing of each year-class typically begins with the receipt of eyed eggs shipped from IDFG's Eagle Fish Hatchery (EFH), Eagle, Idaho, to NWFSC's Burley Creek Hatchery (BCH), Burley, Washington, in early winter. The eggs are then incubated until fry swimup, at which time they are ponded and reared in freshwater circular tanks until they reach the smolt stage. Some of the smolts are retained in freshwater whereas others are sent to NWFSC's Manchester Research Station (MRS), Manchester, Washington, for seawater rearing. The fish are maintained in culture until they begin to show signs of maturation. In most years, slightly more than one-half of the maturing fish are returned to Redfish Lake, where they are allowed to spawn volitionally in a captive rearing program. The remainder of the fish are spawned at BCH with the resulting eggs shipped to restocking programs when they reach the eyed stage. Historically the majority of stocked eggs have gone into egg box programs. The remainder are shipped to production hatcheries where the fish are grown for restocking as summer fry (discontinued), autumn presmolts, and spring smolts.

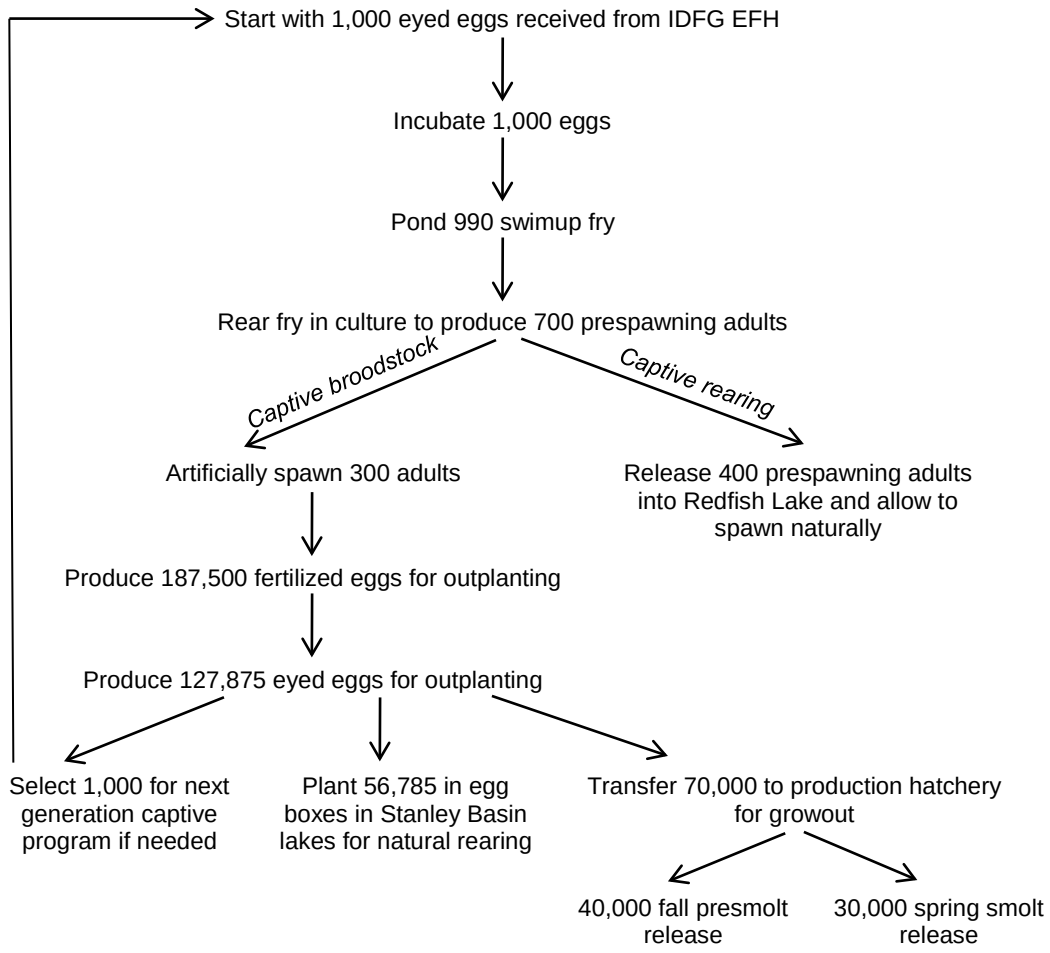

Figure 1. Diagram of the path that fish follow in NMFS's Snake River sockeye salmon captive broodstock and rearing operations. Numbers reflect typical survivals among life stages.

ESA-listed Threatened Snake River Spring/summer Chinook Salmon

Snake River Chinook salmon were listed as threatened under the ESA in 1992 (Mathews and Waples 1991). In spring 1995, NMFS, IDFG, U.S. Fish and Wildlife Service, Oregon Department of Fish and Wildlife (ODFW), Confederated Tribes of the Umatilla Indian Reservation, and Nez Perce Tribe initiated captive broodstocks as part of conservation efforts for ESA-listed stocks of Snake River spring/summer Chinook salmon. Two approaches were identified: a conventional captive broodstock approach (Flagg and Mahnken 1995) and a less conventional captive rearing approach (Berejikian et al. 2004). ODFW choose the traditional captive breeding approach, where maturing fish are spawned and the offspring incubated, reared to the smolt stage, then released into their native watersheds (Figure 2). IDFG adopted a captive rearing approach where maturing adults are released back into their natal streams to spawn naturally (Figure 3). Both approaches shared the same goal of maintaining Snake River Chinook salmon metapopulation structure by preventing year-class failures and local extinctions.

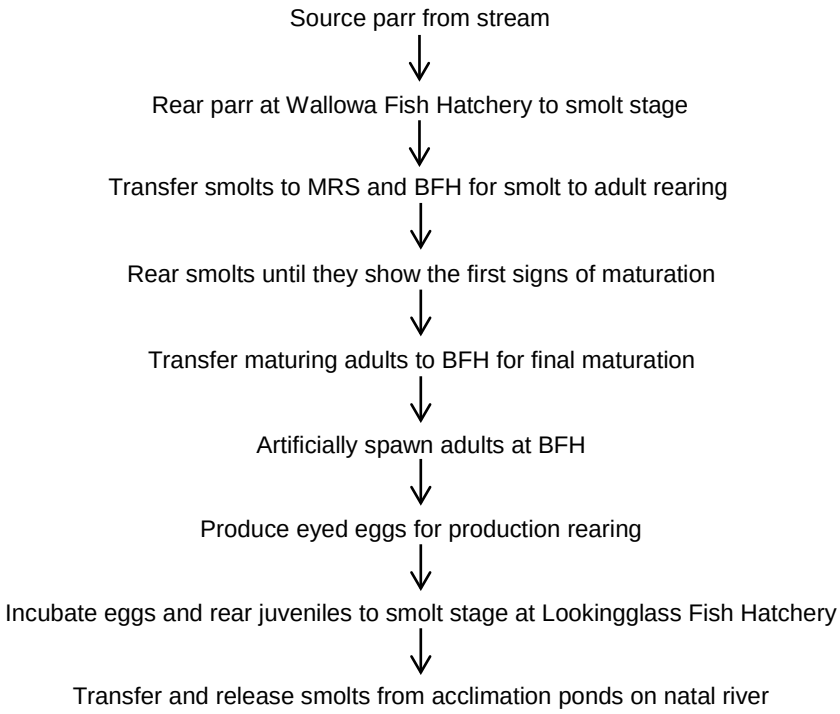

Figure 2. Flow diagram illustrating ODFW's approach of coupling captive broodstock technology with conventional hatchery production to restore Grande Ronde spring Chinook salmon. MRS = Manchester Research Station, BFH = Bonneville Fish Hatchery.

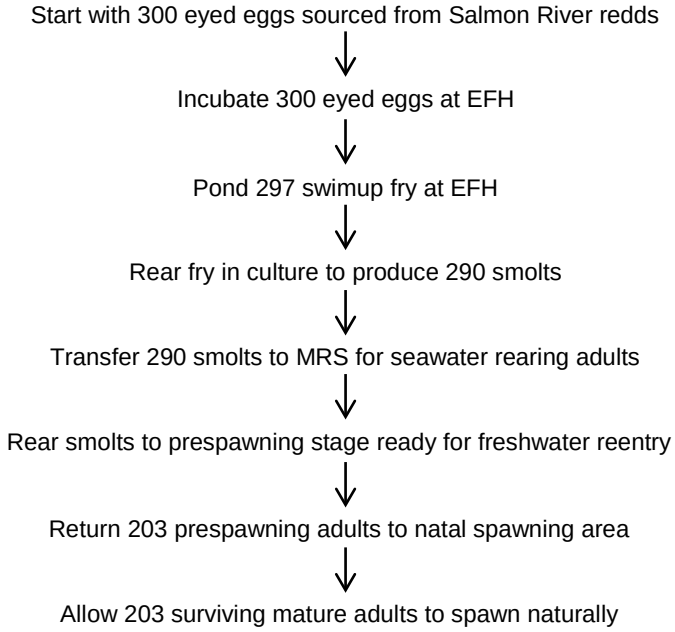

Figure 3. IDFG's captive rearing approach to Salmon River spring/summer Chinook salmon rebuilding. EFH = Eagle Fish Hatchery.

The Oregon captive broodstock program focused on three stocks (upper Grande Ronde River, Catherine Creek, and Lostine River) captured as juveniles from the Grande Ronde River basin. Idaho's Snake River captive broodstock program included three stocks (Lemhi River, East Fork Salmon River, and West Fork Yankee Fork) captured as eyed eggs or juveniles from the Salmon River basin (Hoffnagle et al. 2003, Venditti et al. 2002). After collection, the fish or eyed eggs were transported to the IDFG EFH or the ODFW Lookingglass Fish Hatchery, Elgin, Oregon, for freshwater rearing until they reached the smolt stage. Most smolts from the IDFG program (about 150 stock) and one-third to one-half of the smolts from the ODFW program (about 450 stock) were transferred to the NWFSC marine rearing facilities at MRS. The fish were reared in seawater until they began to show signs of maturity. They were then transferred back to freshwater facilities in Idaho and Oregon for final maturation. The mature fish in the IDFG captive rearing program were released into enclosed stream sections in Salmon River streams to spawn naturally. ODFW artificially spawned the mature fish in its captive broodstock program at its Bonneville Fish Hatchery (BFH), Cascade Locks, Oregon, to produce juveniles for smolt releases in its Grande Ronde Basin supplementation program. ODFW, IDFG, and NMFS investigators are comparing the relative success of the two reintroduction strategies and the effect of freshwater versus seawater rearing.

Broodstock Coordination

The Redfish Lake sockeye salmon captive broodstock program is overseen by the BPA-chaired Stanley Basin Sockeye Technical Oversight Committee. The captive rearing program for Snake River spring Chinook salmon and the Grand Ronde Basin spring Chinook salmon captive broodstock program are overseen by the BPA-chaired Chinook Salmon Captive Propagation Technical Oversight Committee and the Oregon Technical Oversight Team, respectively. All three groups contain representatives of state, federal, tribal, and private groups involved in the conservation of the respective ESA-listed stocks. Members meet regularly to review program activities and provide overall recommendation for collection, rearing, and release strategies.

Fish Culture Facilities

Facilities Selection

At the initiation of the first of the NWFSC captive broodstocks for ESA-listed Columbia River basin fish (Redfish Lake sockeye, 1991), little was known regarding methods to ensure survival of fish to adulthood in captive culture. Most past attempts at captive broodstock culture of Pacific salmon indicated that full-term culture in pathogen-free freshwater generally resulted in much higher survival to spawning and much higher percentages of viable gametes than culture in seawater (Mahnken and Flagg 1995). Therefore, full-term freshwater rearing in pathogen-free water was chosen for initiation of this sockeye endangered species rearing program (Flagg et al. 1995a, Schiewe et al. 1997).

Nonetheless, anadromy with smolt-to-adult rearing in seawater is the normal life history for sockeye and other Pacific salmon. Anadromous spring/summer Chinook and sockeye salmon spawn in freshwater, spend 1 to 2 years rearing in streams and rivers, then migrate to sea as smolts where they spend the majority (2–4 years) of their life (Burgner 1991, Healey 1991). When they begin to mature, adults migrate back to their natal streams, rivers, or lakes to spawn. Salmon are thought to migrate to the sea because it provides better feeding and growing conditions, even though it imposes greater predation risk. Thus potential survival reductions are offset by females producing more eggs and males developing enhanced sexual capabilities (e.g., size and milt volume) compared to population members that remain in freshwater. In addition, anadromous populations, with a portion of their members at sea each year, have an increased chance of survival when natural catastrophes (volcanic ash falls, drought years, etc.) eliminate all freshwater residents.

Anadromy is the key life history characteristic of Pacific salmon that enabled them to colonize the vast vacant areas west of the Continental Divide after glacial recession. The importance of seawater rearing during the marine phase of the salmon life cycle is recognized and called for by the NMFS Interim Standards for the Use of Captive Broodstocks to eliminate possible selection against anadromous traits that might occur with full-term freshwater rearing (Pollard and Flagg 2004). Therefore, tests were initiated using a non-ESA-listed stock to compare freshwater versus seawater culture to adulthood for sockeye salmon and to establish the facilities criteria for the Redfish program (Flagg et al. 1996). At the initiation of these studies in 1992, it appeared probable that many past husbandry problems in seawater were related to culture in net pens exposed to near surface environmental conditions. Several environmental factors critical to survival (e.g., temperature, salinity, toxic plankton blooms) are more variable at the surface than in the deeper marine waters preferred by most salmonids. In addition, fish held in net pens are at risk of escape, natural catastrophes, disease transmission, and predation from marine mammals and birds.

Studies were carried out in seawater at the NMFS MRS and in freshwater at a MRS satellite facility located at the University of Washington's Big Beef Creek (BBC) Fisheries

Research Station near Seabeck, Washington. Studies comparing seawater and freshwater rearing were conducted with two year-classes (1990 and 1991 brood) of Lake Wenatchee sockeye salmon. Three replicates of approximately 300 yearling smolts were placed in each of the following environments: 1) 4.1 m diameter circular fiberglass tanks supplied with fresh well water at 10°C at BBC (freshwater tank [FWT] treatment); 2) 4.1 m diameter circular fiberglass tanks supplied with pumped, filtered, and ultraviolet (UV) radiation–sterilized seawater at MRS (saltwater tank [SWT] treatment); and 3) 4.9 m square seawater net pens at MRS (saltwater pen [SWP] treatment). Water depth in each rearing environment was adjusted to provide approximately 12 m^3 of fish rearing space per container.

As described in Flagg et al. (1998), for the entire 2-year study sockeye salmon reared in the freshwater tanks had generally higher survival and growth rate compared to the seawater groups. Although for the 1990 brood Lake Wenatchee sockeye salmon, there was no significant difference in survival to adult (32% FWT, 35% SWT, and 26% SWP, $P > 0.05$), survival to spawning for the 1991 brood averaged 88% for FWT, 61% for SWT, and 22% for SWP, ($P < 0.02$). The high mortality in all groups of the 1990 brood was attributed to severe infections of *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease (BKD). For the 1991 brood, BKD was noted as a severe problem only in fish reared in net pens. For both broodyears, growth differences ($P < 0.05$) were noted, with the treatments ranked FWT > SWT (69.5% of FWT weight) > SWP (65.9% of FWT weight) for the 1990 brood and FWT > SWT (70.8% of FWT weight) = SWP (78.0% of FWT weight) for the 1991 brood. There were no significant differences ($P > 0.10$) in eyed egg survival for the 1990 brood Lake Wenatchee sockeye salmon (50% FWT, 42% SWT, 45% SWP). Significant differences ($P < 0.001$) in eyed egg survival were noted for 1991 brood spawners between the FWT and SWT and SWP treatments (67% FWT, 43% SWT, 40% SWP).

The test data indicated that culture in seawater net pens was a poor choice for sockeye salmon and probably most other captive broodstock programs. Full-term culture to adult in freshwater appeared to provide lowest disease incidence and highest viability. The study indicated that if seawater rearing was deemed necessary to preserve anadromy, then rearing in filtered and sterilized seawater appeared to be the best choice for captive broodstock programs (Flagg et al. 1998). Therefore, with BPA support, appropriate facilities were constructed at MRS to rear Redfish Lake sockeye salmon captive broodstocks in freshwater and seawater protective culture conditions. This document describes the facilities in their current state.

Over the duration of the project, changes have been made at the facilities as the programs evolved. For example, the seawater portion of the facilities was expanded with the onset of the Chinook salmon captive broodstocks in the mid-1990s. Likewise, the freshwater rearing facility was physically relocated from the University of Washington BBC facility to the MRS BCH. However, the basic design elements and functions have remained the same (For specifics of facilities status during various stages of development, see BPA Web-based annual reports for the NMFS Redfish Lake sockeye salmon and Snake River Chinook salmon captive broodstock programs, online at www.efw.bpa.gov/IntegratedFWP/technicalreports.aspx.).

Seawater Facilities

MRS is located near Manchester, Washington, on Clam Bay, a small bay adjoining the central basin of western Puget Sound. A major advantage of the site is its excellent seawater quality. Clam Bay is a major tidal mixing zone between Sinclair and Dyes inlets to the west and waters of central Puget Sound to the east. Annual seawater temperature at the site normally ranges 7–15°C and salinity ranges 26–29 ppt. The high quality seawater environment, combined with a 250 m pier made available to the station by the U.S. Environmental Protection Agency Region X Laboratory, make MRS an excellent site for the culture of anadromous salmonids during their marine life history phase.

A constant source of processed seawater ensures successful captive rearing survival. A 60 hp centrifugal pump supplies approximately 5,000 L/min (1,250 gal/min) of seawater through a 700 m pipeline from the east end of the pier to the station's land-based facilities. The system is outfitted with a backup 50 hp pump in case of primary pump failure. The pumps are attached to a floating platform that remains at a constant level above the water surface with rising and falling tides. This floating platform is equipped with a 1.1 m × 2.3 m × 1.7 m deep sump located beneath the pumps. The sump is fitted with a 0.6 m diameter and 3.7 m long snorkel intake that draws in seawater 4.9 m below the surface. The intake is equipped with 1.9 cm × 3.2 cm diamond mesh screen to prevent larger objects being sucked into the pump wet end. An alarm system monitors the pumps and electrical supply and is tied into an automatic dialer system linked to cellular telephones. Redundant emergency generators (330 KW each) are automatically serially activated in the event of a power failure.

The seawater supplied to the station is processed to prevent naturally occurring bacterial pathogens and parasites from entering the rearing tanks. Primary filtering consists of six 1.9 m^2 deep bed fiberglass sand filters. The filters use either number 20 grade sand or number 25 glass micro beads as filter media. The sand filters remove all materials greater than 20 microns in diameter, and a portion of smaller materials. Immediately after leaving the sand filters, the seawater enters 2 filter systems, which contain a total of 148 cartridge filters ensuring removal of all particles larger than 5 microns. To control for pathogens, the seawater next passes through stainless steel UV chambers where it is irradiated with a UV dosage of 55,000 to 90,000 microwatts per second per cm^2. After UV filtration, the seawater goes directly to rearing tanks. Sensors monitor water flow and pressure through the seawater filtration system.

Land-based seawater captive broodstock rearing is conducted in two secure buildings with 1,280 m^2 and 400 m^2 of floor space for fish rearing tanks. The 1,280 m^2 facility houses twenty 6.1 m diameter circular fiberglass tanks (Figure 4). The 400 m^2 seawater laboratory contains six 4.1 m diameter circular fiberglass tanks. Water depth in the 6.1 m diameter tanks is generally maintained around 1.5 m and in the 4.1 m tanks near 0.9 m. Before entering fish rearing tanks, the processed seawater passes through packed column degassers which are filled with plastic bio-rings that break up the water, boosting dissolved oxygen (DO) levels and allowing off-gassing of excess nitrogen, which can be present in pumped water situations. Water leaving the packed columns can either go directly to individual tanks within the captive broodstock rearing building or a portion of the seawater can be diverted through chillers. Seawater lines that have been inactive are thoroughly flushed for 24 hours before being used to supply water to culture tanks. This practice eliminates harmful metabolites that can develop in

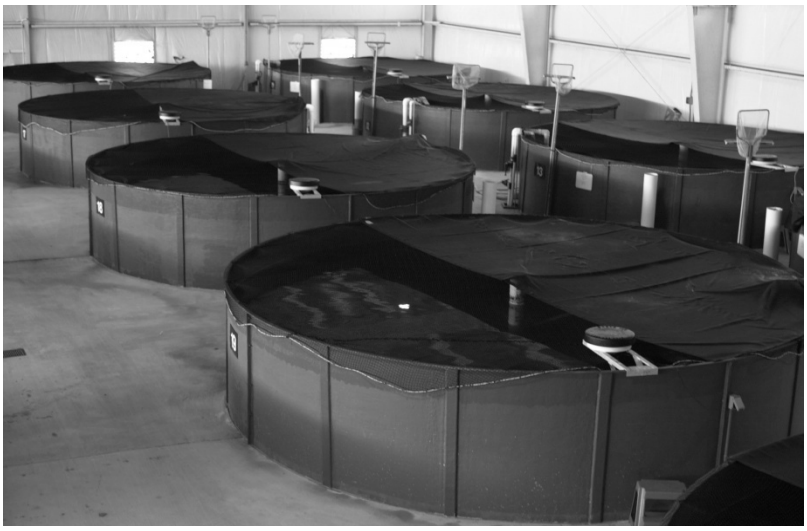

Figure 4. Smolt-to-adult rearing tanks in the main ESA seawater rearing building at MRS.

the lines when they are inactive. Summer water temperature in Clam Bay can reach 15°C, which can be detrimental to maturing salmonids. Generally, two 35-ton chillers with titanium plate heat exchangers are brought online in summer to ensure that water remains below 13°C. In addition, each tank is directly supplied with oxygen to maintain life support in the event of an interruption in water flow.

Effluent seawater from rearing tanks is treated before it is returned to Puget Sound. MRS complies with Washington State Department of Fish and Wildlife (WDFW) quarantine certification standards by depurating all effluent from the captive broodstock rearing areas by ozone treatment. The ozone treatment system consists of four parts, 1) a below ground 3-chamber 25,000 gallon concrete depuration tank, 2) a recirculation pump system to move water from the depuration tank through the ozone building where the water is injected with ozone gas, 3) an air-cooled 6-module ozone generator capable of producing 360 g/hr of ozone, and 4) an ozone destruct system to control ozone off-gassing.

Adopting lighting controls that avoid light shock reactions and maintain fish on a natural photoperiod promote good physiological condition and the entrainment of natural circadian rhythms (see Heinen 1998 for review). Captive broodstock buildings at MRS are equipped with an automated Lutron system (Lutron Electronics Co. Inc., Coopersburg, Pennsylvania) that slowly (over 20 minutes) ramps up incandescent lights at dawn and down at dusk to avoid light shock reactions. All rearing areas have been equipped with either windows or transparent panels to provide some natural light. A series of light readings (four inside and four outside) were taken at midday on 15 June 2010 to compare light levels inside and outside of building 13. The mean light level outside building 13 was 28,775 ±1,242 SD lux and was more than 3 orders of magnitude higher than the 47 ±5 SD lux recorded inside building 13. Interior or exterior lights that might inadvertently light rearing and incubation areas are turned off at dusk and not turned on until dawn to ensure natural photoperiod entrainment. Security measures to protect fish and property include water flow, fire, and intruder alarms. Alarms are monitored through a security

system linked to pagers and cellular telephones. A backup generator automatically activates during power failures.

Freshwater Facilities

Freshwater rearing is conducted at the MRS BCH satellite facility near Burley, Washington (≈21 km from Manchester). The facility includes two buildings and a portable greenhouse for fish rearing. A 613 m^2 building contains nine 3.7 m postsmolt to adult rearing tanks and ten 1.5 m diameter swimup fry to presmolt rearing tanks (Figure 5 and Figure 6). A 256 m^2 building contains four 5.8 m × 1.2 m × 1.2 m tall grey raceways for maturing adult fish and four 3.7 m grey circular tanks for rearing postsmolt to adult fish. The 189 m^2 portable greenhouse building contains ten 5.8 m × 1.2 m × 1.2 m tall grey raceways for holding maturing salmon under near natural light levels (Figure 7). The water depth is maintained at 1.0 m in the 3.7 m tanks, 0.5 m in the 1.5 m tanks, and 0.8 m in the 5.8 m raceways. Each raceway is supplied with hatchery well water and supplemental oxygen and is covered one-quarter to one-third of its length with a dark tarp to provide shelter for the fish to seek refuge. A separate incubation room accommodates aluminum troughs with approximately 300 Novotny et al. (1985) type downwell incubators for isolated egg incubation (Figure 8 and Figure 9).

The hatchery is supplied with approximately 2,000 L/min of high quality 10°C well water pumped from two wells. Two additional wells are on site to allow well cycling and provide backup. Before distribution to the rearing tanks, the water flows through a packed column degassing tower that removes excess nitrogen from the pumped water and raises DO levels to a prefish baseline of about 10.3 ppm (about 90% saturation). Water supplied to incubation and fry rearing can be diverted through a chiller to decrease the water temperature to 5°C to regulate the fish developmental rate. A bulk liquid oxygen tank supplies oxygen through air stones to the rearing tanks for supplementation and for life support in the event of water flow disruption. Effluent from the hatchery is depurated through a settling basin and UV irradiation system, per WDFW quarantine requirements. Entry to BCH is limited by a 2.2 m chain link fence topped with barbed wire.

Low levels of natural lighting and photoperiod are maintained in the rearing areas by screened windows. Measurements made between 9 April and 21 April 2009 indicated area lighting ranged from 32 to 129 lux during daylight hours. The fish rearing areas at BCH are equipped with an automated Lutron fluorescent light system and a sunlight tracking timer (Suntracker, Paragon Technologies, Two Rivers, Wisconsin) that slowly (over 10 minutes) ramps lights up at dawn and down at dusk. Interior or outside lights that might inadvertently light rearing and incubation areas are turned off at dusk and not turned on until dawn to ensure natural photoperiod entrainment. Security measures to protect fish and property include water flow, fire, and intruder alarms. Alarms are monitored through a security system linked to cellular telephones. A backup generator automatically activates during power failures.

Figure 5. Fry-to-presmolt rearing room at BCH.

Figure 6. Smolt-to-adult rearing tanks at BCH.

Figure 7. Raceways inside the greenhouse type building used for final freshwater maturation of sockeye salmon.

Figure 8. Incubation buckets in aluminum troughs at BCH.

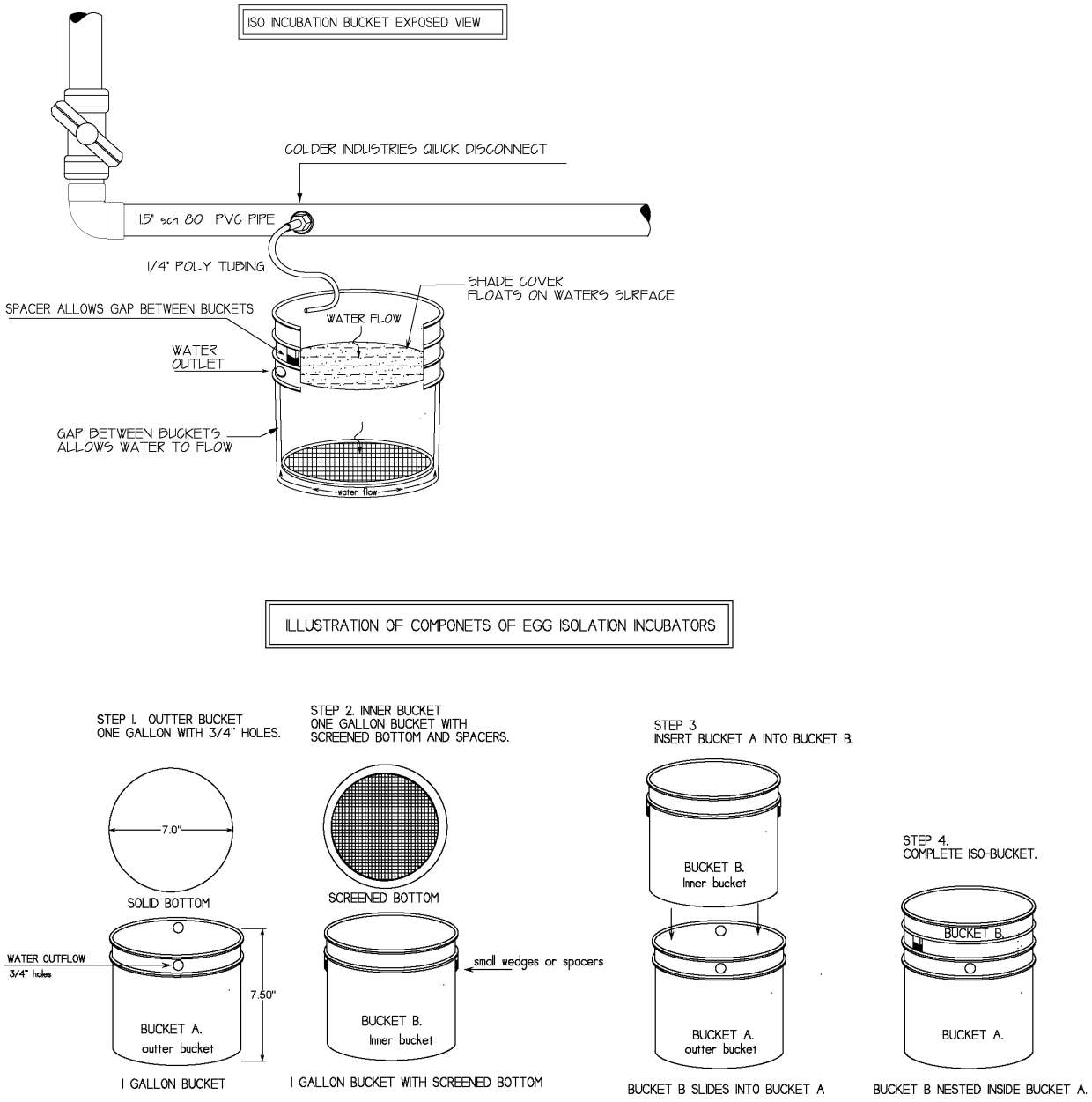

Figure 9. Illustrations of the isolation egg incubator system and its individual components.

Fish Culture Practices

Captive broodstock fish are reared at BCH and MRS using standard fish culture practices and approved therapeutics (for a general overview of methods, see Leitritz and Lewis 1976, Piper et al. 1982, FRED 1983, McDaniel et al. 1994, Schreck et al. 1995, Pennell and Barton 1996). Fish culture practices conform to the husbandry requirements detailed in ESA Section 10 Propagation Application for Permit 1148 for NMFS rearing of ESA-listed Snake River sockeye salmon. This permit specifies the number of fish that can be reared and released. It requires the use of prudent fish culture practices that maximize survival and minimize the risk of artificial selection in the hatchery environment. These include maintaining fish in water to the maximum extent possible during handling, providing adequate water replenishment in holding units, and not handling fish when water temperatures exceed 20°C. The permit specifies that NWFSC must conduct coordinated planning under the Stanley Basin Sockeye Technical Oversight Committee and that fish taken into the program be selected to represent the entire genetic spectrum of the founding population.

Biosecurity Protocols

Biosecurity practices are in place to prevent the spread of diseases from local fish to the ESA stocks and from one stock to another within the ESA program (Table 1). Housing fish within a fully enclosed building and rearing them on treated water is essential to ensure that pathogens from wild fish do not reach the ESA captive broodstocks. For the seawater system, an insight developed over the course of operations was to ensure a direct linkage (both on or both off) between the pumps and the UV treatment to ensure that untreated water was not pumped into ESA tanks. In recent years, we prohibited the holding of salmonids in facility net pens located near the intake pumps to reduce potential salmonid pathogens from entering the water supply.

Biosecure culture practices form the basic approach to prevent pathogens from being spread from one cultured stock to another. Separate brushes and nets are provided for each pool; staff use a new pair of disposable gloves per pool when brushing pools or removing mortalities. All equipment is disinfected in 100 ppm iodophore for a minimum of 30 minutes before being moved to a new pool. Disinfection includes crowder screens, nets, transfer tubes, anesthetic tank, tables, weighing pan, scale, passive integrated transponder (PIT) tag reader equipment, waders, and rain gear. Personnel are expected to change rain gear as they move between pools to provide proper disinfection time. Shower curtains are placed around anesthetic tanks during fish sampling and transfer to prevent splashed water from reaching adjacent pools. Adjacent pools may be temporarily covered with disinfected plastic when there is a risk of cross-contamination. After fish handling, the floor is sprayed with an iodophore disinfectant.

In general, the fish are handled with extreme care and kept in water to the maximum extent possible during transport and processing procedures. This includes the use of a water-filled fish transfer tube for moving fish between buildings at BCH. The 3.7 m diameter rearing

Table 1. NMFS biosecurity protocols for facilities operation. These procedures are performed on a continuous basis throughout the program.

- Maintain stocks in fully enclosed buildings.
- For seawater systems, filter incoming water to 5 microns then treat with 55,000–90,000 microwatts/sec of UV radiation. Do not culture salmonids in net pens near pump intake.
- For freshwater systems, use well (ground) water supplies.
- Maintain rearing densities below 8 kg/m^3 and loading densities below 0.84 kg/L/min.
- Provide chilled water (below 12°C) during summer high water temperatures.
- Provide backup power supply for pumps and disinfection system tied together to ensure seawater does not flow to tanks unless UV system treatment system is active.
- Maintain zoned rearing areas for individual stock groupings (e.g., Idaho sockeye vs. Chinook salmon).
- Use foot baths with 100 ppm iodophore at all building access points and between individual zoned areas.
- Use separate crowder screens for each stock grouping (e.g., Idaho sockeye vs. Chinook salmon).
- Use separate waders and raingear for each stock grouping (e.g., Idaho sockeye vs. Chinook salmon).
- Use separate brushes and nets for each pool.
- Use a new pair of disposable gloves per pool when brushing pool or picking mortalities.
- Iodophore disinfect rearing pools (100 ppm for 30 min).
- Iodophore disinfect all sampling equipment between pools (100 ppm for 10 min), including:
  - Crowder screens
  - Nets and hauling boots
  - Anesthetic tank
  - Table
  - Weighting pan, scale, and table
  - PIT tag reader equipment
  - Rain gear—it will be changed between pools to increase disinfection time in iodophore.
- Place shower curtain around anesthetic tanks during sampling to control splash contamination.
- Spray down floor areas and working surfaces with idiophore after fish handling.

tanks are constructed to hold at least 15 cm of water when the external standpipes are removed. This minimized the chance of fish being accidentally dewatered during tank draining or flushing. As experience has taught us, this safety device that prevents complete dewatering of the tank must be removed to allow 100% drawdown when tanks are disinfected to ensure all disinfectant is flushed from the tank. Low levels of natural and artificial lighting and natural photoperiod are maintained in the rearing areas by screened windows and a sunlight-tracking timer on the rearing area lighting system.

Fish health is monitored in several ways. Fish are observed daily for feeding response, external condition, and behavior as initial indicators of developing problems. Indicators include signs of lethargy, erratic swimming, side swimming, flashing, unusual respiratory activity, body surface abnormalities, and unusual coloration. Dead or morbid fish are removed immediately, bagged, PIT tags read, and submitted for pathology screening. A fish pathologist performs necropsies to determine cause of death. Infectious disease screening includes collection of kidney tissue, which is subjected to enzyme-linked immunosorbent assay (ELISA) to determine

BKD infection. Virology screening is performed on the kidney samples using a Chinook salmon epithelial cell tissue culture–based assay in order to detect infectious hematopoietic necrosis virus (IHNV) and viral hemorrhagic septicemia virus (VHSV). Any tissue culture monolayer showing signs of cytopathic effects is then subjected to polymerase chain reaction–based identification of the virus species. Samples from other overt lesions are also cultured on a variety of laboratory medium and subjected to microbial analysis.

Other common bacterial pathogens that may be encountered include furunculosis (*Aeromonas salmonicida*), vibriosis (*Vibrio anguillarum*), and members of the *Flavobacterium* genus. Typically, when a treatable pathogen is either detected or suspected, the NMFS fish pathologist prescribes appropriate prophylactic and therapeutic drugs (e.g., oxytetracycline or erythromycin). Azithromycin (an erythromycin derivative) is also used to treat BKD in *R. salmoninarum*–infected populations. Medication is either mixed with feed or injected, with dosage based on fish weight. Prior to transfer to seawater, fish in the adult release group receive an injection of the *V. anguillarum* bacteria vaccine. In addition, maturing fish are injected with erythromycin as a prophylactic approximately 1 month prior to spawning. The injection is administered interperitoneally at the base of the ventral fins at a dose of 20 or 30 mg/kg of fish wet weight. Ovarian fluid and milt from spawning adults are also screened with the BKD-ELISA and for IHNV and VHSV.

Rearing Densities

At our ESA captive broodstock culture facilities, rearing density is maintained less than 8 kg/m^3 (0.5 lb/ft^3) for most of the juvenile-to-adult period. This value is on the low end of the 4.8 to 32.0 kg/m^3 (0.3 to 2.0 lb/ft^3) maximum allowable range Senn et al. (1984) established for production hatcheries. Senn et al. (1984) production hatchery criteria cover fish ranging in size from 0.38 to 64.4 g and allow rearing density to increase as fish grow within this size range. Given the increased postrelease survival derived from rearing juvenile production fish at lower densities (see Maynard et al. 1995 for review), it was determined that setting the rearing density for immature ESA-listed fish in a safety net conservation program at or below the lowest values used in typical production programs would be the conservative approach. Therefore, we set the immature rearing density criteria at 8 kg/m^3 (0.5 lb/ft^3).

As the fish approach maturity, rearing density is allowed to increase to 15 kg/m^3 (1 lb/ft^3). This increase in allowable holding density results from the lower metabolic wastes associated with maturing fish that are no longer feeding. As with juveniles, a conservative approach is taken, with the established value being about one-half to two-thirds of the accepted 30 kg/m^3 (1.9 lb/ft^3) and 24 kg/m^3 (1.5 lb/ft^3) density established for the respective holding of ocean-returned spring Chinook and sockeye salmon adults in flow through raceways and rectangular ponds at production hatcheries (Senn et al. 1984).

Loading Densities

Based on practical experience and published literature, loading densities for ESA captive broodstocks should not exceed 0.84 kg/L/min (7 lb/gal/min), except for nonfeeding maturing adults with oxygen supplementation. In practice, loading densities at BCH in freshwater tanks ranged from 0.24 kg/L/min (2 lb/gal/min) to 0.84 kg/L/min (7 lb/gal/min) for active feeding fish,

while raceway loading densities at BCH reached a maximum of 1.85 kg/L/min (15.4 lb/gal/min) with nonfeeding maturing adults and supplemental oxygen. Seawater loading densities reached a maximum of 1.08 kg/L/min (9 lb/gal/min).

Loading density targets were derived based on the following literature review. Based solely on the theoretical oxygen requirements, loading densities for large salmon (0.5–5 kg, 1–10 lb) in seawater should be set in the 0.84 to 1.44 kg/L/min (7–12 lb/gal/min) range according to Liao (1971). However, the presence of metabolic wastes, decaying food, and the control of pathogenic organisms lead most authorities to recommend lower loading densities. Piper et al. (1982) recommend that enhancement facilities rearing Chinook salmon for smolt release should be maintained at loading densities in the 0.14 to 1.56 kg/L/min (1.2–13 lb/gal/min) range. These loading density values were derived from Wedemeyer and Wood (1974) and vary with fish size and water temperature. Wedemeyer and Wood's loading density recommendations for the largest (15 fish/lb) size category they considered are 0.66 kg/L/min (5.5 lb/gal/min) at 17.2°C (63°F) and 1.56 kg/L/min (13 lb/gal/min) at 3.3°C (38°F).

Senn et al. (1984) also consulted Wedemeyer and Wood (1974) for generating their loading density recommendations. However, Senn et al. (1984) modified the recommendations so that they can be applied to larger juveniles. Senn et al. (1984) recommend that loading density not exceed 0.72 kg/L/min (7.2 lb/gal/min) for Chinook salmon that are 75.6 g (6 fish/lb) at 14.4°C (58°F).

Loading density recommendations for Chinook salmon reared in enhancement facilities are also provided in Pennell and McClean (1996), who established flow needs based on fish size, water temperature, feed rate, and fish quality. Their A level recommendations for fish to be released as ocean-ranched smolts range from a low of 0.20 kg/L/min (1.67 lb/gal/min) for 20 g fish being fed 2.23% body weight per day at 15°C up to 1.16 kg/L/min (9.68 lb/gal/min) for 20 g fish that are not being fed and being held at 10°C. These numbers are lower than those presented in Piper et al. (1982). Piper et al. (1982), Senn et al. (1984), and Pennell and McClean (1996) loading density recommendations should be reduced in seawater because oxygen content at any given temperature is lower in seawater than in freshwater.

Wood (1979) observed that significant water quality problems develop when Chinook salmon are reared in loading densities above 0.48 kg/L/min (4 lb/gal/min). Wood (1979) observed bacterial gill disease infections in Chinook salmon reared in ponds when loading densities exceed 0.48 kg/L/min (4 lb/gal/min). Bacterial gill disease infections were observed at loading rates of 0.60 to 0.72 kg/L/min (5−6 lb/gal/min) in standard raceways when fish were at a size of 90 to 110 fish/lb (5.0−4.1 g). Wood (1979) suggested that larger fish were more tolerant of higher loading rates and avoided developing bacterial gill disease. Piper et al. (1982) noted that parasite infections become uncontrollable when loading density in steelhead (*Oncorhynchus mykiss*) ponds exceeded 0.84−0.96 kg/L/min (7−8 lb/gal/min).

A general relationship exists between water flow, feeding rate, and sanitary conditions in fish rearing vessels. Prolonged feeding rates in excess of 0.019 kg/day/L/min (0.150 lb/day/gal/min) of inflow can lead to the outbreak of bacterial gill disease in juvenile Chinook salmon in freshwater (Fowler 1989). The relationship between feed and flow limits the rate at which fish can be grown when water flow is limited.

Several loading density recommendations have been made for holding nonfeeding adult salmon that have returned from the sea and are maturing in freshwater. Pepper (1984) recommended 2.5 L/min/kg (0.30 gal/min/lb) as a satisfactory flow rate for holding nonfeeding Atlantic salmon (*Salmo salar*) brood. Unspecified lower loading densities were recommended for aquaculture brood that are feeding. Senn et al. (1984) recommended 1.80 kg/L/min (15 lb/gal/min) for nonfeeding adult Pacific salmon being held for maturation. Schreck et al. (1995) recommended that returning ocean-ranched Pacific salmon brood be held at 1 kg for every 3.8 L/min of flow, which is equivalent to 0.26 kg/L/min (2.2 lb/gal/min).

Based on this review, it seems prudent to rear ESA captive broodstocks at loading densities no greater than 0.84 kg/L/min (7 lb/gal/min). Although lower loading rates are preferred, it is recognized that they may limit captive broodstock production and significantly increase operational costs.

Turnover Rates

The most rapid turnover rate for the 6.1 m diameter circular tanks at MRS is 0.38 times/hour and 0.80 times/hour for the 4.1 m diameter circular tanks. At BCH the quickest turnover rate is 0.53 times/hour for the 3.7 m diameter circular tanks, 1.17 times/hour for the raceways, and 2.46 times/hour for the 1.5 m diameter circular tanks. When the tanks are lightly loaded, the turnover rate can be as low as one-third of these values. WDFW (1996) recommends turnover rates be greater than 1.0 for rearing ponds and large raceways and greater than 2.0 for standard raceways. In most cases, water availability and the drain design of our circular tanks precludes our meeting these standards. The adoption of the above lower rearing and loading density standards helps compensate for these reduced turnover rates.

Tank and Raceway Cover

All tanks and raceways used for sockeye captive broodstock rearing are completely covered with a taut 2.5 × 2.5 cm or smaller mesh nylon netting to prevent fish from escaping. The energy absorbing properties of the nylon mesh minimizes injuries that might occur to fish when they leap against it. In addition to the mesh covering, one-half of each tank is covered with solid black fabric that provides a shaded area for fish to take refuge. Raceways are one-fourth to one-third covered with dark plastic tarps.

Feed

Fish at BCH are reared on commercial feeds produced by Bio-Oregon or Skretting (Vancouver, British Columbia). Beginning at swimup at BCH, fry are fed a semimoist starter mash. As they grow, the fish are transitioned through standard pelleted semimoist or dry grower feeds and progressed through brood ration sizes (6 mm, 9 mm). Fish reared at MRS receive Skretting commercial feed in dry pellet sizes appropriate for fish size, dispensed by disk and belt automatic feeders. The pellet size utilized follows the feed manufacturer's recommendations, based on current guidelines for commercial aquaculture and guidance provided in Fowler (1989). However, pellet size is adjusted from the recommendation to ensure that the smallest fish in the population are able to feed. Daily ration ranges from 5.6% body weight per day for swimup fry

to 0.4% for adults, depending on fish size and water temperature (Iwama 1996). The fish are grown according to the profile described in Figure 10, which is based on periodic sample weights of past broodyears. In order to determine adherence to the growth profiles, the fish are sampled one to three times per year to assess weight and length.

Feeding swimup fry is initiated with ad libitum hand feeding in 1.8 m diameter circular tanks. After the fish are transferred to 3.7 m diameter circular tanks, their diet is either hand fed or rationed by belt feeders. Prior to loading the feeders, a portion of the day's ration is broadcast over the surface to observe the fish's feeding response. Feeding frequency varies with day length, feeder type, and fish size, as suggested by Fowler (1989).

Spawning Procedures

All Chinook salmon captive broodstock are transferred to states of origin as prespawning adults. Therefore, only Redfish Lake sockeye salmon spawn at NMFS facilities. The peak spawning period for Redfish Lake sockeye salmon is October to mid-November. As fish approach maturation (age-3 and age-4), feeding is reduced by one-half through May and discontinued in June to coincide with the time when ocean run sockeye typically begin entering the Columbia River. Ultrasonic examinations in June, July, and August allow estimates of sex and maturity by detecting gonad development. The ultrasound determinations aid in early

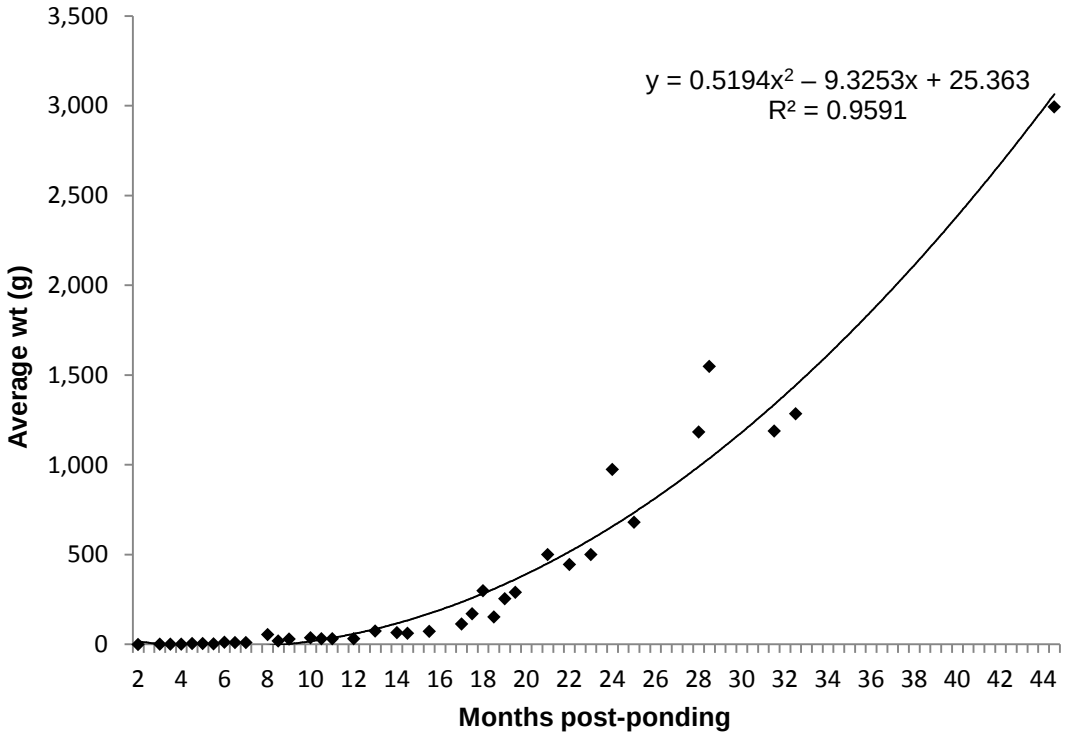

Figure 10. Growth rate projection for Redfish Lake sockeye salmon reared in 10°C freshwater at BCH. Growth profile based on historical data for sockeye reared in freshwater by NMFS.

separation of maturing from nonmaturing fish to prevent feed deprivation for an extended period in nonmaturing fish. Maturing adults for release in Idaho are transferred from seawater at MRS to freshwater at BCH in June and early July for final freshwater maturation. In September, maturing adult fish are transported from BCH to Idaho's Stanley Basin where they are released for volitional spawning.

Gamete crosses are structured to maintain genetic diversity. Eggs from each female are divided into two lots. Each lot is paired with a different male to decrease the risk of all eggs of one female being crossed with an infertile male. Currently, an inbreeding avoidance matrix using allele-sharing coefficients guides breeding decisions within the sockeye salmon captive broodstock program. The matrix, produced by IDFG geneticists,[1] is a listing of preferential mates based on the proportion of shared alleles at 14 microsatellite loci. The lower the matrix number, the better the cross because the individuals share fewer alleles in common. The use of an inbreeding avoidance matrix minimizes losses of genetic diversity that might occur in a random mating system.

During the captive broodstock spawning season, mature salmon are anesthetized (a 50 g/L stock solution of tricaine methanesulfonate [MS-222] is added to a handling tank to produce a final anesthetic environment of approximately 26 mg/L) and females are checked for ripeness on a weekly basis, or more frequently as the fish mature. In most years at the onset of the spawning season, males may have had low milt volumes when live spawned, and sometimes the milt lacked sufficient motility. Hormone implants consisting of 100 to 150 µg gonadotropin releasing hormone analog (GnRHa) pellets supplied by the Center for Marine Biotechnology (University of Maryland, Baltimore) are injected into the dorsal musculature of many of the males to expedite spermiation to coordinate spawning timing between males and females (Swanson 1995). The GnRHa implants subsequently increase the volume of milt produced. The implants also aid in ensuring the availability of a sufficient number of spermiating males to pair with ovulating females at the outset of spawning for desirable matrix crosses containing fewer shared alleles. At the end of the spawning season, a few late ripening females may also be implanted to ensure that their eggs reach the eyed stage in time to be transported and placed in the Stanley Basin lakes before ice-over.

Female fish that are ready to spawn, as determined by egg expression and ventral softness (FRED 1983), are anesthetized, killed, and their PIT tag, fork length, and weight recorded. The females are bled for 3 to 5 minutes by severing the caudal peduncle to the depth of the caudal blood vessels. The bleeding procedure limits the amount of blood that might accumulate with the eggs and interfere with fertilization. Females are then abdominally incised with a sterile spawning knife. The free flowing eggs are then gently stripped and collected into a preweighed 4 L zipper-locking plastic bag. The eggs from each female are weighed, divided into two lots, and held on an insulating layer of plastic placed over ice in a cooler until they are fertilized. All spawned fish are analyzed for common bacterial and viral pathogens by analysis of tissue and fluid samples that are collected from the kidney, spleen, and pyloric caeca of each fish and ovarian fluid from each female. The samples are placed on ice until they can be transported to the fish health facility at the NWFSC's Montlake Laboratory in Seattle, Washington.

[1] C. Kozfkay, Idaho Dept. Fish and Game, Eagle, ID. Pers. commun., September 2006.

All available males (producing motile milt) are used at least once in spawning, and many are used twice. Males are selected based on their ripeness and ranking on the spawning matrix. Males are live-spawned by ventral compression, and the milt collected into preweighed 4 ml Whirl-Pak bags (Nasco, Fort Atkinson, Wisconsin). The milt is weighed and a spermatocrit sample extracted with a standard hematocrit tube. Milt motility is then qualitatively assessed using a microscope (40×) and classified as very good (near 100% motility) good (80% motility), fair (about 50% motility), poor (less than 20% motility), or no good (0% motility). To ensure consistency, the qualitative milt analyses are typically performed by the same individual. There is no statistically detectable ($P > 0.05$) difference in viability of milt classified as very good, good, or fair. Therefore, the use of fair motility milt, while held to a minimum, occurs when a better alternative is not available or to allow an individual male his only opportunity to contribute genetic material. On the day they are collected, bags of milt are inflated with oxygen, sealed, and chilled until used. Once spawning is completed for the season, all males are killed and tissue samples are collected for health analysis.

Eggs are fertilized following dry method procedures (Piper et al. 1982). A measured amount of milt (0.5 to 5 ml) from an individual male is transferred by a sterile pipette into the plastic bag containing one egg lot. The eggs and milt are mixed for 1 full minute by gently palpating the bag. Enough water is added to just cover the eggs and activate the sperm, and the eggs are lightly agitated to distribute the activated milt. The bags are left undisturbed for approximately 5 minutes for fertilization to take place. The eggs are water hardened in a 1 ppm free iodine solution (buffered to obtain a pH of 6.5–7.0) for 20 minutes, then poured from the bags into downflow containers for isolated incubation.

Egg Incubation

Individual lots of eggs are placed into isolation containers in the incubation troughs at BCH and covered with heavy black plastic to eliminate light. Beginning two days after fertilization, the eggs are treated with a formalin drip into the hatchery head tank at 1,668 ppm for 15 minutes on alternating days for control of *Saprolegnia* spp. (water mold). The eggs are left undisturbed during the sensitive period beginning 48 hours after fertilization until they reach the eyed stage (30 days at 10°C). Eyed eggs are shocked and dead or unfertilized eggs are removed and counted or weighed to determine eyed egg viability rates. The eggs are then packed into plastic mesh tubes (AquaSeed Corp., Seattle, Washington), packaged, and shipped to IDFG for outplanting (see below).

Captive broodstock eggs that are received from IDFG and the subsequent sac fry are periodically checked for mortalities. Early growth is regulated by temperature to bring emergence timing into closer synchrony with the wild sockeye salmon, reducing the need for limiting post-ponding feeding. In 2002 the incubation water temperature was chilled from the ambient 10°C to 7.5°C to more closely equate the temperature units of the individual groups of eggs. Beginning in 2003 the water temperature was chilled to 5°C to align the egg and fry development with that of their wild counterparts in the Stanley Basin lakes. At swimup stage, when the yolk sac is completely absorbed and the fish are off the bottom of the bucket, the inner flow-through containers with the swimup fry are moved from the incubation stacks and suspended in floating foam rings in blue plastic 1.8 m diameter tanks (Figure 5). To assure that fry are feeding and thriving, they remain in the containers or are transferred to larger flow-

through suspended containers until they reach approximately 0.7 g, at which time they are released from the containers into the tank. The water temperature is normally increased to 10°C (ambient well water temperature) on or around April 1.

The fish are reared in the 1.8 m diameter tanks until they are tagged with individually identifying PIT tags (Prentice et al. 1990) and combined into the 3.7 m diameter rearing pools (Figure 6). At tagging or within a few months after tagging, a tissue sample is taken from the adipose fin for DNA analysis by IDFG to construct a spawning matrix for when the fish mature. Adult release fish are also DNA sampled for comparison with outmigrating smolt offspring and adults returning to Stanley Basin lakes.

Egg and Fish Transportation

Several types of egg and fish transfers are conducted for ESA-listed sockeye and Chinook salmon captive broodstocks at the MRS seawater rearing facility and BCH freshwater satellite.

1. For ESA-listed endangered Redfish Lake sockeye salmon, 1) small egg lots of approximately 400–500 eggs each are transferred to BCH by IDFG to establish rearing lineages (Baker et al. 2005, 2006a, 2006b, 2007), 2) smolt-to-adult stages are transferred within and between facilities, 3) approximately 400–500 prespawning adults are transferred to Idaho annually for release into Redfish Lake habitats to spawn naturally (Flagg et al. 2004), and 4) approximately 150,000–200,000 eyed eggs are transferred to IDFG annually for use in recovery efforts (Flagg et al. 2004).
2. For ESA-listed threatened Snake River spring/summer Chinook salmon from the Salmon River basin in Idaho, 1) groups of approximately 150 smolts from select lineages are usually transferred to the MRS seawater facilities and 2) all prespawning adults are transported to Idaho for release to natal streams to spawn naturally.
3. For ESA-listed threatened Snake River spring/summer Chinook from the Grande Ronde Basin in Oregon, 1) groups of up to 450 smolts from select lineages are usually transferred to the MRS seawater facilities and 2) all prespawning adults are transported to ODFW's BFH, where they are spawned by ODFW personnel and the eggs used for recovery efforts.

Transfer protocols for each species and life history stage are similar, but differ in travel time and destination environment.

Snake River Sockeye Salmon

All smolt transfers from BCH to MRS are made by NMFS. Protocols for transferring and receiving smolts revolve around the transition from freshwater to seawater. Smolts are generally taken off feed several days prior to transfer. Transfer takes place in 100% freshwater. On arrival at MRS, smolts (≈100 g average weight) are netted a few at a time out of the transfer tank and into the receiving pool. The pool is filled with freshwater with oxygen aeration to saturation level. Small aquarium powerhead-type submersible pumps are used to create a small current to circulate the water. The temperature difference between transfer water and receiving water should be within 2°C. Generally the freshwater at MRS is warmer than the transport water, so ice is added to bring the temperature differential within specifications. After all smolts are unloaded, a low flow of seawater is added (≈7 gal/min) to the pool and the smolts are slowly transitioned to full strength seawater (29 ppt) over approximately 12 hours. Feeding is initiated as soon as the fish exhibit a feed response, usually within 2 days of transfer.

The intersite transportation of adult fish is done by IDFG and NMFS (Appendix A). IDFG and NMFS use standard fish hauling trucks and equipment. All transportation conducted

by NMFS is done in a manner that emphasizes fish health and safety. Transfer protocols for adults involve transition from seawater back to freshwater. All transportation occurs in (265–7,950 L) insulated transfer tanks and the temperature is not allowed to rise more than 3°C. The transport tanks are supplied with a continuous oxygen supply that maintains DO at full saturation. The oxygen reservoir, two K cylinders (≈4,500 L), contain at least twice the oxygen needed to make the entire trip. Oxygen is metered in at a rate of 0.25–0.50 L/min and the oxygen level is maintained at 10–20 mg/L. Temperature and oxygen levels are checked at departure and every 2–3 hours during transport. Fish transport densities are kept at or below 0.06 kg/L (0.5 lb/gal). Transport temperature is typically approximately 10°C. Additionally, drivers are equipped with cell phones and have backup personnel ready to respond in event of equipment failure.

Seawater-reared adults (≈3 kg average weight) are transferred to two different locations between June and September. In June adult fish are sorted for maturity at MRS. Fish are taken off feed 2 weeks prior to sorting. Adults are sorted for maturity and transferred back to freshwater (BCH) on the same day. Fish are lightly crowded in the pool, anaesthetized, length measured, weighed, examined for reproductive status (using ultrasound), and loaded directly into the transfer tank. Water in the transfer tank is 25% seawater and 75% freshwater. When loading density is reached, typically 1–2 hours, the fish are then trucked (0.5 hour trip) to BCH. Transition from seawater to freshwater occurs during the transfer with the adults being unloaded directly into 100% freshwater. Total transition time from seawater to freshwater is approximately 2 hours. Approximately 1 month after the first transfer of maturing adults, the remaining fish at MRS are re-sorted for maturity to ensure that all maturing adults are transferred back to freshwater.

Maturing adults are held in freshwater at BCH until early September. Typically the day after Labor Day the fish are loaded into IDFG and NMFS transport tanks and transferred from BCH to Redfish Lake near Stanley, where they are released into the lake for volitional spawning. Loading is done with the fish being netted directly out of the raceways and into the transfer tanks. The fish are transported in 100% freshwater at 10°C for approximately 10 hours to IDFG's EFH near Boise, Idaho. On arrival, approximately half of the transfer water is exchanged with fresh well water at the hatchery. After approximately 1 hour of water exchange, the fish are then trucked another 3 hours to Redfish Lake where they are released. If necessary, water is again exchanged to achieve temperature acclimation. Liberation is done via discharge tube from the IDFG truck and via dip net from the NMFS truck. Total transport time from BCH to Redfish Lake is approximately 14 hours.

Eggs from sockeye salmon spawned at BCH to be shipped to Idaho are placed in open-mesh perforated plastic AquaSeed egg tubes (27.7 cm long × 6.4 cm diameter) at approximately 2,500 eggs per tube. Each packed tube is wrapped in wet paper toweling to contain moisture and placed in a small shipping container (cooler). A small amount of ice is placed in a top layer of toweling to keep the eggs cool and moist during shipment. Shipment to Boise is by common carrier flight of approximately 2 hours. At Boise, the eggs are picked up at the airport by IDFG personnel and transferred to the IDFG EFH (Baker et al. 2005, 2006a, 2006b, 2007).

Snake River Chinook Salmon

Transport of all smolts to MRS is conducted by ODFW and IDFG (Baker et al. 2005, 2006a, 2006b, 2007, Hoffnagle et al. 2003). Protocols for transferring and receiving Chinook smolts are virtually identical to the protocols used for sockeye (see Snake River Sockeye Salmon subsection above).

The majority of intersite transportation of adult fish is done by ODFW and IDFG with assistance from NMFS (Appendix A). All three agencies use standard fish hauling trucks and equipment. Transfer protocols for Chinook salmon adults from MRS (seawater) back to ODFW and IDFG facilities (freshwater) is also virtually identical to the protocols used for sockeye adults (see Snake River Sockeye Salmon subsection above).

Adult fish are taken off feed a minimum of 1 week prior to transfer. Adults being transferred from MRS back to Oregon and Idaho are transferred in 25% seawater and 75% freshwater. Transition from seawater to freshwater occurs during the transfer, with the adults being unloaded directly into 100% freshwater. Depending on the destination, this transfer acclimation time can be as short as 4 hours or as long as 22 hours.

Adults (≈2 kg average weight) from the Oregon captive brood program are sorted for maturity and transferred back to freshwater (BFH) on the same day in late April and again in late May. Fish are lightly crowded in the pool, anaesthetized, and measured for metrics and reproductive status (using ultrasound). Maturing fish are injected with erythromycin (40 mg/kg) and loaded directly into the transfer tank. When loading density is reached, typically 1–2 hours, the fish are then trucked to BFH. Total transition time from seawater to freshwater is approximately 5 hours. Adults from subsequent pools are sorted and held overnight for transfer the next day. Transfer protocols are the same with the exception that the fish are loaded into the transfer tank without anesthesia.

Adults (≈2.5 kg average weight) from the Idaho captive brood program are sorted for maturity 1 to 5 weeks prior to transfer back to freshwater in Idaho. The procedure is the same as for the Oregon program. Fish are lightly crowded, anaesthetized, measured for metrics and reproductive status, and returned to a holding pool for later transfer.

Program Production

Snake River Sockeye Salmon

The main goal of the NMFS component of the Redfish Lake sockeye salmon captive broodstock program is to supply maturing adults and eyed eggs to Idaho's Stanley Basin. This production and the numbers of gametes and juvenile fish received to generate this production are described in Figure 11 through Figure 15.

The first transfer of maturing adults began in 1996. From 1996 through 2008, 11 transfers were made for a total program production of 2,210 maturing adults (Figure 11). Transfers of eyed eggs to Idaho began in 1994. From 1994 to 2008, 14 transfers totaling 1,922,700 eyed eggs were made (Figure 12).

In addition to producing eyed eggs for Idaho, the program supplies eyed eggs for continuation of the NMFS portion of the program. A total of 3,277 eyed eggs were retained for the years 1996–2008, with 814 used in the captive broodstock program (Figure 13) and 2,463 used in the adult release program (Figure 14). The NMFS program receives eyed eggs from Idaho to support the program. The number of eyed eggs received from Idaho is shown in Figure 15.

Snake River Spring/summer Chinook Salmon

The main goal of the MRS Chinook salmon marine rearing program is to receive smolts from the Snake River basin, rear these fish in seawater when they would naturally be at sea, then supply prespawning adults to Idaho and Oregon (Figure 16 through Figure 19). As of July 2008, a total of 3,523 prespawning adults have been returned to Idaho for release back into their natal streams for natural spawning (Figure 16). By the same date, a total of 4,031 prespawning adults had been returned to Oregon for that state's stock rebuilding actions (Figure 17). The number of smolts brought onto the station for each of these broodyears is shown in Figure 18 and Figure 19.

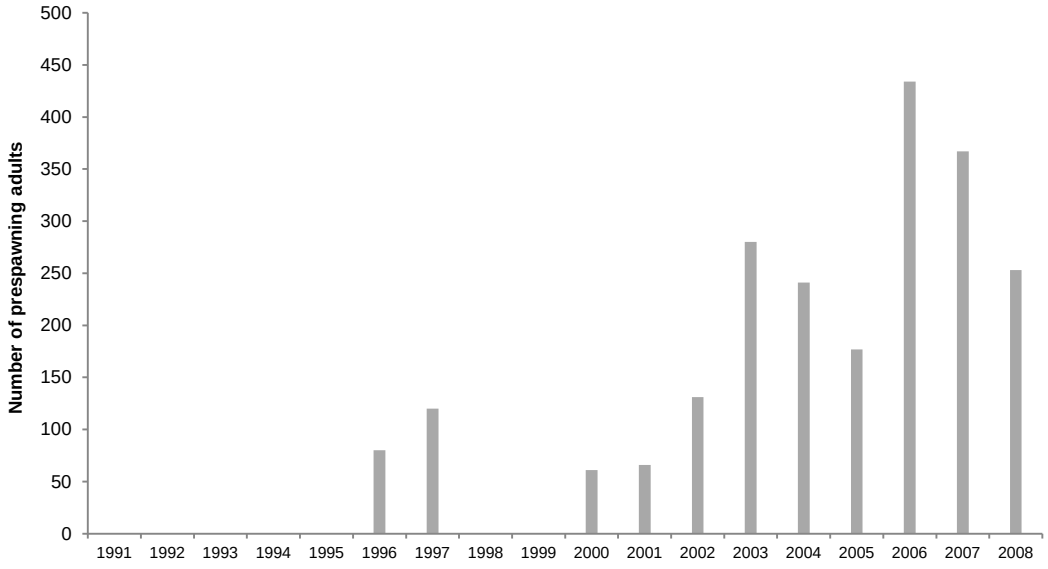

Figure 11. NMFS annual production of prespawning sockeye salmon adults for release in Stanley Basin lakes.

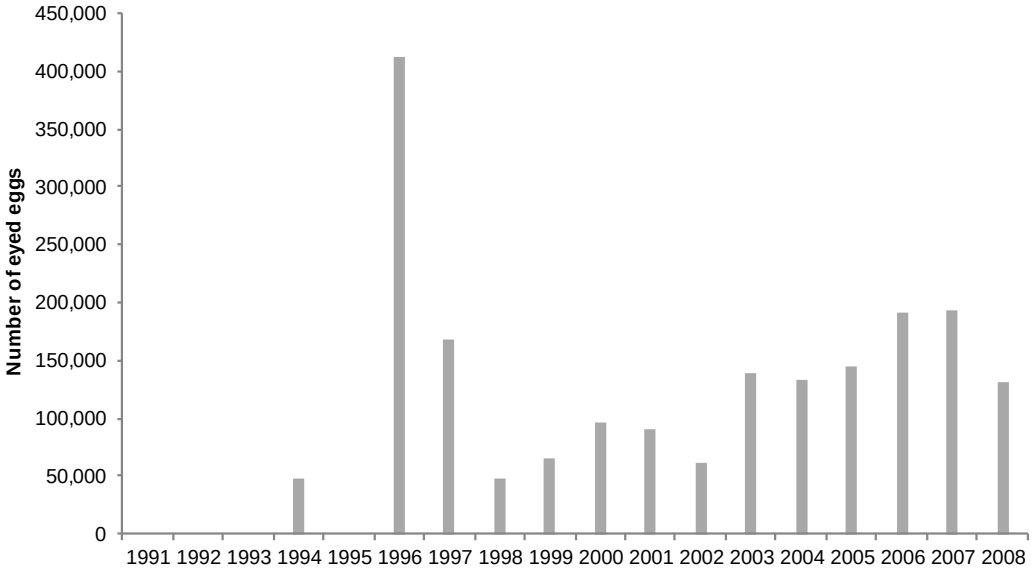

Figure 12. NMFS sockeye salmon eyed egg production available for recovery actions.

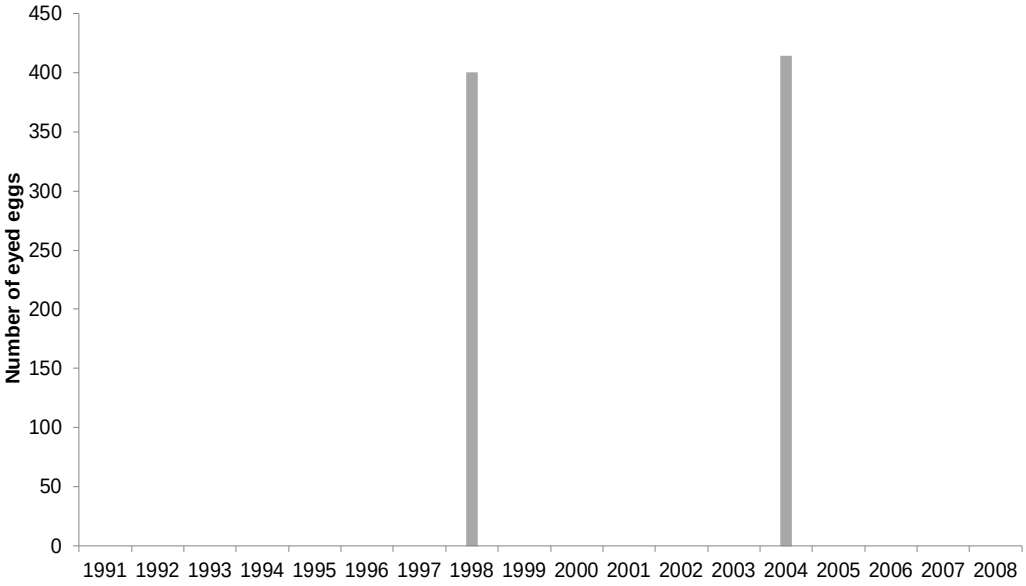

Figure 13. Number of eggs NMFS retained each year for incorporation into its captive broodstock program.

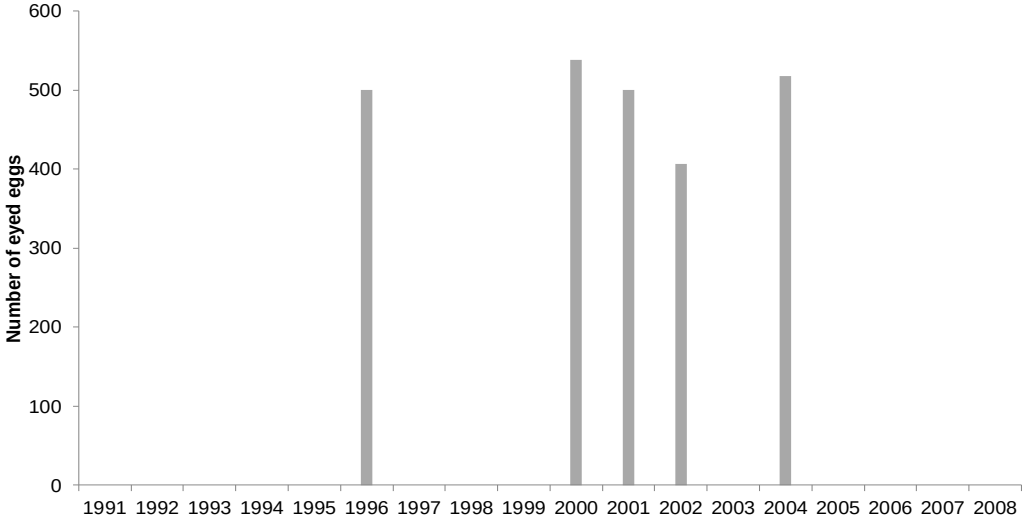

Figure 14. Number of eggs NMFS retained for incorporation into adult release production.

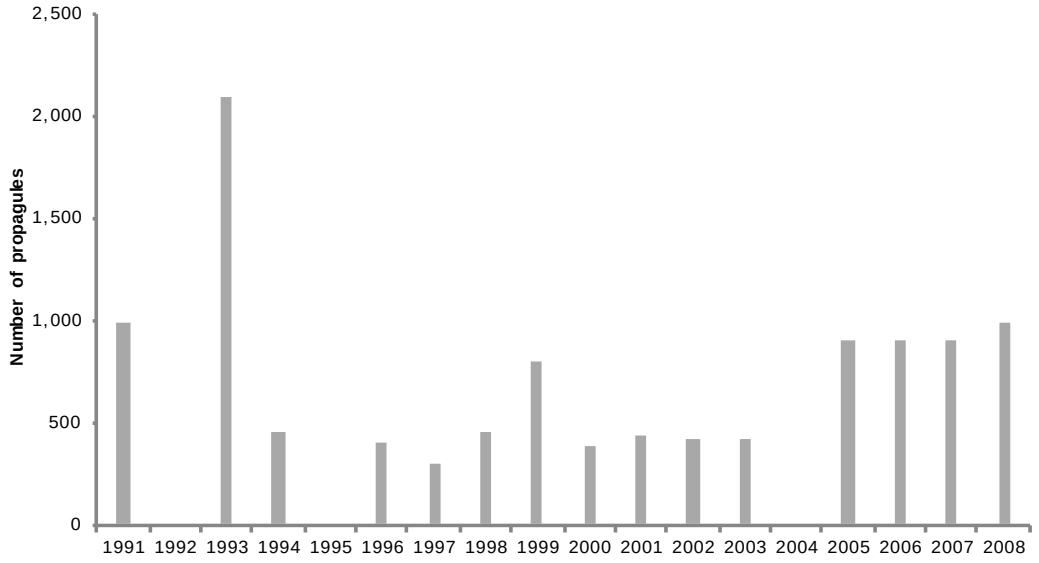

Figure 15. Number of eggs and juvenile fish IDFG supplied each broodyear for incorporation into NMFS captive broodstock and rearing programs.

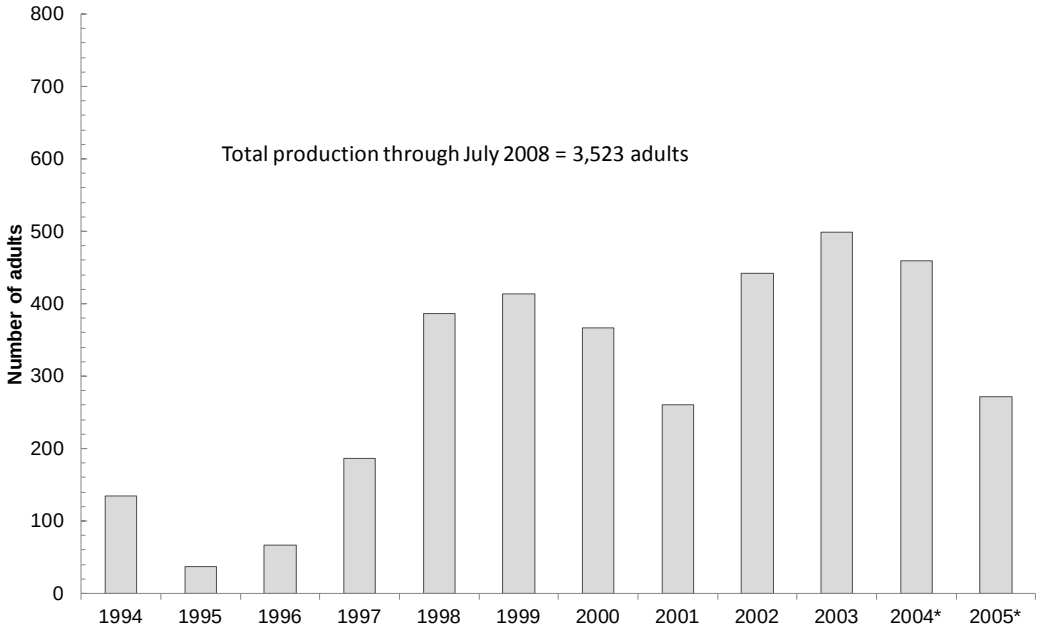

Figure 16. Number of Salmon River spring/summer Chinook salmon adults produced from each broodyear for stock rebuilding activities in Idaho. Asterisk (*) indicates maturation of broodyear not yet complete.

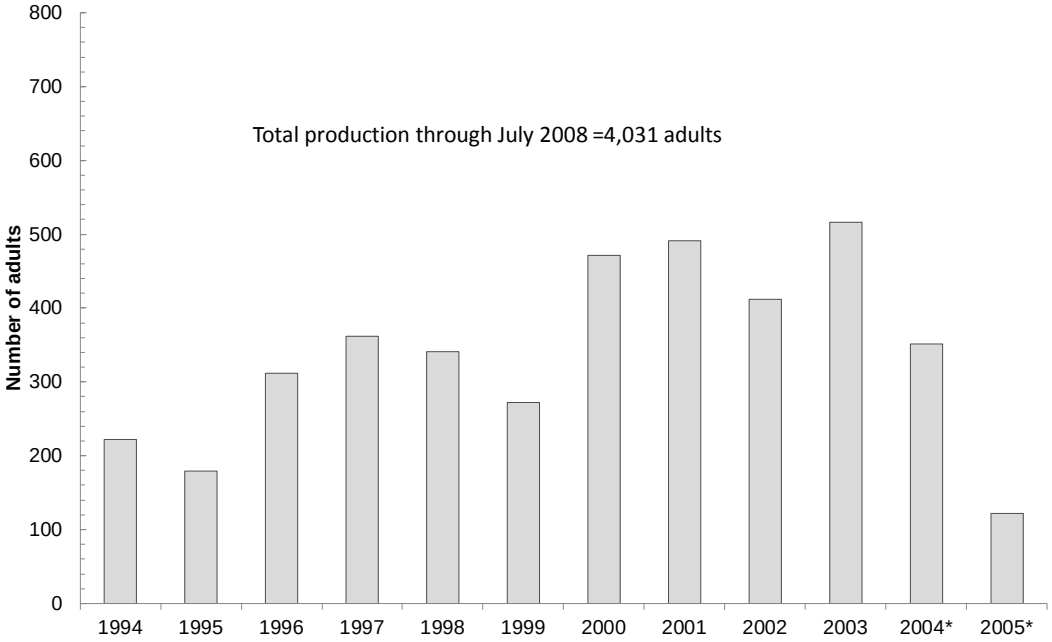

Figure 17. Number of Grande Ronde River spring/summer Chinook salmon produced from each broodyear for stock rebuilding activities in Oregon. Asterisk (*) indicates maturation of broodyear not yet complete.

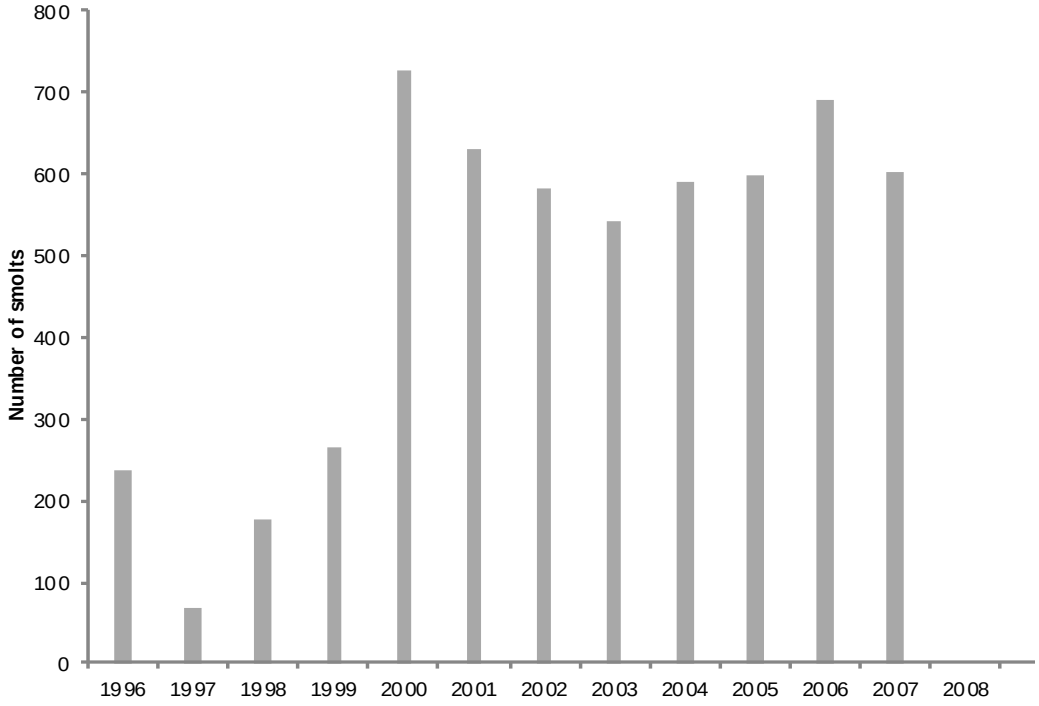

Figure 18. Number of Salmon River spring/summer Chinook salmon smolts transferred to MRS from Idaho.

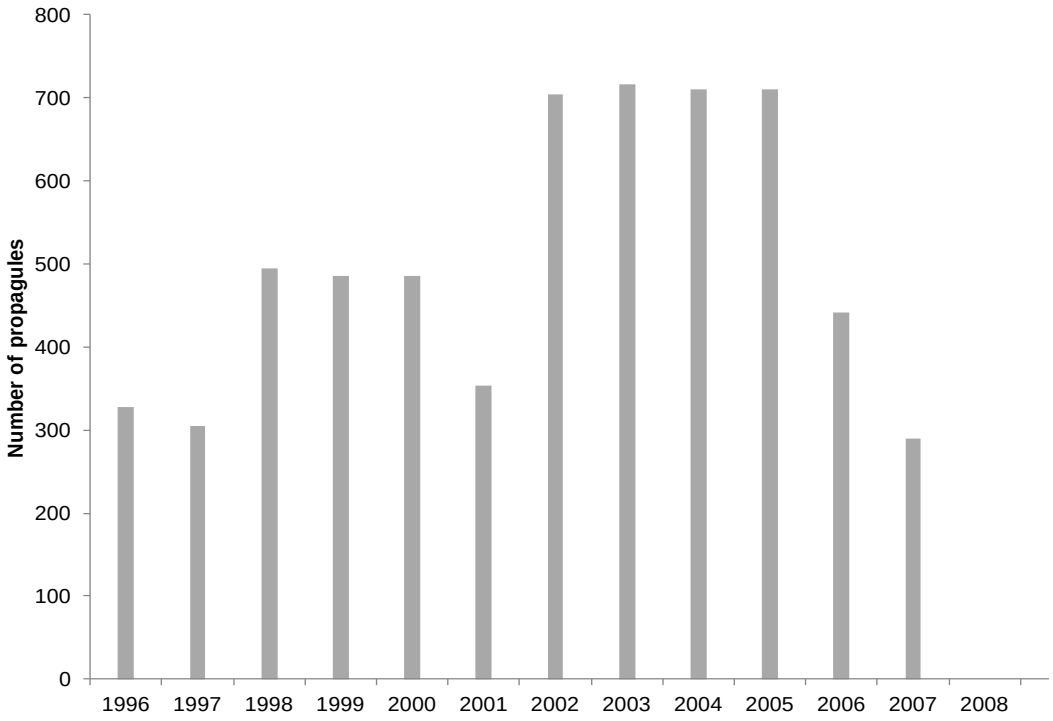

Figure 19. Number of Grande Ronde River spring/summer Chinook salmon smolts transferred to MRS from Oregon.

Biological Metrics of NMFS Captive Reared Fish

Snake River Sockeye Salmon Culture

The survival of sockeye salmon at NMFS facilities covers two conjoining, but distinct, portions of the entire life cycle. The earliest portion of the life cycle, from green egg (unfertilized) to the eyed stage, is the product of the fish that personnel spawn and incubate to the eyed stage at NMFS facilities. When these fish reach the eyed stage, they are shipped to other facilities for outgrowing or stocking. The remainder of the life cycle—from eyed egg to mature adult—represents NMFS's success in rearing eyed eggs received from IDFG's EFH. Although NMFS has sourced fish for the adult release program from spawned eggs, these fish are released into Redfish Lake for volitional spawning before they complete their full life cycle. As it is rare for a cohort to be taken through the whole life cycle at NMFS facilities, the results are presented for the two distinct periods and entire life cycle survival is estimated from these data.

The survival of sockeye salmon from the green-to-eyed-egg stage at NMFS facilities between 1994 and 2008 averaged 62.2% (±3.2) (Figure 20). The lowest green-to-eyed egg survival (32.5%) occurred in 1999 and the highest survival (78.3%) in 2002. Green-to-eyed egg survival fluctuates from year to year and linear regression indicates there is a significant (P = 0.000), although slight, increase in survival with time. The r value is 0.189 with the equation (egg viability = −2791.150 + 1.425 spawn year), explaining 3.6% of the variation in the data. The average survival during this period is less than would be expected for ocean-returned Chinook and coho salmon adults in typical production hatcheries (i.e., 90%+) and provides the greatest opportunity for improvement, given the brief portion of life cycle it represents.

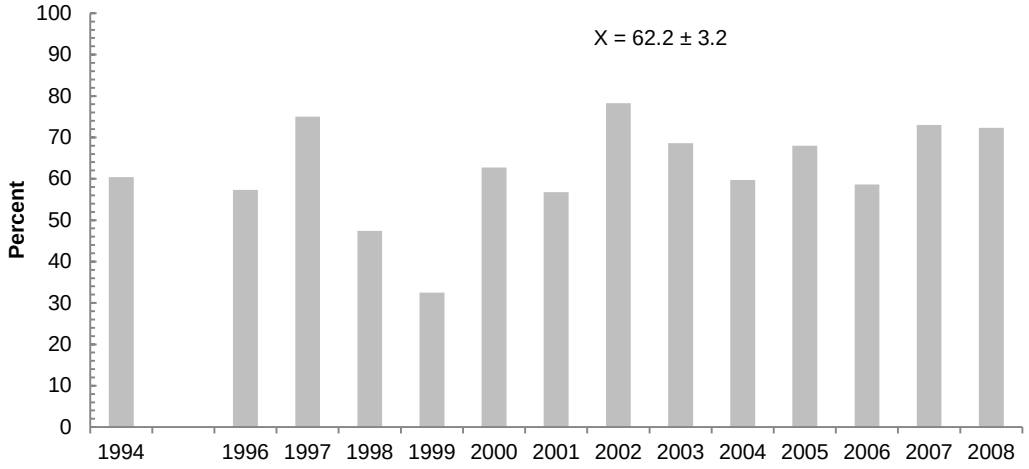

Figure 20. Average survival from green egg to eyed egg stage of Redfish Lake sockeye salmon by spawn year at NWFSC facilities, 1994–2008.

The average survival of captive broodstock during the longer eyed-egg-to-maturation life stage averaged 61.7% (±5.5) for broodyear cohorts completing their life cycle between 1991 and 2008 (Figure 21). Linear regression of eyed-egg-to-adult survival against broodyear yielded a statistically significant relationship (eyed-to-adult survival = 6,422.75 + 3.245 broodyear, r = 0.762) that explained 58.0% of the variation. Thus eyed-to-adult survival appears to have improved over the course of the program, with the lowest survivals of 22.7% (broodyear 1991) and 35.0% (broodyear 1996) occurring in the early years of the program. The survival of all cohorts since 1997 never dipped below 59.1% and achieved a maximum of 85.0% in broodyear 2003. As with the green-to-eyed egg data, eyed-egg-to-mature-adult survival data appears to become less variable over time. Eyed-egg-to-mature-adult survival is orders of magnitude greater than the fish are likely to experience in the wild during this same portion of their life cycle.

As mentioned above, full life cycle survival can only be estimated as NMFS data cover two conjoined, but nonetheless separate, portions of the entire life cycle. This estimate can be derived by multiplying the percentage of fish surviving from green-to-eyed-egg stage by the proportion of fish surviving from the eyed-egg-to-mature-adult stage. Using this approach, the above 62.2% green-to-eyed egg survival can be multiplied by 0.617 to estimate an overall green-egg-to-adult survival of 38.4%. However, with the improvement in eyed-to-adult survival seen over the last decade, one might choose to use a higher proportion to account for the advances in fish culture. When the 0.753 mean proportions for the last 5 broodyears (broodyear 2000 to broodyear 2004) is substituted into the equation, estimated full life cycle survival increases to 46.84%. This number, which accounts for program improvements, is probably the better tool for predicting future performance of this or other captive broodstocks initiated for rearing sockeye salmon. In setting up a new program, at least 38.4 mature adults should be produced for every 100 green eggs taken into culture. If the program can benefit from the improvements we experienced, it will likely produce 46.8 adults from the same 100 green eggs. If these 100 green eggs were left in nature, one would expect about 10% of them to survive to smolt and about

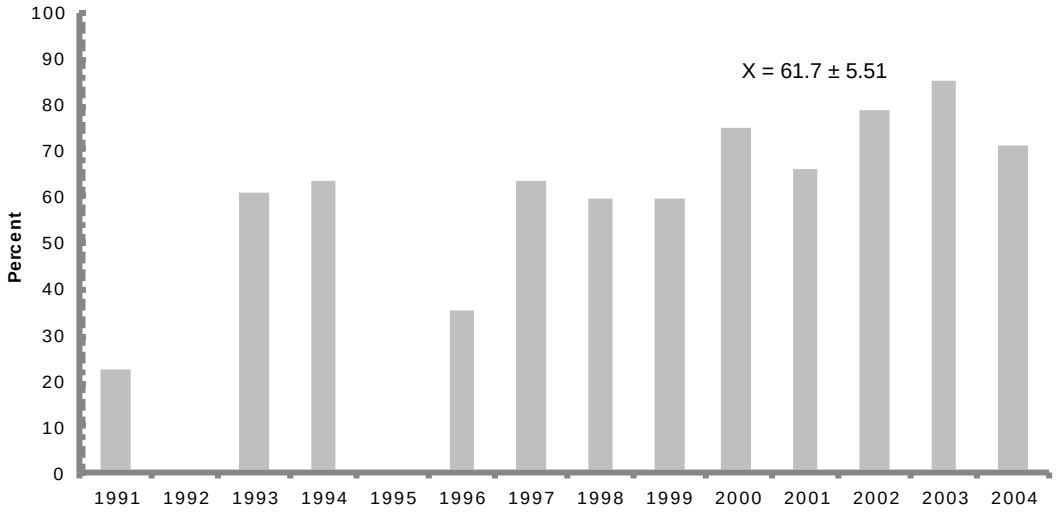

Figure 21. Average survival by broodyear from eyed egg stage to mature adult for Redfish Lake sockeye salmon captive broodstock reared at NWFSC facilities.

1–3% of the outmigrating smolts to survive to maturation. This would produce less than one (0.1–0.3) fish recruiting to the spawning population. Thus even with the lower value (38.4%), population amplification from one generation to the next will be more than two orders of magnitude higher than if the fish were reared in the wild (Flagg et al. 2004).

Sockeye salmon in NMFS freshwater captive broodstocks typically spawn at a younger age than the 4- and 5-year-old anadromous sockeye that returned to Stanley Basin lakes in the 1960s (Bjorn et al. 1968). The age of maturation for male fish in broodyear 2000, 2001, 2002, and 2003 cohorts is illustrated in Figure 22. In these four broodyears, a few males matured as 2-year-olds, most matured as 3-year-olds, with very few males maturing as 4-year-olds. No 5-year-old males were produced in any of these broodyears. The presently available data set for female sockeye salmon is more extensive, covering broodyears from 1991 to 2004 (Figure 23). No freshwater-reared females matured as 2-year-old fish, most matured as 3-year-old fish, a few matured as 4-year-old fish, and none matured as 5-year-old fish. The maturation age of females appears to be shifting with the passage of time, as the percentage of females maturing as 4-year-olds in the freshwater captive broodstock has decreased in recent years. We have observed that adult release sockeye reared in seawater produce many more fish that mature as 4-year-olds than their freshwater-reared counterparts. Flagg et al. (1998) speculated that this phenomenon of delayed maturation age in seawater was a result of slower and less uniform growth due to the cyclic nature of seawater temperature compared to the constant temperature freshwater at NWFSC facilities.

Bjorn et al. (1968) reported the average length of 4- and 5-year-old sockeye salmon females returning to Stanley Basin lakes to be 546 mm. In the freshwater captive broodstock program, the average length of mature 3-year old females ranged from a cohort low of 438 mm to a cohort high of 487 mm (Figure 24). As expected, given their younger age, these 3-year-old females matured at a smaller size than the 4- and 5-year-old anadromous adults that historically returned to the Stanley Basin lakes. The average fork length of 4-year-old females in the NMFS

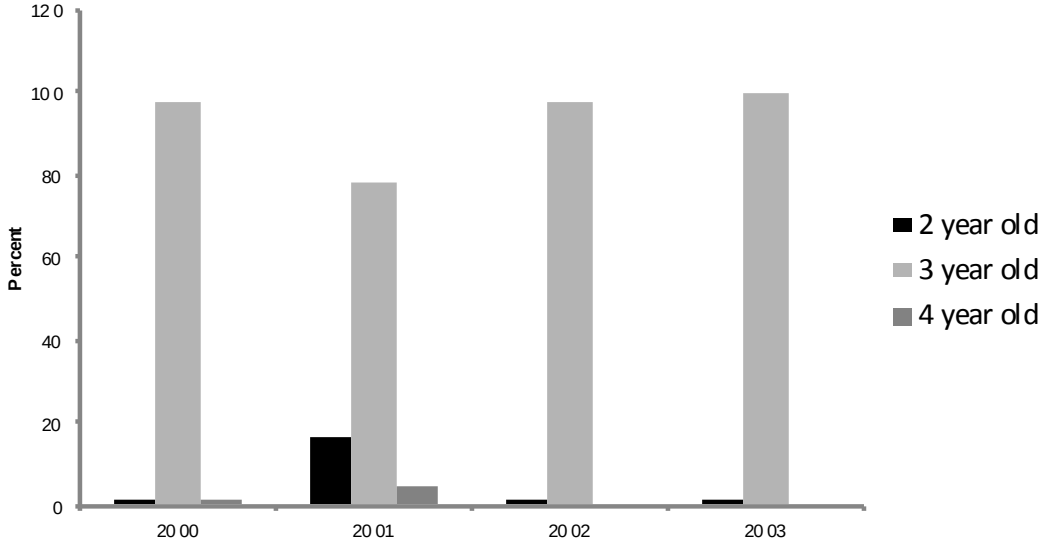

Figure 22. Percent by broodyear of Redfish Lake sockeye salmon males in the captive broodstock program maturing as 2-, 3-, and 4-year-old adults.

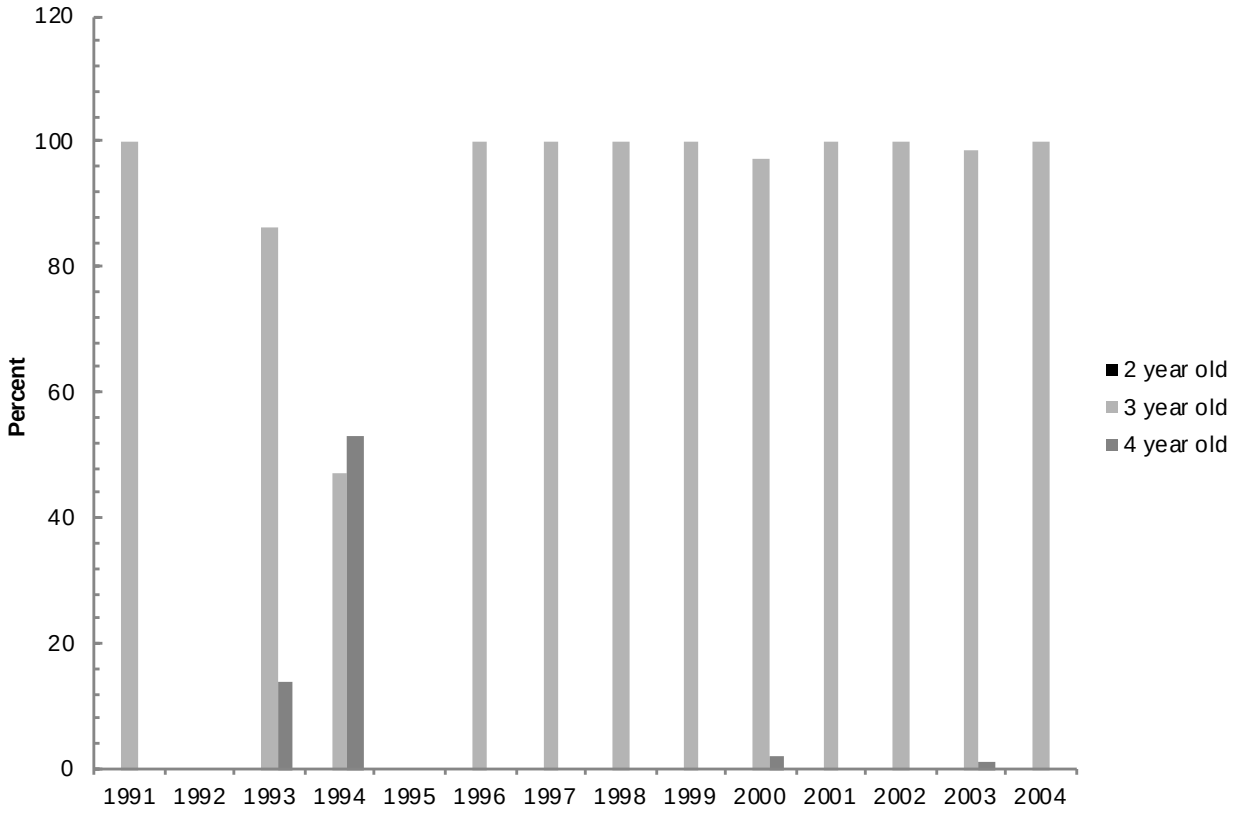

Figure 23. Percent of broodyear 1991–2004 female Redfish Lake sockeye salmon reared in freshwater at NWFSC facilities maturing as 2-, 3-, and 4-year-old fish.

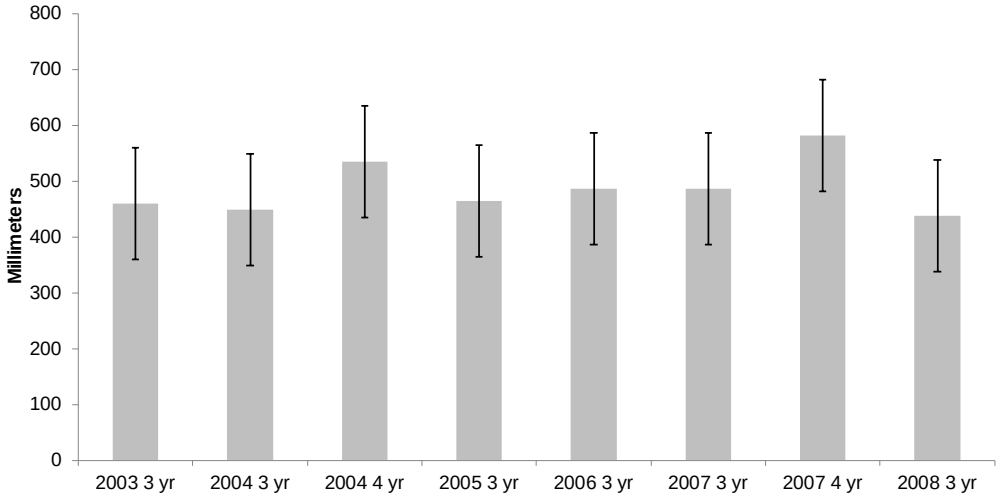

Figure 24. Average fork length (±SE) for mature 3- and 4-year-old female Redfish Lake sockeye salmon spawned at NWFSC freshwater facilities, 2003–2007.

captive broodstock ranged from a cohort low of 534 mm to a cohort high of 582 mm (Figure 24), bracketing the value reported by Bjorn et al. (1968).

The fecundity of 3-year-old Redfish Lake sockeye salmon captive brood females ranged from a low of 1,565 to a high of 2,218 eggs per female and averaged 1,765.5 ±84.8 eggs for all spawning conducted between 1994 and 2008 (Figure 25). During this same time frame, the average fecundity of 4-year-old females averaged 2,349.8 ±359.6 eggs per female and ranged from an annual low of 1,788 eggs per fish to a high of 3,275 eggs per female (Figure 26). As is apparent, the fecundity of sockeye salmon in the captive broodstock program increases with age. Linear regression indicates the fecundity of 3-year-old females significantly increases (P = 0.000) with spawn year (fecundity = –67,003.387 + 34.321 spawn year). The r value was 0.315, indicating that regression only explained about 10% of the variation in the data. Regressing fecundity against spawn year did not yield a significant (P = 0.185) relationship for 4-year-old females.

When fecundity is evaluated on female weight, there is a trend for the number of eggs produced per gram of fish to increase with the passage of time (Figure 27). Linear regression yields an equation where egg number per gram of 3-year-old fish = −57.218 + 0.029 broodyear that is a statistically significant (P = 0.000) relationship with an r value of 0.300 that explains about 9.0% of the variation in the data. This same linear regression on the data for 4-year-old fish did not yield a statistically significant relationship (P = 0.232). The number of eggs produced per gram of 3-year-old fish averaged 1.337 ±0.049 with an annual low of 0.985 egg/gram of fish in spawn year 2000 and an annual high of 1.524 eggs per gram of fish in spawn year 2003.

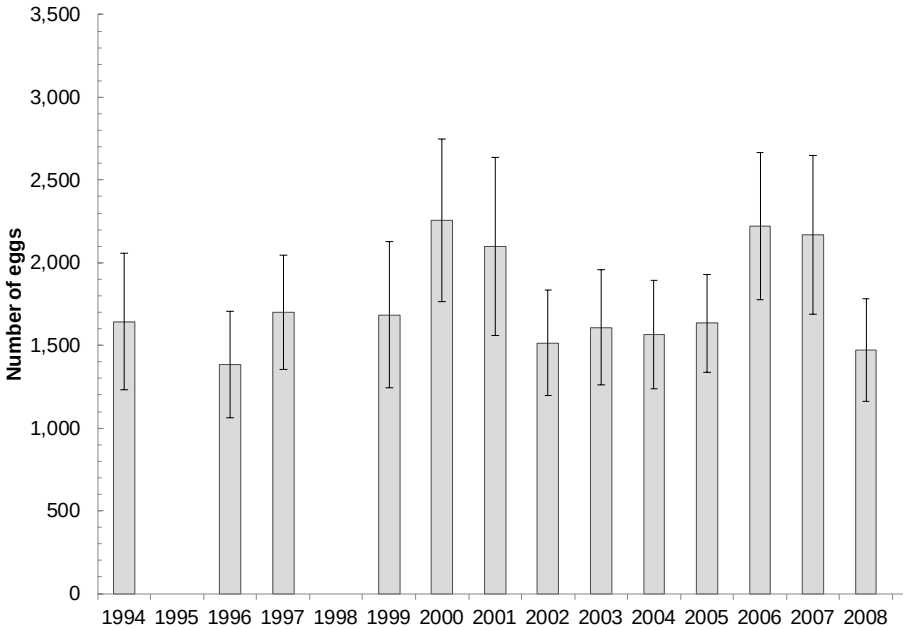

Figure 25. Average fecundity (±SD) of 3-year-old Redfish Lake sockeye salmon captive broodstock females spawned at NWFSC facilities, 1994–2008.

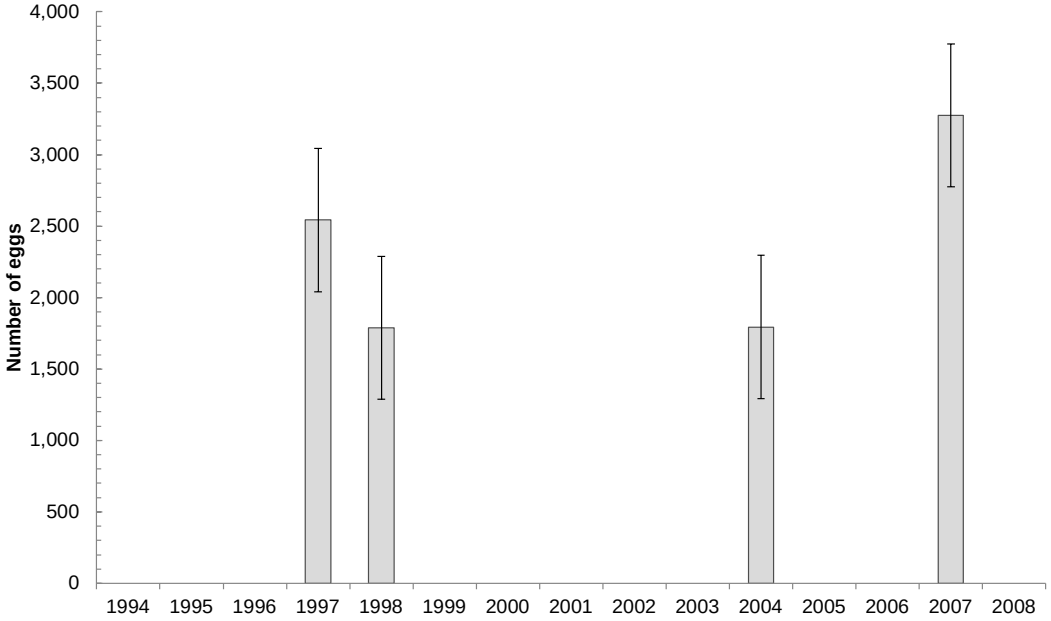

Figure 26. Average fecundity (±SD) of 4-year-old Redfish Lake sockeye salmon captive broodstock females spawned at NWFSC facilities, 1994–2008.

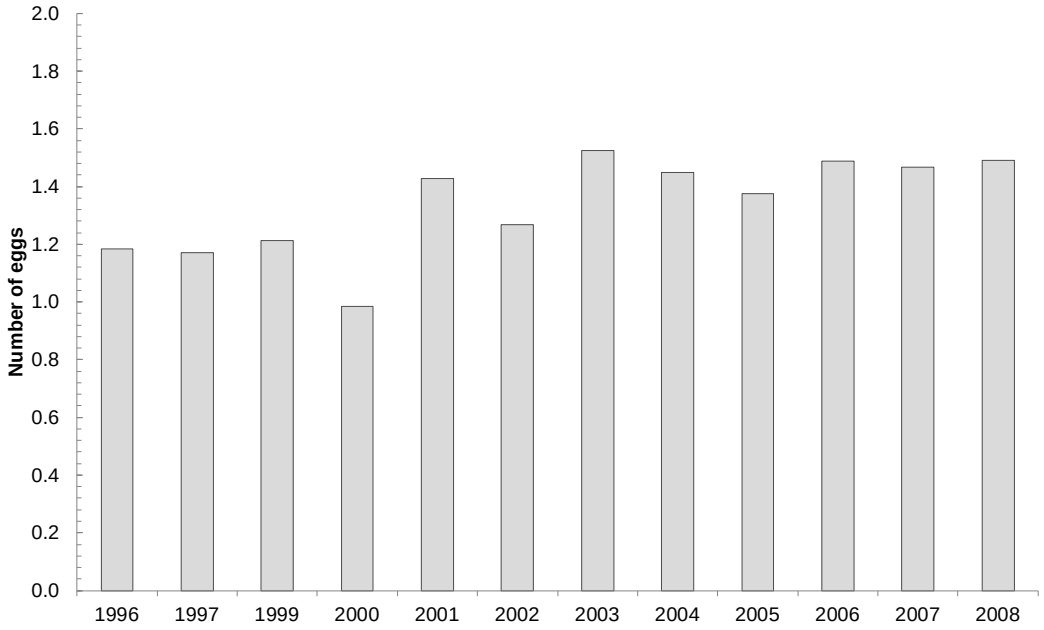

Figure 27. Average number of eggs produced per gram of 3-year-old spawning Redfish Lake sockeye salmon captive broodstock, 1996–2008.

Two types of data are available to assess average egg weight. Collection of green egg weight, which is a good surrogate for energetic reproductive investment, began in 2003 to compare the relative performance of fish in two different maturation environments. Eyed egg weight data has been collected as a fish culture tool since 1991 to evaluate female fecundity. However, eyed egg weight is a less valuable metric for estimating the energy content of an egg,

as it is affected by the amount of water taken during the water hardening process and the amount of organic material lost during respiration. Although not the best proxy for evaluating reproductive effort, eyed egg data that are collected in a consistent manner from year to year can be used to examine temporal changes in egg weight. Therefore, we present both types of data to get a better understanding of how project practices have affected egg weight.

Green eggs from 3-year-old female Redfish Lake sockeye salmon captive broodstock averaged 0.084 ±0.001 g. The smallest (0.079 g) green eggs were observed in 2003 and the largest (0.087 g) in 2006 (Figure 28). Linear regression indicated no significant ($P = 0.285$) trends in green egg size with the passage of time over the relatively short time frame that these data are available.

The average eyed egg weight for 3-year-old females from 1991 to 2005 was 0.107 ±0.004 g, with a range from 0.085 g for broodyear 2005 females to 0.130 g for broodyear 1997 (Figure 29). As illustrated in Figure 29, there is a tendency for eyed egg weight to decrease over time. Linear regression analysis indicates this trend is statistically significant ($P < 0.0005$) with an r value of 0.563 with regression accounting for 31.7% of the variation in the data. The regression equation is eyed egg weight = 4.516 – 0.002 broodyear. Remarkably, average eyed egg weight of 4-year-old fish during this same period was an identical 0.107 ± 0.004 g and ranged from 0.095 to 0.115 g (Figure 30). Linear regression indicates there is also a significant ($P = 0.013$) trend for decreasing eyed egg weight over time for 4-year-old females. The equation generated by this regression is eyed egg weight = 16.482 − 0.008 broodyear with an r value of 0.213, which explains approximately 4.5% of the variation observed in the data.

Chinook Salmon Culture

The survival of broodyear 1994–2003 Salmon River spring/summer Chinook reared at MRS averaged 58.8% ±4.3. The survival of Grande Ronde spring/summer Chinook salmon was higher, averaging 67.9% ±3.4 during this same period.

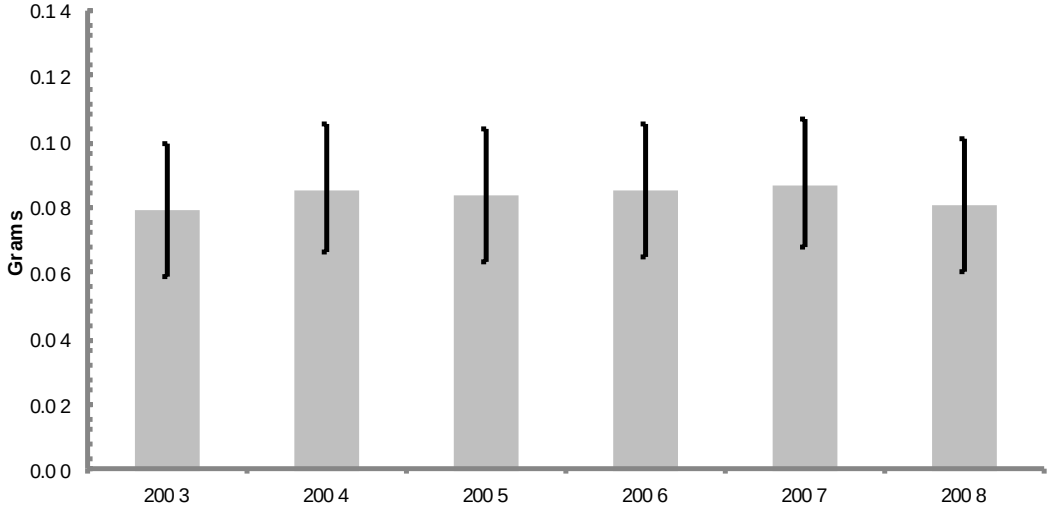

Figure 28. Average green egg weight (±SE) by spawn year for 3-year-old spawning Redfish Lake sockeye salmon captive broodstock, 2003–2008.

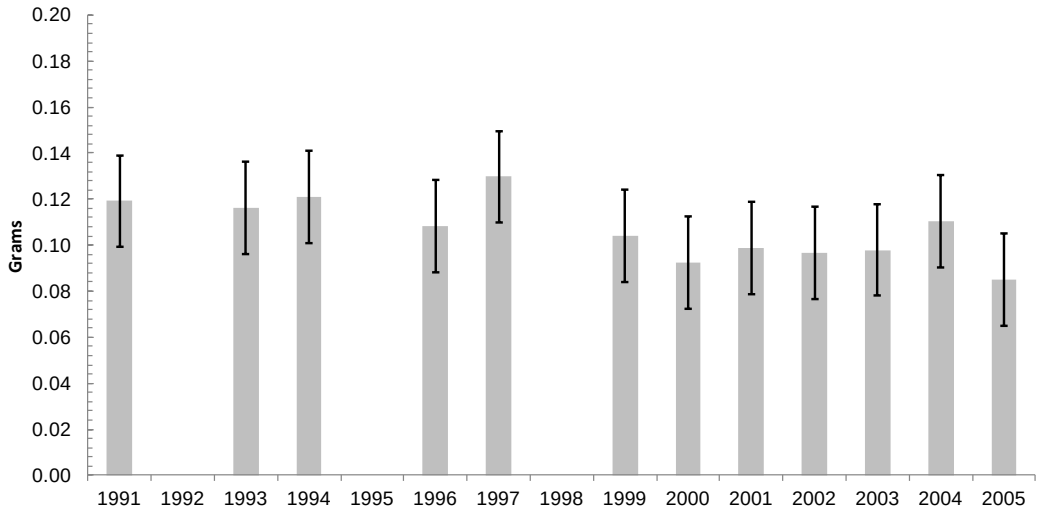

Figure 29. Average eyed egg weight (±SD) for 3-year-old spawning Redfish Lake sockeye salmon captive broodstock, broodyear 1991–2008.

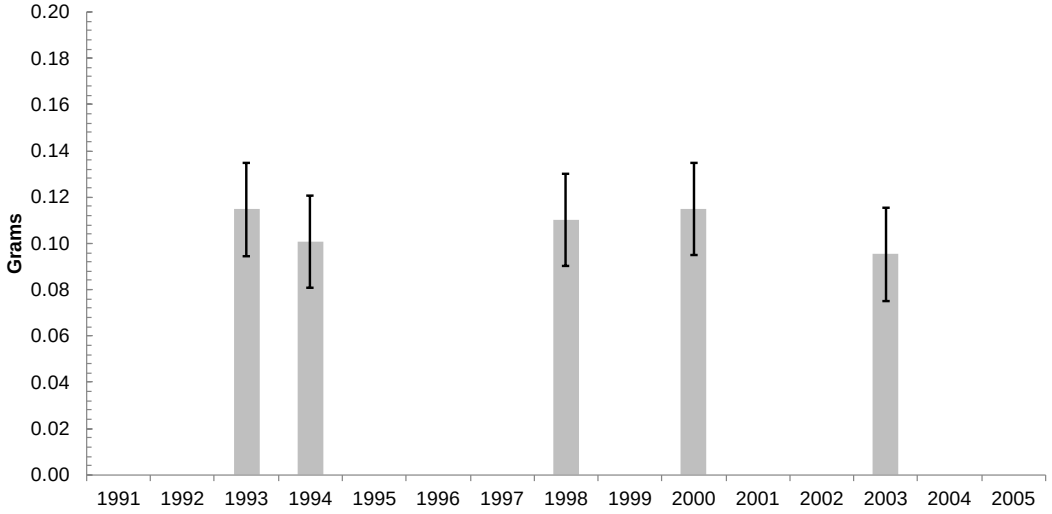

Figure 30. Average eyed egg weight (±SD) for 4-year-old spawning Redfish Lake sockeye salmon captive broodstock, 1993–2008.

Figure 31 through Figure 33 illustrate the individual survival of the three Salmon River stocks of spring/summer Chinook salmon sent to MRS by IDFG. There is a significant amount of broodyear-to-broodyear variation in these data and only one statistically detectable trend over time. Lemhi River broodstocks have a slightly higher survival (65.3 ±5.7%) than broodstocks from the West Fork Yankee Fork (58.0 ±6.7%) or East Fork (57.4 ±9.5%) of the Salmon River. Lemhi River broodstocks are the only stock in which linear regression detected a significant ($P <$ 0.0005) relationship between in-culture survival and broodyear. The linear equation fit to the data is percent survival = 772.631 – 0.354 broodyear with r = 0.048 and the regression explaining a minute amount (0.23%) of the variation in the data. Linear regression of West Fork

Yankee Fork and East Fork Salmon River stocks survival data by broodyear yielded *P* values of 0.292 and 0.235, respectively. The variation in in-culture marine survival of these Salmon River tributary stocks sourced from three different tributaries within the Salmon River basin provides comparative information on how different spring/summer Chinook salmon stocks respond to marine culture.

Figure 34 through Figure 36 illustrate the individual survival of the three Grande Ronde River spring/summer Chinook salmon stocks sent to MRS by IDFG. The Catherine Creek stock showed the highest average survival (73.9% ±7.8), followed by the Lostine River stock (69.0% ±4.9), and the Grande Ronde River stock (63.4% ±7.8). The survival of the three Grande Ronde River stocks from Oregon also shows no clear trends over time. As with the Salmon River stocks, the tributary habitat of the Grande Ronde stocks noticeably differ and the response of these stocks to in-culture marine survival at MRS provides guidance on the types of variation one might expect in see in other spring/summer Chinook salmon stocks within upriver mountain basins.

The Snake River spring/summer Chinook salmon reared in seawater at MRS appear to be smaller than their wild-reared counterparts. Columbia River stream-type Chinook salmon returning from the sea are about 500 mm long when maturing as 3-year-olds and 710 mm long when maturing as 4-year-olds (Healey 1991). More specifically in the Grande Ronde River, Boe et al. (2005, 2006, 2007a, 2007b, 2009, 2010) report the average fork length ranges 39.6–61.8 cm for natural 3-year-old jacks, 68.4–76 cm for natural 4-year-old adults, and 80.4–90.4 cm for natural 5-year-old returning fish. Sampling conducted by IDFG at Lower Granite Dam indicates fork lengths of the majority of 3-year-old adults are 55–60 cm long, 4-year-old adults are 70–85

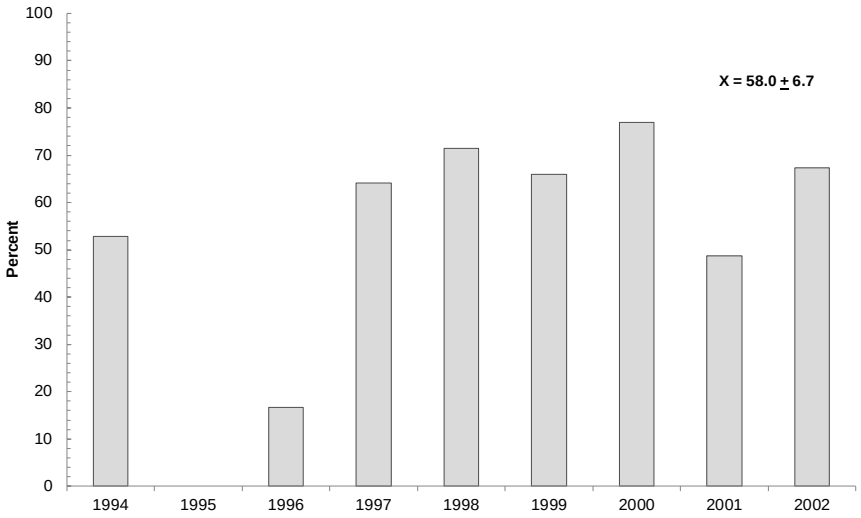

Figure 31. Percent survival by broodyear for West Fork Yankee Fork stock of Salmon River spring/summer Chinook salmon at MRS.

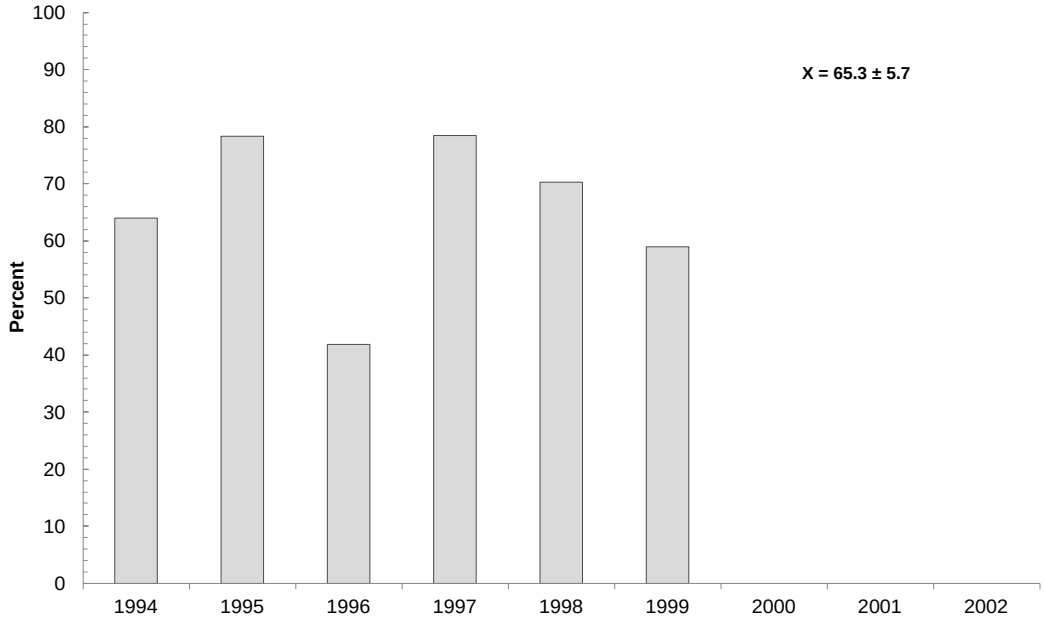

Figure 32. Percent survival by broodyear for Lemhi River stock of Salmon River spring/summer Chinook salmon at MRS.

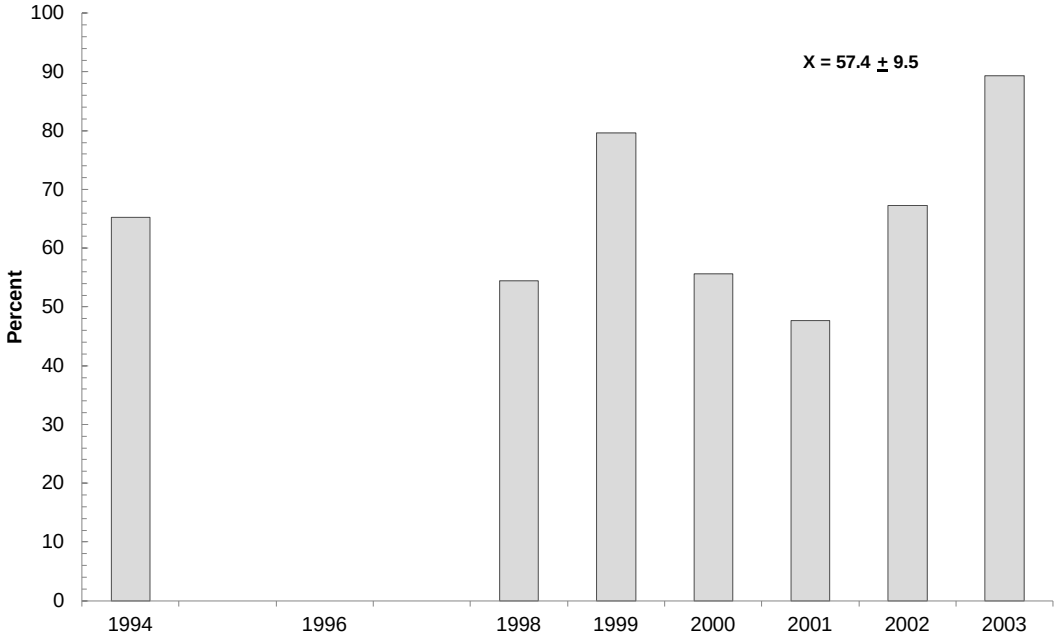

Figure 33. Percent survival by broodyear for East Fork stock Salmon River spring/summer Chinook salmon at MRS.

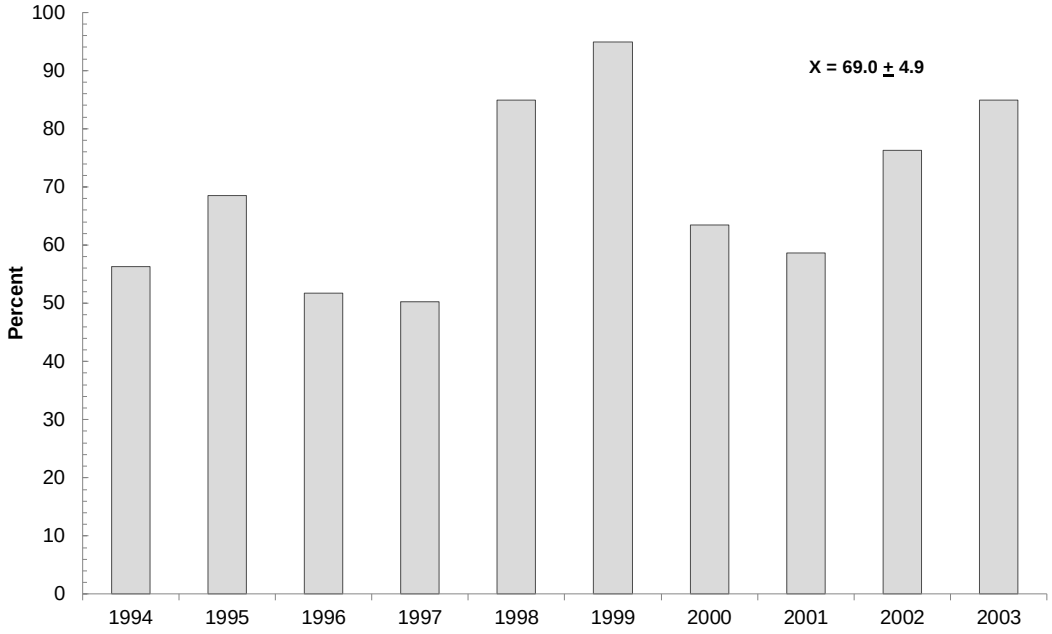

Figure 34. Percent survival by broodyear for Lostine River stock of Grande Ronde River spring/summer Chinook salmon at MRS.

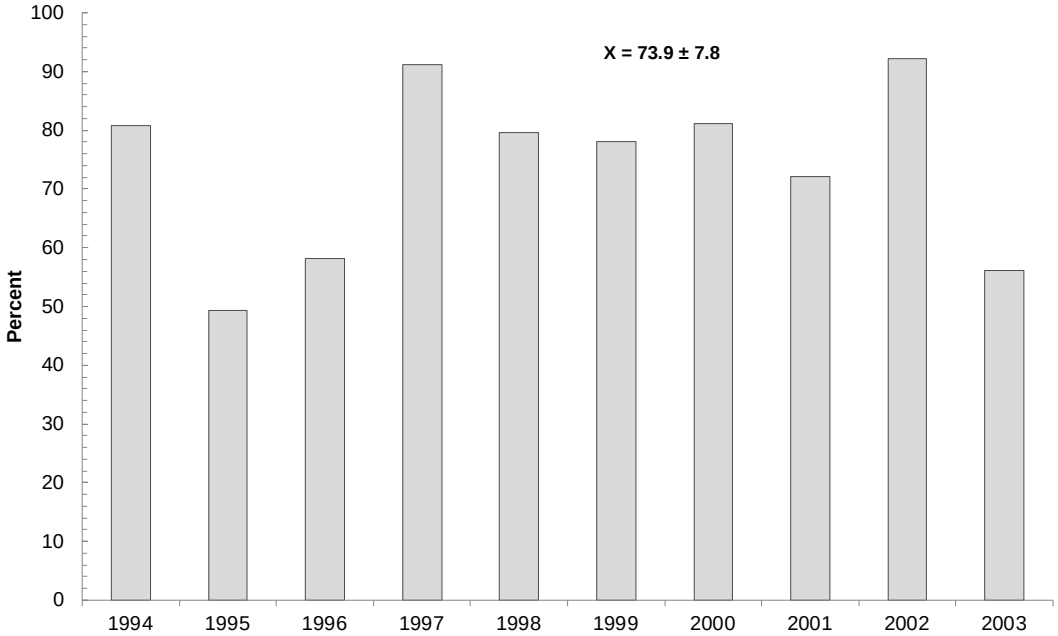

Figure 35. Percent survival by broodyear for Catherine Creek stock of Grande Ronde River spring/summer Chinook salmon at MRS.

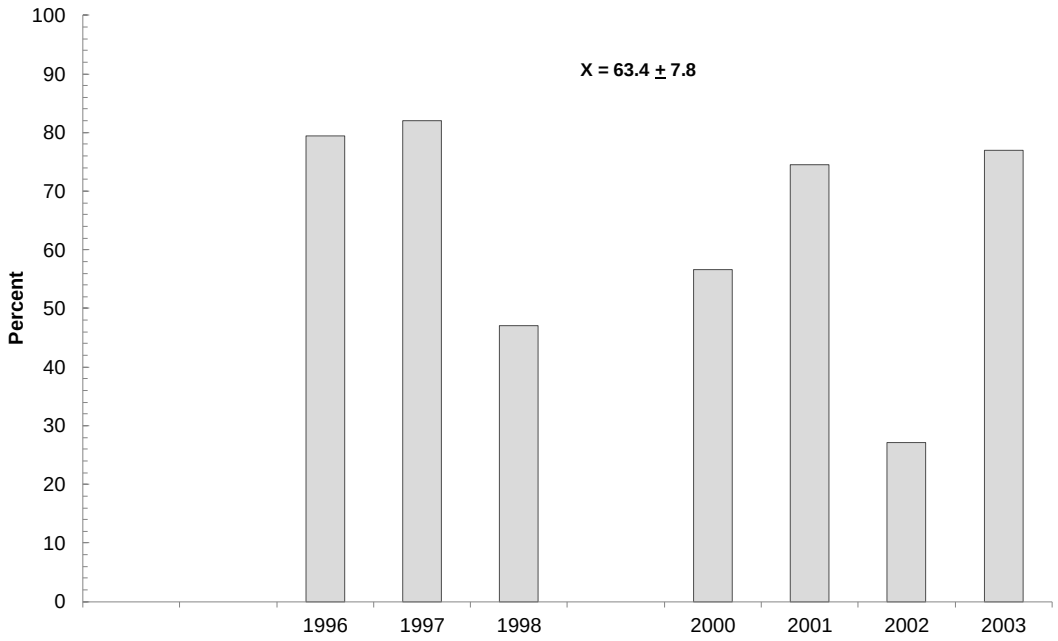

Figure 36. Percent survival by broodyear for Grande Ronde River stock of Grande Ronde River spring/summer Chinook salmon at MRS.

cm long, 5-year-old adults are 85–97 cm long, and most 6-year-old adults 88–99 cm long (Copeland et al. 2009). The average fork length of prespawning 3- and 4-year-old adult Salmon River fish at Manchester was 374 ±5mm and 489 ±7mm, respectively (Figure 37). Grande Ronde 3- and 4-year-old prespawning adults averaged 350 ±30 mm and 470 ±28 mm, respectively (Figure 38).

The average weight of 3- and 4-year-old maturing Salmon River spring Chinook salmon at MRS was 844.4 ±39.6 g for fish that would spawn as 3-year-olds and 1,811.3 ±86.0 g for fish that would spawn as 4-year-olds. Grande Ronde River maturing fish were generally heavier, weighing an average of 910.3 ±84.6 g for fish that would spawn as 3-year-olds and 2,060.1 ±159.3 g for fish that would spawn as 4-year-olds.

The maturation schedules (Figure 39 and Figure 40) for the Salmon and Grande Ronde groups of Snake River spring/summer Chinook salmon stocks reared at MRS suggest that fish may be maturing at a younger age than if they had gone to sea. For reference, data collected on natural spring Chinook salmon returning to weirs on the Grande Ronde River (Boe et al. 2005, 2006, 2007a, 2007b, 2009, 2010) indicate few fish mature as 3-year-old jacks (0–16%), most as 4-year-olds (49–85%), and often many as 5-year-olds (0–47%). Although precocious 2-year-old minijacks have been observed in the Grande Ronde, little data is available as to their origin (hatchery vs. natural) and numbers. Information collected at Lower Granite Dam on returning natural origin Snake River spring/summer Chinook salmon in 2008 indicates 15.7 % returned as 3-year-olds, 70.5% returned as 4-year-olds, 13.4% returned as 5-year-olds, and 0.5% returned as 6-year-olds (Copeland et al. 2009).

The 29.735% (±5.238) of the Salmon River fish that matured as precocious 2-year-old males seems higher than expected for naturally rearing fish. Age-3 jacks make up 24.461%

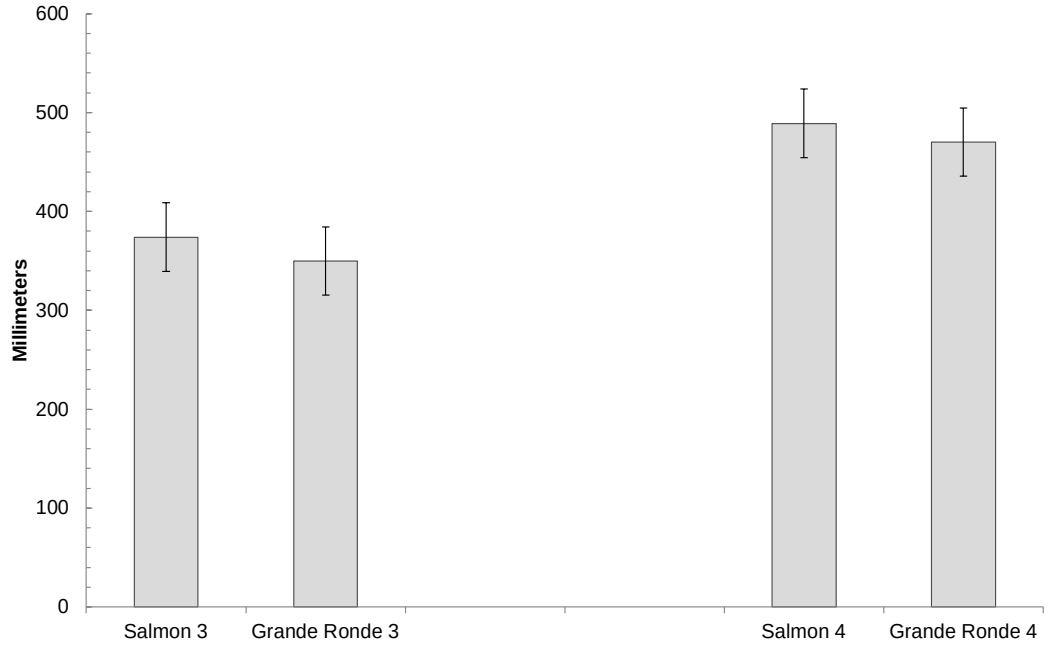

Figure 37. Average fork length (±SE) of prespawning 3- and 4-year-old adult Salmon River and Grande Ronde River spring/summer Chinook salmon at MRS.

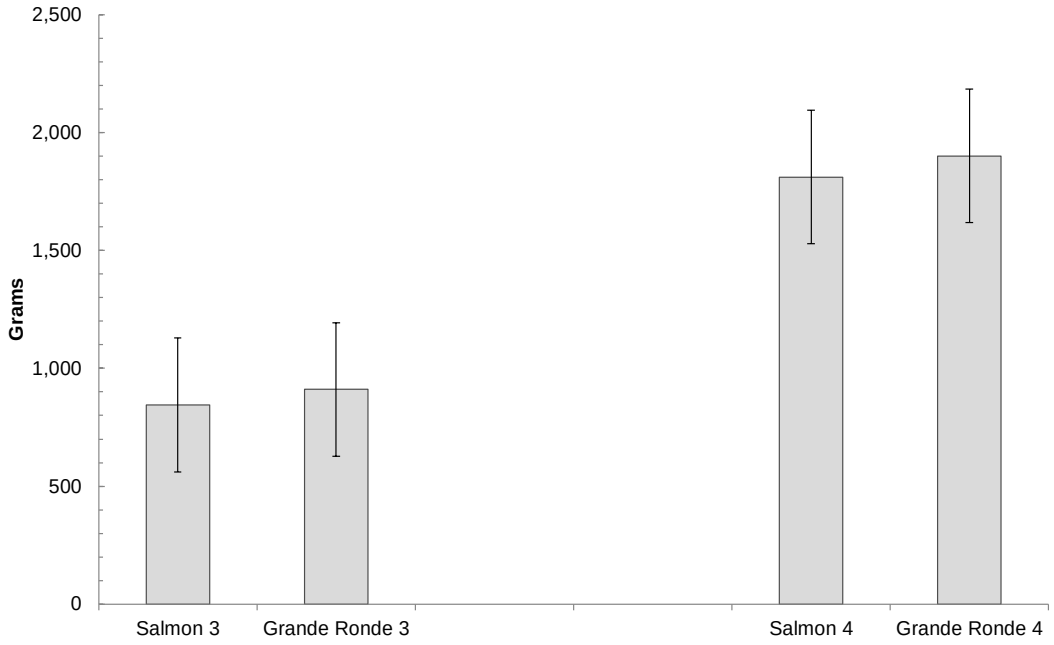

Figure 38. Average weight (±SE) of prespawning 3- and 4-year-old adult Salmon River and Grande Ronde River spring/summer Chinook salmon at MRS.

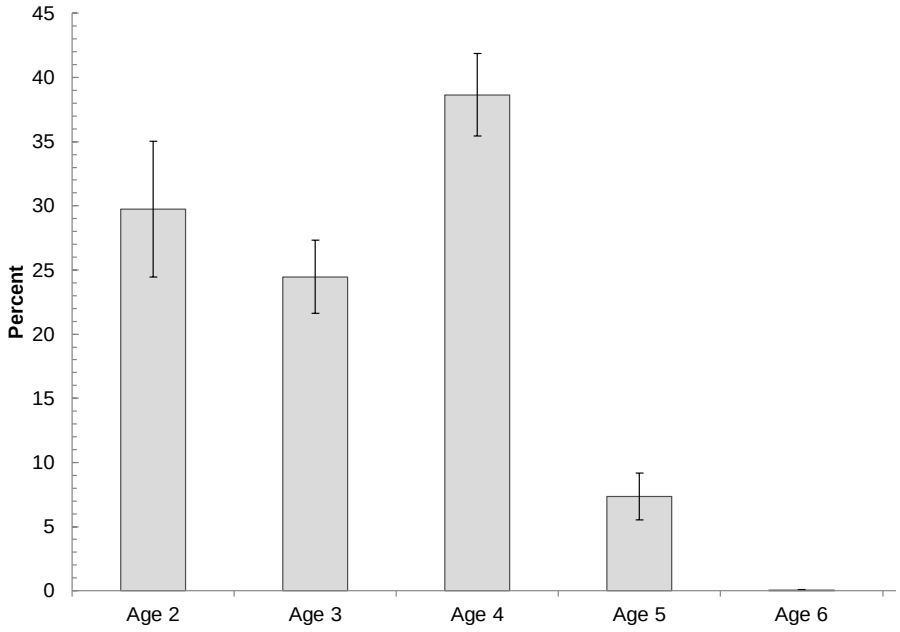

Figure 39. Percent of Salmon River stocks of Snake River spring/summer Chinook salmon reared at MRS maturing as 2-, 3-, 4-, 5-, and 6-year-old fish. Data based on broodyears 1994–2003.

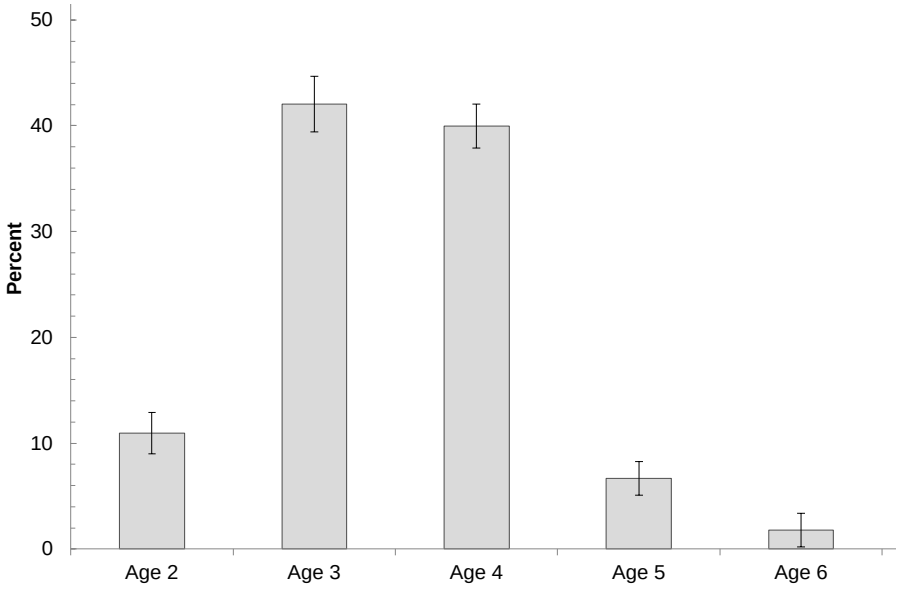

Figure 40. Percent of Grande Ronde River stocks of Snake River spring/summer Chinook salmon reared at MRS maturing as 2-, 3-, 4-, 5-, and 6-year-old fish. Data based on broodyears 1994–2003.

(±2.86) of the fish that survived to maturity in the Salmon River stocks, which also seems higher than expected for naturally rearing or ocean-ranched Chinook salmon. The percentage of Salmon River fish maturing as 4-, 5-, and 6-year-olds was 38.6% ±3.2, 7.3% ±1.828, and 0.1% ±0.04, respectively. The data for Grande Ronde stocks are less skewed towards younger fish, with only 10.5% ±1.9 maturing as 2-year-old precocious males. A greater percentage of the younger maturing fish in the Grande Ronde populations became 3-year-old adults (42% ±2.6) than in the Salmon River populations. The percentage of Grande Ronde Chinook maturing as 4-year-olds (40% ±2.1) was similar to that in the Salmon River stocks. As with the Salmon River stocks, only a few matured as 5-year-old (6.7% ±1.6) or 6-year-old (1.8% ±1.6) fish. The greater percentage of fish maturing at a younger age in both Snake River captive broodstock groups may be the result of higher growth.

Adult Fish Transportation

Snake River Sockeye

From 1996 through 2008, maturing adults from the Snake River captive broodstock groups at NWFSC facilities were sorted for maturity in June and July. Seawater-reared adults from MRS were transferred back to NMFS freshwater facilities at sorting and held in freshwater with the freshwater-reared component until transfer back to the Stanley Basin lakes of central Idaho via IDFG or NMFS transport trucks. Adults were released into their natal lakes in September of each year for volitional spawning in October as part of sockeye salmon restoration efforts by IDFG. During 12 years of adult transfers from NMFS facilities to Redfish Lake (1996−2008), there have been no mortality events (Table 2 and Appendix A).

Table 2 provides information on adult transfers from 1996 to 2008. For Redfish Lake sockeye salmon, 11 transfers (1 per year) were conducted using the protocols described above (see Appendix A for details). Transport container size ranged from 1,900 to 10,220 L (500 to 2,700 gal) for these transfers. The adults were transported for approximately 10 hours to EFH where half of the transfer water was exchanged for fresh well water. After about 1 hour of water exchange, the fish were transported another 3 hours to the release site at Redfish Lake. Total transport time from NMFS hatcheries to Redfish Lake was approximately 14 hours. At the time of release, a small number of adults were radio tagged for tracking fish movements in the lake during the approximate 4–6 weeks from release to spawning. Numbers of fish per transfer ranged from 61 to 434, transport density ranged 16–60 g/L (0.13−0.50 lb/gal) and had a geomean of 31 g/L (0.26 lb/gal). During the 5-year period documented in Appendix A for these transfer protocols, only one mortality event was associated with adult fish transfers.

Salmon River Chinook Salmon

From 1997 to 2005, maturing adults from Salmon River captive broodstock groups at MRS were sorted for maturity in late April and transferred back to IDFG's EFH 1 to 2 weeks later via IDFG transport truck. The remaining fish were sorted again in late May and transferred to EFH in early June via NMFS transport truck. Maturing adults from these transfers were held at the hatchery and tagged for release by IDFG into their natal Salmon River system streams for volitional spawning in August of each year as part of Chinook salmon restoration efforts.

Table 2. Summary of adult fish transfer success from MRS facilities to Oregon and Idaho. Fish transfers were conducted by either NMFS personnel with NMFS tanks and trucks or by ODFW or IDFG with state agency tanks and trucks and with NMFS personnel assisting. See Appendix A for complete data.

Stock transfers	No. of transfers	Numeric values	No. of fish	Average weight (g)	Fish (lb)	Transport tank (gal)	Density (lb/gal)	Transport duration	Survival (%)
Snake River sockeye									
To Stanley area,	11	Range	61–434	450–4,039	133–1,734	500–2,700	0.13–0.50	14	98.5–100
Idaho		Average	201	1,660	740	1,740	0.28	14	98.8
		Geomean	665	1,620	588	1,490	0.26	14	99.7
Salmon River Chinook									
To Boise area,	21	Range	12–245	118–3,440	15–648	180–2,700	0.08–0.55	10	100
Idaho		Average	67	1,495	207	707	0.28	10	100
		Geomean	45	1,202	120	492	0.25	10	100
To Stanley area,	9	Range	30–288	150–2,460	108–957	250–2,100	0.33–0.62	18–22	96.4–100
Idaho		Average	103	1,645	350	805	0.47	19.8	99.6
		Geomean	74	1,622	265	572	0.46	19.7	99.6
Grande Ronde Chinook									
To Cascade Locks	61	Range	3–204	60–2,699	4–651	70–2,250	0.22–1.11	5	100
area, Oregon		Average	71	1,210	194	575	0.30	5	100
		Geomean	50	940	104	475	0.22	5	100

Table 2 provides information on these transfers from 2002 to 2006, during which a total of 21 transfers (2−4 per year) were conducted using the above protocols (see Appendix A for details). The transport container size ranged 681−10,220 L (180–2,700 gal). The total transition time (transport time) from seawater to freshwater was approximately 10 hours. The numbers of fish per transfer ranged from 12 to 245, transport density ranged 10−66 g/L (0.08−0.55 lb/gal) and had a geomean of 30 g/L (0.25 lb/gal). During the 5-year period documented in Appendix A for the transport protocols described above, no direct mortality events were associated with the adult fish transfers (Table 2 and Appendix A). Additionally, no posttransfer mortalities were noted for these groups of fish at EFH.

In 2006−2008, the maturity sorting and transfer procedure was altered due to hatchery construction at EFH. Adults were sorted for maturity in early June, tagged, and held for an additional 5 weeks in seawater. In mid-July, fish were loaded on IDFG and NMFS transport trucks, transferred to their natal streams, and released for volitional spawning. Nine transfers were conducted using the above protocols (Table 2 and Appendix A). Transport container size ranged 681–10,220 L (180–2,700 gal). Adults were transported for approximately 10 hours to EFH where half of the transfer water was exchanged for fresh well water. After approximately 2 hours of water exchange, the fish were transported another 4 hours to release sites on the West Fork Yankee Fork and the East Fork of the Salmon River (total transfer time 18–22 hours). Numbers of fish per transfer ranged from 30 to 288, transport density ranged 40−75 g/L (0.33–0.62 lb/gal) and had a geomean of 55 g/L (0.46 lb/gal). During the 3-year period documented in Appendix A for these transfer protocols, only one mortality event occurred (Table 2 and Appendix A). In 2007 when the fish were released in the West Fork Yankee Fork during clear water conditions, senior IDFG staff noticed they were doing a poor job of holding position in the stream. A severe storm event occurred several hours after the release that unleashed into the water a tremendous amount of silt and ash from a fire the previous year, which resulted in an approximate 35% postrelease mortality of fish released into the West Fork Yankee Fork. Other than this instance, no posttransfer mortalities were noted for Snake River Chinook salmon.

Grande Ronde River Chinook Salmon

From 1996 through 2008, maturing adults from Grande Ronde River captive broodstock groups were sorted for maturity in spring and early summer and transferred to ODFW's BFH on the same day via ODFW or NMFS transport trucks. Maturing adult fish from these transfers were held and spawned at BFH and offspring used by ODFW for Chinook salmon restoration efforts in the Grande Ronde River system.

Table 2 provides information on these transfers from 2002 to 2006; during this period a total of 61 transfers (1–7 per year) were conducted using the above protocols (see Appendix A for details). Transport container size ranged 265–8,520 L (70–2,250 gal). Total transition time (transport time) from seawater to freshwater was approximately 5 hours. Numbers of fish per transfer ranged from 3 to 204, transport density ranged 2–133 g/L (0.02–1.11 lb/gal) and had a geomean of 26 g/L (0.22 lb/gal). During the entire 12 years of adult transfers from MRS to BFH (1997–2008), there have been no mortality events (Table 2 and Appendix A). Additionally, no posttransfer mortalities were noted for these groups of fish at BFH.

Insights and Recommendations

Fish culture technologies described in this report successfully maintained ESA-listed sockeye and Chinook salmon stocks in safety net programs. This has prevented the loss of stocks with unique genetic characteristics and generated large numbers of fish that were used in restoration actions. The value of broodstock technology can be demonstrated by following the theoretical fate of 100 green sockeye salmon eggs. If left for natural spawning and rearing, one would expect no more than a single recruit to the next generation (one-to-one replacement in a static population). In contrast, if 100 eggs are placed in a safety net captive broodstock program using the technology described here, they should produce 46 adults that could be released back into their native waters for natural spawning.

The decision to take the population into a safety net program produces a 46-fold amplification of the population within a single generation. If a decision is then made to retain these 46 mature adults in the safety net program and spawn them to produce juveniles for reintroduction, one would expect to produce approximately 40,606 green eggs, assuming 23 females spawned at age 3 with an average fecundity of 1,765.5 eggs/female. If the 40,606 eggs were fertilized and reared to the smolt stage with a 50% survival in captivity, assuming a 0.1% smolt to adult survival, they would produce more than 20 recruits to the next generation. This is a 20-fold amplification over what would occur if 100 green eggs were left in nature. The ability to rapidly amplify a population is the main strength of safety net fish culture technology (see Flagg and Mahnken 1995 for more information on amplification potentials).

Key factors leading to the success of safety net fish culture technology include pathogen free water, onshore tanks, fully enclosed buildings, and a rigorous biosecurity protocol. These factors improve fish health and provide higher survivals than when Pacific salmon are reared in untreated surface water. In addition, in our case these key factors help ensure out-of-basin stocks do not contract local Puget Sound pathogens that could prohibit their return to natal areas under existing interstate fish transfer permit requirements. The onshore tanks and fully enclosed building not only protect against avian and aerosol-borne pathogens, but also avoid predation that may occur when captive broodstocks are reared in net pens (Flagg and Mahnken 1995).

Rearing Chinook and sockeye salmon at low densities in large tanks is critical for producing the high survival experienced in this captive broodstock program. Low fish densities and large tanks help buffer the low turnover rates associated with the water quantity limitations at our freshwater and seawater rearing facilities. They are also crucial in ensuring oxygen, ammonia, and temperatures remain adequate when pump failures or other life support emergencies develop. Ramped lighting and energy adsorbing netting help prevent fish from injury. Rearing Pacific salmon in seawater during the marine portion of their life cycle should help promote the retention of anadromous traits within the population. Additionally, we have noted that seawater rearing for Chinook salmon in particular produces fish with color and shape similar to their naturally reared counterparts. Likewise, when sockeye salmon are placed in

outdoor or greenhouse facilities for final maturation, they develop red coloration similar to ocean-returning fish. More natural body shape and coloration should benefit safety net fish when they are used in adult reintroductions.

Appropriate rearing water temperature is critical for successful captive broodstock programs. The 10°C ground water used to rear sockeye salmon is pathogen free and improves in-culture survival and gamete quality, but results in accelerated growth to produce fish that mature at a younger age than in nature. We have been able to reduce some precocity in sockeye by chilling eggs so that fry emerge near the natural time and grow less overall during their first year. The increased precocity observed at MRS in Idaho Chinook salmon stocks may result from previous rearing at a higher temperature and for longer times in freshwater at IDFG's EFH compared to the Oregon Chinook salmon. The seawater at MRS has more annual variation in water temperature than does the ground water at Burley, and does not provide the cool environment that sockeye or Chinook salmon experience during their natural oceanic migrations. Nonetheless, either the temperature profile or some other characteristic of seawater is sufficient to increase the percentage of sockeye salmon maturing as 4-year-olds in adult release groups reared at MRS.

Reducing the risk of extinction by maintaining duplicate genetic groups in separate facilities is a proven strategy. Duplicate facilities provides insurance that at least one facility remains pathogen free if the other experiences disease problems. Duplicate facilities also provide an opportunity to expand production if one facility experiences abnormally low survival. It is recommended that, as the importance of retaining the stock increases (e.g., has endangered vs. threatened listing status), the number of duplicate groups be increased.

Transport protocols for ESA-listed stocks should follow relatively conservative guidelines, although little information is available concerning precise requirements for transport of maturing salmonids, especially those listed under ESA. Piper et al. (1982) and Pennell and Barton (1996) indicate transport limits of about 60−120 g/L (0.5−1.0 lb/gal) for fry size salmonids. Piper indicates that loading density can be increased with size to about 300–420 g/L (2.5–3.5 lb/gal) for smolt size steelhead (*O. mykiss*) for 8−10 hour transports. Our loading densities were very conservative compared to those described above, ranging 2.4−133 g/L (0.02–1.11 lb/gal) with a geomean below 30 g/L (0.25 lb/gal). In total we documented 91 successful transfers of adult size Chinook salmon and 11 transfers of adult size sockeye salmon under our transport protocols, with transports ranging 5−22 hours. Only two minor mortality events were associated with any of these transfers, with geomeans for the transfers ranging from 99.6 to 100% survival. Additionally, no posttransfer transportation-associated mortalities were noted for these groups of fish.

In summary, the captive broodstock fish culture technologies described in this report can be safely utilized to prevent extinction and amplify populations of salmon for recovery efforts. If the results described above for ESA-listed endangered Redfish Lake sockeye salmon can be used as a guide, it can be expected that for every 100 green eggs taken into a safety net captive broodstock program, more than 40,000 green eggs could be produced to the next generation. Those initiating a safety net captive broodstock project for Chinook salmon should expect that for every 100 smolts sent to a marine facility for seawater rearing, 58 prespawning adults can be produced for use in restoration efforts. At a 1% return rate, this is 58 times the number of

prespawning adults that would be expected if these same 100 smolts had been left in nature to complete their marine migration.

At the initiation of present captive broodstock programs for Pacific salmon, Flagg and Mahnken (1995) indicated that the technology was in its infancy, without well-described standards and with full-term rearing to adult stage problematic. We believe information described in the present report provides standards for conducting a successful captive broodstock effort. Egg-to-adult survivals of 47% or better can be obtained if captive broodstocks are reared in biosecure situations with adequate life support at densities below approximately 1 kg/L/min (9 lb/gal/min). Salmonid captive broodstocks can safely be reared to adult in either fresh well water or sterilized seawater. Although for some species (e.g., Chinook salmon), rearing to adult in seawater appears to enhance adult fish quality and body confirmation.

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Appendix A: Transport Details for Intersite Fish Transfers

This appendix consists of three tables listing transport details for transfers from NWFSC's Manchester Research Station in Washington to sites in Oregon and Idaho.

Table A-1. Transport details for intersite fish transfers.

Date	Transfer[a]	Transfer time (h)	Broodyear	No. fish	Average weight (g)	Fish (lb)	Transfer tank (gal)[b]	Density (lb/gal)	Load temp.	Release temp.	DO[c] (mg/L)	Survival (%)	Comment[d]
1996 transfer													
8/16/96	MRS to BFH	5	1994	20					10°C	10°C	10–15	100	B, C
1997 transfers													
7/24/97	MRS to BFH	5	1994	53					10°C	11°C	15–20	100	B, C
8/19/97	MRS to BFH	5	1994, 1995	16					10°C	11°C	10–20	100	B, C
9/17/97	MRS to BFH	5	1995	1					10°C	11°C	10–20	100	B, C
1998 transfers													
6/17/98	MRS to BFH	5	1994, 1995	125	445–1,684	308	750 ODFW	0.41	10°C	11°C	10–15	100	B, C
7/14/98	MRS to BFH	5	1994, 1995	24	445–1,684	67	250 NMFS	0.27	10°C	11°C	10–20	100	B, C
8/17/98	MRS to BFH	5	1994, 1995, 1996	14	100–1,684	28	500 NMFS	0.06	10°C	12°C	10–20	100	B, C
9/2/98	MRS to BFH	5	1996	5			140 NMFS		10°C	12°C	10–20	100	B, C
1999 transfers													
6/2/99	MRS to BFH	5	1994, 1995, 1996	204	746–2,280	651	1,500 ODFW	0.43	10°C	11°C	15–20	100	B, C
6/23/99	MRS to BFH	5	1994, 1995, 1996	22	840–1,943	70	500 NMFS	0.14	10°C	11°C	10–20	100	B, C
7/30/99	MRS to BFH	5	1995, 1996	3	820–1,994	8	140 NMFS	0.06	10°C	12°C	10–20	100	B, C
8/12/99	MRS to BFH	5	1997	34	131	10	180 NMFS	0.06	10°C	12°C	10–20	100	B, C
2000 transfers													
5/24/00	MRS to BFH	5	1996	118	2,153–2310	583	525 ODFW	1.11	9°C	10°C	15–20	100	B, C
5/25/00	MRS to BFH	5	1994, 1995, 1997	97	1,161–1923	290	525 ODFW	0.56	9°C	10°C	15–20	100	B, C
5/26/00	MRS to BFH	5	1994, 1995, 1996, 1997	76	1,087–2,155	277	525 ODFW	0.53	9°C	10°C	15–20	100	B, C
6/22/00	MRS to BFH	5	1995, 1996, 1997	14	1,133–2,570	62	500 NMFS	0.12	10°C	11°C	10–20	100	B, C
7/19/00	MRS to BFH	5	1998	42			210 NMFS		10°C	12°C	10–20	100	B, C
2001 transfers													
5/21/01	MRS to BFH	5	1997	71	2,699	422	750 ODFW	0.56	9°C	10°C	15–20	100	B, C
5/22/01	MRS to BFH	5	1996, 1997	80	2,120–2,618	395	750 ODFW	0.53	9°C	10°C	15–20	100	B, C
5/23/01	MRS to BFH	5	1995, 1996, 1997, 1998	80	1,374–2,462	280	750 ODFW	0.37	9°C	10°C	15–20	100	B, C

Table A-1 continued. Transport details for intersite fish transfers.

Date	Transfer[a]	Transfer time (h)	Broodyear	No. fish	Average weight (g)	Fish (lb)	Transfer tank (gal)[b]	Density (lb/gal)	Load temp.	Release temp.	DO[c] (mg/L)	Survival (%)	Comment[d]
2001 transfers (continued)													
5/24/01	MRS to BFH	5	1998	69	1,066–1,141	166	750 NMFS	0.22	9°C	10°C	10–20	100	B, C
6/29/01	MRS to BFH	5	1997	28	1,017–2,108	115	750 NMFS	0.15	10°C	12°C	10–20	100	B, C
7/2/01	MRS to BFH	5	1998	14	1,093–1,212	36	360 NMFS	0.10	10°C	12°C	10–20	100	B, C
8/9/01	MRS to BFH	5	1999	26			140 NMFS		10°C	12°C	10–20	100	B, C
2002 transfers													
4/8/02	MRS to BFH	5	1996, 1997, 1998, 1999	196	699–2,088	520	2,250 ODFW	0.23	9°C	10°C	15–20	100	B, C
4/11/02	MRS to BFH	5	1999	41	378	34	180 NMFS	0.19	9°C	10°C	10–20	100	B, C
5/20/02	MRS to BFH	5	1998	70	1,202–2,069	278	750 ODFW	0.37	9°C	10°C	15–20	100	B, C
5/20/02	MRS to BFH	5	1997, 1999	31	637–1,817	73	750 NMFS	0.10	9°C	10°C	10–20	100	B, C
7/22/02	MRS to BFH	5	2000	43			210 NMFS		10°C	12°C	10–20	100	B, C
2003 transfers													
4/9/03	MRS to BFH	5	1999, 2000				750 ODFW		9°C	10°C	15–20	100	B, C
4/10/03	MRS to BFH	5	1998, 2000				750 ODFW		9°C	10°C	15–20	100	B, C
4/11/03	MRS to BFH	5	1998				750 ODFW		9°C	10°C	15–20	100	B, C
5/28/03	MRS to BFH	5	1999, 2000	9	671–1,187	21	360 NMFS	0.06	10°C	11°C	10–20	100	B, C
7/23/03	MRS to BFH	5	2001	36	60–138	9	180 NMFS	0.05	10°C	12°C	10–20	100	B, C
2004 transfers													
4/7/04	MRS to BFH	5	2000	108	1,376–1,465	330	750 ODFW	0.44	9°C	10°C	15–20	100	B, C
4/7/04	MRS to BFH	5	2000	51	1,681	190	350 ODFW	0.54	9°C	10°C	15–20	100	B, C
4/8/04	MRS to BFH	5	2001	152	649–721	229	750 ODFW	0.31	9°C	10°C	15–20	100	B, C
4/8/04	MRS to BFH	5	2001	82	732	132	350 ODFW	0.38	9°C	10°C	15–20	100	B, C
4/9/04	MRS to BFH	5	1999	6	1,787	24	140 NMFS	0.17	10°C	10°C	10–20	100	B, C
5/26/04	MRS to BFH	5	2000, 2001	21	255–1,357	48	220 NMFS	0.22	10°C	10°C	10–20	100	B, C
7/30/04	MRS to BFH	5	2002	59	86	12	500 NMFS	0.02	10°C	12°C	10–20	100	B, C
2005 transfers													
4/4/05	MRS to BFH	5	2001	92	1,102–1,277	231	750 ODFW	0.31	9°C	10°C	15–20	100	B, C
4/5/05	MRS to BFH	5	2000, 2001	90	1,197–1,277	235	750 ODFW	0.31	9°C	10°C	15–20	100	B, C
4/13/05	MRS to BFH	5	2002	164	582–789	274	750 NMFS	0.37	10°C	10°C	10–20	100	B, C

Table A-1 continued. Transport details for intersite fish transfers.

Date	Transfer[a]	Transfer time (h)	Broodyear	No. fish	Average weight (g)	Fish (lb)	Transfer tank (gal)[b]	Density (lb/gal)	Load temp.	Release temp.	DO[c] (mg/L)	Survival (%)	Comment[d]
2005 transfers (continued)													
5/25/05	MRS to BFH	5	2000, 2001, 2002	39	225–1,453	85	440 NMFS	0.19	10°C	10°C	10–20	100	B, C
7/18/05	MRS to BFH	5	2003	85	105–112	20	210 NMFS	0.10	10°C	12°C	10–20	100	B, C
2006 transfers													
4/24/06	MRS to BFH	5	2002, 2003	136	793–1,366	338	750 ODFW	0.45	9°C	10°C	15–20	100	B, C
4/25/06	MRS to BFH	5	2002, 2003	133	829–1,287	307	750 ODFW	0.41	9°C	10°C	15–20	100	B, C
4/27/06	MRS to BFH	5	2001, 2002, 2003	82	721–891	158	750 NMFS	0.21	10°C	10°C	10–20	100	B, C
6/9/06	MRS to BFH	5	2002, 2003	28	793–1,365	77	250 NMFS	0.31	10°C	10°C	10–20	100	B, C
7/26/06	MRS to BFH	5	2004	38	88–100	8	140 NMFS	0.06	10°C	12°C	10–20	100	B, C
2007 transfers													
4/24/07	MRS to BFH	5	2003, 2004	139	1,123–1,681	434	750 ODFW	0.58	9°C	10°C	15–20	100	B, C
4/25/07	MRS to BFH	5	2003, 2004	164	909–1,965	472	750 ODFW	0.63	9°C	10°C	15–20	100	B, C
4/27/07	MRS to BFH	5	2002, 2003	62	1,004–1,965	245	750 NMFS	0.33	10°C	10°C	10–20	100	B, C
5/24/07	MRS to BFH	5	2002, 2003, 2004	20	909–1,965	55	500 NMFS	0.11	10°C	11°C	10–20	100	B, C
7/25/07	MRS to BFH	5	2005	21	82	4	70 NMFS	0.06	10°C	12°C	10–20	100	B, C
2008 transfers													
4/22/08	MRS to BFH	5	2004	62	1,786	244	750 ODFW	0.33	9°C	10°C	15–20	100	B, C
4/23/08	MRS to BFH	5	2005	87	988	190	500 ODFW	0.38	9°C	10°C	15–20	100	B, C
4/24/08	MRS to BFH	5	2004	89	2,014	395	750 ODFW	0.53	9°C	10°C	15–20	100	B, C
5/2/08	MRS to BFH	5	2002, 2003	15	1,362–1,497	47	360 NMFS	0.13	10°C	11°C	10–20	100	B, C

[a]MRS = Manchester Research Station, Washington; BFH = Bonneville Fish Hatchery, Oregon.
[b]ODFW = Oregon Department of Fish and Wildlife; NMFS = National Marine Fisheries Service.
[c]DO = dissolved oxygen.
[d]B = Stop every 2–3 hours to check dissolved oxygen level, temperature, and fish condition; C = Fish transferred in 25% seawater and 75% freshwater.

Table A-2. Transport details for intersite fish transfers, Salmon River spring Chinook salmon (*Oncorhynchus tshawytscha*).

Date	Transfer[a]	Transfer time (h)	Broodyear	No. fish	Average weight (g)	Fish (lb)	Transfer tank (gal)[b]	Density (lb/gal)	Load temp	Release temp	DO[c] (mg/L)	Survival (%)	Comment[d]
2002 transfers													
4/23/02	MRS to EFH	10	1998	52	3,073	352	1,000 IDFG	0.35	10°C	11°C	10–15	100	A, C
4/23/02	MRS to EFH	10	1997, 1998	98	2,305	498	900 IDFG	0.55	10°C	11°C	10–15	100	A, C
4/23/02	MRS to EFH	10	1997, 1999	113	790–2,088	229	800 IDFG	0.29	10°C	11°C	15–20	100	A, C
4/23/02	MRS to EFH	10	1998	17	1,263–2,309	70	440 NMFS	0.16	10°C	11°C	10–20	100	A, B, C
4/23/02	MRS to EFH	10	1999	23	841	43	180 NMFS	0.25	10°C	11°C	10–20	100	A, B, C
6/11/02	MRS to EFH	10	1998	15	2,169	72	250 IDFG	0.29	10°C	12°C	10–15	100	A, C
6/11/02	MRS to EFH	10	1998, 1999	49	409–2,375	164	690 NMFS	0.24	10°C	12°C	10–20	100	A, B, C
2003 transfers													
5/6/03	MRS to EFH	10	1999	135	1,730–2,100	583	2,700 IDFG	0.22	10°C	12°C	10–15	100	A, C
5/6/03	MRS to EFH	10	1998	22	1,870	91	250 IDFG	0.36	10°C	12°C	10–15	100	A, C
5/6/03	MRS to EFH	10	2000	37	1,690	138	250 IDFG	0.55	10°C	12°C	10–15	100	A, C
5/6/03	MRS to EFH	10	1998, 2000	40	1,580–3,440	235	750 NMFS	0.31	10°C	12°C	10–15	100	A, B, C
6/12/03	MRS to EFH	10	1999, 2000	12	1,209–1,541	36	360 NMFS	0.10	10°C	12°C	10–15	100	A, B, C
2004 transfers													
5/6/04	MRS to EFH	10	2000	116	1,992–2,906	648	1,900 IDFG	0.34	10°C	11°C	10–15	100	A, C
5/6/04	MRS to EFH	10	1999, 2001	13	670	19	250 IDFG	0.08	10°C	11°C	10–15	100	A, C
5/6/04	MRS to EFH	10	1998, 1999, 2001	20	718–2,386	72	250 IDFG	0.29	10°C	11°C	10–15	100	A, C
7/8/04	MRS to EFH	10	2002	49	143	15	180 NMFS	0.08	10°C	12°C	10–20	100	A, B, C
2005 transfers													
5/4/05	MRS to EFH	10	2001, 2002	33	1,018	74	250 IDFG	0.30	10°C	11°C	10–15	100	A, C
5/4/05	MRS to EFH	10	2001, 2002	32	1,022	72	250 IDFG	0.29	10°C	11°C	10–15	100	A, C
5/4/05	MRS to EFH	10	2001, 2002	151	782	260	750 NMFS	0.34	10°C	11°C	10–20	100	A, B, C
7/21/05	MRS to EFH	10	2003	125	174	48	360 NMFS	0.13	10°C	12°C	10–20	100	A, B, C
2006 transfers													
7/12/06	MRS to Stanley, Idaho	20	2002, 2003, 2004	245	118–2,100	621	2,100 IDFG	0.30	10°C	14°C	10–15	100	A, C
7/12/06	MRS to Stanley, Idaho	20	2002	81	1,587	283	750 NMFS	0.38	10°C	14°C	10–20	100	A, B, C

Table A-2 continued. Transport details for intersite fish transfers, Salmon River spring Chinook salmon.

Date	Transfer[a]	Transfer time (h)	Broodyear	No. fish	Average weight (g)	Fish (lb)	Transfer tank (gal)[b]	Density (lb/gal)	Load temp	Release temp	DO[c] (mg/L)	Surviv-al (%)	Comment[d]
2007 transfers													
7/11/07	MRS to Stanley, Idaho	20	2002, 2003, 2004	288	150–2,460	957	2,100 IDFG	0.46	10°C	16°C	10–15	100	A, C
7/11/07	MRS to Stanley, Idaho	22	2002, 2003, 2004, 2005	192	150–2,111	446	750 NMFS	0.59	10°C	14°C	10–20	96.4	A, B, C, D
7/11/07	MRS to Stanley, Idaho	22	2003, 2004	39	1,100–2,068	156	250 IDFG	0.62	10°C	16°C	10–15	100	A, C, D
7/11/07	MRS to Stanley, Idaho	22	2003, 2004	38	1,100–2,068	152	250 IDFG	0.61	10°C	16°C	10–15	100	A, C, D
2008 transfers													
7/9/08	MRS to Stanley, Idaho	18	2003, 2004, 2005	160	990–1,960	632	1,900 IDFG	0.33	10°C	12°C	10–15	100	A, C
7/9/08	MRS to Stanley, Idaho	18	2003, 2004, 2005	71	990–1,960	306	750 NMFS	0.41	10°C	12°C	7–24	100	A, C
7/9/08	MRS to Stanley, Idaho	18	2004	30	1,635	108	250 IDFG	0.43	10°C	12°C	10–15	100	A, C
7/9/08	MRS to Stanley, Idaho	18	2004	30	1,635	108	250 IDFG	0.43	10°C	12°C	10–15	100	A, C

[a]MRS = Manchester Research Station; EFH = Eagle Fish Hatchery.
[b]IDFG = Idaho Department of Fish and Game; NMFS = National Marine Fisheries Service.
[c]DO = dissolved oxygen.
[d]A = Water exchange at Eagle Hatchery ID after 10 hour drive; B = Stop every 2–3 hours to check dissolved oxygen level, temperature, and fish condition; C = Fish transferred in 25% seawater and 75% freshwater; and D = Postrelease mortality was 45% due primarily to severe environmental conditions at time of release.

Table A-3. Transport details for intersite fish transfers, Snake River (Redfish Lake) sockeye salmon (*O. nerka*).

Date	Transfer[a]	Transfer time (h)	Broodyear	No. fish	Average weight (g)	Fish (lb)	Transfer tank (gal)	Density (lb/gal)	Load temp	Release temp	DO[b] (mg/L)	Survival (%)	Comment[c]
1996 transfer													
9/10/96	MRS to Stanley, Idaho	14	1993	80	1,510	266	2,000 IDFG	0.13	10°C	14°C	10–15	100	A, B, E
1997 transfer													
9/11/97	MRS to Stanley, Idaho	14	1993, 1994	120	1,866	493	2,000 IDFG	0.25	10°C	14°C	10–15	100	A, B, E
2000 transfer													
9/5/00	MRS to Stanley, Idaho	14	1997	61	990	133	500 NMFS	0.27	10°C	14°C	10–15	100	A, B, E
2001 transfer													
9/9/01	MRS to Stanley, Idaho	14	1997	66	2,498	363	2,000 IDFG	0.18	10°C	14°C	10–15	98.5	A, B, E
2002 transfer													
9/11/02	MRS to Stanley, Idaho	14	1999	131	1,390	401	2,000 IDFG	0.20	10°C	14°C	10–15	100	A, B, E
2003 transfer													
9/16/03	MRS to Stanley, Idaho	14	2000	280	1,395–1,743	1,001	2,700 IDFG 750 NMFS	0.29	10°C	14°C	10–15	100	A, B, E
2004 transfer													
9/7/04	MRS to Stanley, Idaho	14	2000, 2001	241	1,437–3,413	1,007	2,700 IDFG 750 NMFS	0.29	10°C	14°C	10–15	100	A, B, E
2005 transfer													
9/7/05	MRS to Stanley, Idaho	14	2000, 2001, 2002	177	1,200–2,244	671	2,700 IDFG 750 NMFS	0.19	10°C	14°C	10–15	100	A, B, E
2006 transfer													
9/8/06	MRS to Stanley, Idaho	14	2002, 2003, 2004	434	450–3,300	1,734	2,700 IDFG 750 NMFS	0.50	10°C	14°C	10–15	100	A, B, E
2007 transfer													
9/5/07	MRS to Stanley, Idaho	14	2002, 2003, 2004	367	1,253–3,200	1,341	2,100 IDFG 750 NMFS	0.47	10°C	14°C	10–15	98.9	A, B, E
2008 transfer													
9/10/08	MRS to Stanley, Idaho	14	2003, 2004, 2005	253	982–4,039	726	2,100 IDFG	0.35	10°C	14°C	10–15	100	A, B, E

[a]MRS = Manchester Research Station.
[b]DO = dissolved oxygen.
[c]A = Water exchange at Eagle Hatchery, ID, after 10-hour drive; B = Stop every 2–3 hours to check dissolved oxygen level, temperature, and fish condition; and E = Fish transferred in 100% freshwater.

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