National Marine Fisheries Service (NMFS)
Application for a Scientific Research/Enhancement Permit under Section 10(a)(1)(A) of the Endangered Species Act of 1973

A. Title: Application for Permit for Scientific Purposes and to Enhance the Propagation or Survival of Listed Species under the Endangered Species Act of 1973

B. Species: Snake River Sockeye Salmon (*Oncorhynchus nerka)*

C. Date: 1 December 2002

D. Applicant Identity:

Dr. Walton W. Dickhoff, Acting Division Director
National Marine Fisheries Service
Northwest Fisheries Science Center
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E. Information on Personnel, Cooperators, and Sponsors.

***1. Principal Investigators*:** Thomas A. Flagg 360-871-8306
Dr. Desmond J. Maynard 360-871-8313

***Field Supervisors*:** Dr. Lee Harrell
Carlin McAuley
Deborah A. Frost
Michael Wastel

***2. Field Personnel*:** James Hackett
Dr. William Fairgrieve
Kelly Henderson
Bryon Kluver
Gail McDowell

***3. Project Funding*:** Bonneville Power Administration
Division of Fish and Wildlife – KEWU-4
P.O. Box 3621
Portland, Oregon 97208-3621
Dr. Jeffery Gislason (COTR)
Phone: 503-230-3594

***4. Contractor Activities*:** none

5. Disposition of tissue samples, dead specimens, or other remains:

Select mortalities are frozen or preserved as appropriate for genetic or other analyses. Select specimens may also be preserved (e.g., preserved in formalin, tanned, preserved by taxidermy, etc.) for future use for research or educational purposes. NMFS and other sponsors and cooperating institutions involved for the Redfish Lake Sockeye salmon captive broodstock program may display preserved specimens in government offices, universities, and other public places for purposes of education and public outreach. Specimens not vital to analysis, outreach education, or restoration are incinerated, rendered, or buried.

6. Qualifications and experience of all staff responsible for care without supervision:

CURRICULUM VITAE--Thomas Alvin Flagg

Current Employer: Northwest Fisheries Science Center, National Marine Fisheries Service

Education:

- B.S., Fisheries Biology, University of Washington, Seattle, WA, 1976.
- M.S., Fisheries Biology, University of Washington, Seattle, WA, 1981.
- Ph. D. program, University of Idaho, Moscow, ID, present.

Professional Experience:

Position	Organization	City	State	Dates
Fisheries Research Biologist	NMFS	Manchester	WA	1/78-present

Current Responsibilities: Program Manager, Salmon Enhancement Projects. Responsibilities include: development of captive broodstock programs to conserve depleted gene pools of salmonids; development of supplementation techniques for restoration of depleted stocks of salmonids to their native habitats; and development of fish husbandry technology to produce wild-type juvenile salmon for release from hatcheries.

Expertise: Mr. Flagg has participated in a number of captive broodstock programs for Pacific and Atlantic salmon. His current research focuses on reform of fish husbandry strategies for conservation hatcheries. Past research included: determination of status of depleted stocks of fish including those proposed for listing as threatened or endangered under the Endangered Species Act; development of the passive integrated transponder (PIT) tagging system for salmonids; development of freshwater and seawater net-pen aquaculture husbandry and captive broodstock techniques for Atlantic and Pacific salmon (including research in the areas of aquaculture systems design and development, stock rearing strategies, nutrition, disease investigations, maturation and spawning, hormonal sex reversal, smoltification, and stock performance); investigation of fish-collection and transportation related mortalities in juvenile salmonids in the Columbia River system; evaluation of the impact of the 1980 Mt. St. Helens eruption on juvenile salmonids in the Columbia River system; and investigation of the relationship between swimming behavior, smoltification status, and seawater survival for coho salmon.

Selected Publications:

Flagg, T. A., B. A. Berejikian, J. E. Colt, W. W. Dickhoff, L. W. Harrell, D. J. Maynard, C. E. Nash, M. S. Strom, R. N. Iwamoto, and C. V.W. Mahnken. 2000. Ecological and behavioral impacts of artificial production strategies on the abundance of wild salmon populations. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-NWFSC-41, 91 p.

Flagg, T. A. and C.V.W. Mahnken. 2000. Endangered species recovery: captive broodstocks to aid recovery of endangered salmon stocks. Encyclopedia of Aquaculture, J. Wiley and Sons, p. 290-292.

Schiewe, M. H., T. A. Flagg, and B. A. Berejikian. 1997. The use of captive broodstocks for gene conservation of salmon in the western United States. Bull. Natl. Res. Inst. Aquacult., Suppl. 3:29-34.

Flagg, T. A., C. V. W. Mahnken, and K. A. Johnson. 1995. Captive broodstocks for recovery of Snake River sockeye salmon. Am. Fish. Soc. Symp. 15:81-90.

Flagg, T. A. and C. V. W. Mahnken (editors). 1995. An assessment of captive broodstock technology for Pacific salmon. Report to Bonneville Power Administration, Contract DE-AI79 93BP55064, 299 p.

CURRICULUM VITAE--Dr. Desmond J. Maynard

Current Employer: Northwest Fisheries Science Center, National Marine Fisheries Service

Education:

- A.A., Business management, Cape Cod Community College, Hyannis, MA, 1971.
- B.S., Marine Biology, University of Massachusetts, North Dartmouth, MA, 1974.
- M.S., Fisheries Science, University of Washington, Seattle, WA, 1980.
- Ph.D., Fisheries Science, University of Washington, Seattle, WA, 1987.

Professional Experience:

Position/Title	Organization	City	State	Dates
Fish. Research Biologist	NMFS	Manchester	WA	6/88-present
Fish & Wildlife Instructor	Grays Harbor College	Aberdeen	WA	9/87-6/88
Consulting Biologist	Island County	Clinton	WA	1/80-12/81
Fisheries Biologist	NMFS	Seattle	WA	9/77-9/79
Teaching Assistant	University of Washington	Seattle	WA	9/75-6/78
Research Associate	Survey Chemistry	Dartmouth	MA	6/74-1/75

Current Responsibilities: Hatchery Technology team leader and principal investigator on the BPA sockeye salmon captive broodstock, BPA chinook salmon captive broodstock , BPA NATURES project, and HSRG NATURES project. Dr. Maynard's responsibilities include overseeing captive broodstock culture; developing captive broodstock and NATURES rearing protocols; designing experiments to evaluate the effect of these protocols on salmon behavior, morphology, growth, and survival; oversight of experimental activities; data analysis; publishing finding in annual reports and journal articles; and participation in interagency technical meeting.

Expertise: Dr. Maynard's primary expertise is in fish behavior and culture. He has taught graduate level courses on fish sociobiology and behavioral ecology, conducted research on the social behavior of salmon, and investigated the effects of petroleum on salmon homing and migration. Dr. Maynard has been a member of the Animal Behavior Society since 1977, where he has served on the applied animal behavior and film committees. He has taught college level courses on Aquaculture and his research since 1992 has focused on developing culture techniques to increase the postrelease survival of hatchery salmon. Dr. Maynard also has expertise in fish taxonomy and evolution and has been a member of the NMFS Biological Review Teams for several petitioned listings. In addition, he has expertise in fish tagging and has led several investigations comparing the effects of tags on fish survival.

Selected Publications:

Maynard, D. J., T. A. Flagg, and C. V. W. Mahnken, and S. L. Schroder. 1996. Natural rearing technologies for increasing postrelease survival of hatchery-reared salmon. Bull Natl. Res. Inst. Aquacult., Suppl. 2:71-77.

Maynard, D. J., G. C. McDowell, E. P. Tezak, and T. A. Flagg. 1996. The effect of diets supplemented with live-food on the foraging behavior of cultured fall chinook salmon. Prog. Fish-Cult. 58:187-191.

Maynard, D. J., D. A. Frost, F. William Waknitz, and Earl F. Prenctice. 1996. Vulnerability of marked age-0 steelhead to a visual predator. Trans. Am. Fish. Soc. 125:130133.

Maynard, D. J., T. A. Flagg, and C. V. W. Mahnken. 1995. A review of semi-natural culture strategies for enhancing the postrelease survival of anadromous salmonids. American Fisheries Society Symposium 15:307-314.

Maynard D. J., and D. D. Weber. 1981. Avoidance reactions of juvenile coho salmon (*Oncorhynchus kisutch*) to monocyclic aromatics. Can. J. Fish. and Aquat. Sci

CURRICULUM VITAE--W. Carlin McAuley

Current Employer: Northwest Fisheries Science Center, National Marine Fisheries Service

Education:

- B.A. Zoology, University of Washington, Seattle, WA, 1973.

Professional Experience:

Position/Title	Organization	City	State	Dates
Endangered Species Biologist	NMFS	Manchester	WA	10/91-present
Hatchery Biologist	Domsea Farms Inc.	Gorst	WA	10/73-3/91

Current Responsibilities: Hatchery Manager for NMFS Manchester Research Station captive broodstock programs for recovery of ESA-listed endangered Redfish Lake sockeye salmon and threatened Snake River spring/summer chinook. Responsible for every aspect of life cycle including feed rations, disease detection and control, detailed record database, and spawning.

Expertise: Mr. McAuley has participated in a number of captive broodstock programs for Pacific salmon. He has conducted research into captive broodstock programs using non-endangered Lake Wenatchee sockeye salmon captive broodstock (BY 1990 and 1991) reared in three different environments. Responsible for daily care including feed, disease detection and control, detailed record database, spawning, and egg incubation. Provided support for other researchers investigating physiological developments in these same fish. Specialized skills include various fish tagging techniques (coded wire tag, PIT tag, elastomer tag, freeze branding), and Fluorescent Antibody Technique for detection of fish disease pathogens. Provided aquaculture consulting to Domsea Farms Inc. Primarily responsible for continuation of 13 year genetics breeding program for captive broodstocks of Pacific salmon at Domsea Farms. Duties consisted of management of captive breeding program of fish reared in freshwater, including collection and input of data into genetics database, tracking and maintaining separate identity of 40 families, and decision making for various program steps

Selected Publications:

Flagg, T. A., W. C. McAuley, D. A. Frost, M. R. Wastel, W. T. Fairgrieve and C. V. W. Mahnken. 2001. Redfish lake sockeye salmon captive broodstock rearing and research, 1995-2000. Report to Bonneville Power Administration, Contract DE-AI79 92BP41841, 66 p.

Flagg, T. A., W. C. McAuley, M. R. Wastel, D. A. Frost, C. V. W. Mahnken, and J. C. Gislason. 1998. Redfish Lake Sockeye Salmon Captive Broodstock Program, NMFS. Proceedings of the 48th Annual Northwest Fish Culture Conference, Gleneden Beach, Oregon, p. 127-135.

Flagg, T. A., and W. C. McAuley. 1993. Redfish Lake sockeye salmon captive broodstock rearing and research, 1991-1993. Report to Bonneville Power Administration, Contract DE-A179-92BP41841.

McAuley, W. C. 1981a. DOMSEA coho broodstock program. *In* T. Nosho (editor), Salmonid broodstock maturation, p.23-24. Proceedings of the salmonid broodstock maturation workshop. University of Washington Sea Grant Pub. WSG-WO-80-1.

McAuley, W. C. 1981b. DOMSEA coho broodstock program-update. *In* T. Nosho (editor), Salmonid broodstock maturation, p.65-66. Proceedings of the salmonid broodstock maturation workshop. University of Washington Sea Grant Pub., WSG-WO-80-1.

CURRICULUM VITAE--Deborah A. Frost

Current Employer: Northwest Fisheries Science Center, National Marine Fisheries Service

Education:

- A.A., Lower Columbia College, Longview, WA, 1984.
- B.S., Zoology, University of Washington, Seattle, WA, 1986.

Professional Experience:

Position/Title	Organization	City	State	Dates
Fisheries Research Biologist	NMFS	Manchester	WA	5/94-present
Fisheries Biologist	NMFS	Manchester	WA	5/90-5/94
EKG Technician	Swedish Hosp.	Seattle	WA	10/87-5/90

Current Responsibilities: Site Manager for freshwater rearing facility. Directly involved with the daily culture of captive Redfish Lake sockeye salmon through all life phases. Includes maintaining strict isolation procedures for these fish, administering medicated feed on schedule, analyzing mortalities and conducting fluorescent antibody technique (FAT) procedures for bacterial kidney disease. Organized the PIT tagging of captive broodstock. Organizes spawning and incubation activities. Maintains daily records of feed, mortality, and water temperature for all fish.

Expertise: Fisheries Biologist, Manchester Research Station and Burley Creek Hatchery. Assisted in the development and evaluation of various passive integrated transponder (PIT) tag systems for fish. Involved with conducting captive broodstock culture and growth research on non-endangered Lake Wenatchee sockeye salmon (organized the PIT tagging of this group of fish as well as the monthly collection of growth data). Observed salmon behavior with respect to various PIT-tag interrogation passageway models in lab and stream. Conducted various comparative tagging predation and survival studies in rearing pools and in a natural stream. Involved in the initial natural feed and habitat rearing methods for the NMFS NATURES program. Involved in a study to test a model for survival estimates for juvenile chinook salmon passage through Snake River dams and reservoirs. This consisted of assembling a PIT tagging station and relocating it to different sites on the Snake River, PIT tagging chinook salmon, teaching others to PIT tag salmon and operate digitizer board, and maintaining and uploading tagging data to the database.

Selected Publications:

Flagg, T. A., W. C. McAuley, D. A. Frost, M. R. Wastel, W. T. Fairgrieve and C. V. W. Mahnken. 2001. Redfish lake sockeye salmon captive broodstock rearing and research, 1995-2000. Report to Bonneville Power Administration, Contract DE-AI79 92BP41841, 66 p.

Flagg, T. A., W. C. McAuley, M. R. Wastel, D. A. Frost, C. V. W. Mahnken, and J. C. Gislason. 1998. Redfish Lake Sockeye Salmon Captive Broodstock Program, NMFS. Proceedings of the 48th Annual Northwest Fish Culture Conference, Glenden Beach, Oregon, p. 127-135.

Flagg, T. A., W. C. McAuley, M. R. Wastel, D. A. Frost, and C. V. W. Mahnken. 1996. Redfish Lake sockeye salmon captive broodstock rearing and research, 1994. Report to Bonneville Power Administration, Contract DE-AI79 92BP41841, 98 p.

Maynard, D. J., D. A. Frost, F. W. Waknitz, and E. F. Prentice. 1996. Vulnerability of marked age-O Steelhead to a visual predator. Transactions of the American Fisheries Society 125:330-333.

Prentice, E. F., D. J. Maynard, S. L. Downing, D. A. Frost, M. S. Kellett, D. A. Bruland, P. Sparks-McConkey, F. W. Waknitz, R. N. Iwamoto, K. McIntyre, and N. Paasch. 1994. A Study to Determine the Biological Feasibility of a New Fish Tagging System (1990-93). Report to Bonneville Power Administration, Contract DE-AI79-84BP11982, 209 p. + appendices.

CURRICULUM VITAE--Michael R. Wastel

Current Employer: Northwest Fisheries Science Center, National Marine Fisheries Service

Education:

- A.S. Fisheries - Peninsula College, Port Angeles, WA, 1977.

Professional Experience:

Position/Title	Organization	City	State	Dates
Fisheries Biological Technician,	NMFS	Manchester	WA	92-present
Fisheries Technician	WDFG	Olympia	WA	91-92
Hatchery Manager	Domsea Farms Inc	Gorst	WA	80-91

Current Responsibilities: Provide technician support for NMFS Manchester Research Station captive broodstock programs for recovery of ESA-listed endangered Redfish Lake sockeye salmon and threatened Snake River spring/summer chinook. Lead contact for construction of captive broodstock research facilities for freshwater and seawater rearing at Manchester. Responsible for aspect of life cycle culture including feed rations, disease detection and control, detailed record database, and spawning.

Expertise: Mr. Wastel has participated in a number of captive broodstock projects for Pacific salmon. 1991-1992--Fisheries Technician, Steelhead Stock Assessment Program, Washington Department Fish and Wildlife, Olympia, Washington. Responsible for the collection of data on steelhead life histories for 22 Washington State rivers, all tributaries of the Columbia River. 1980-1991--Hatchery Manager, Fisheries Research Division, Domsea Farms Inc. Gorst Creek Hatchery, Gorst, Washington. Supervised and participated in all phases of hatchery operations. Responsible for all fish rearing activities. Worked on both production and broodstock programs. Supervised six employees. Assigned work schedules. Formulated fiscal budget. Produced monthly reports. Maintained hatchery systems including; wells, pumps, generators and alarm system. Expanded and upgraded hatchery incubation. Installed additional tanks. Assisted in the formation of coho salmon broodstock program which produced fish with superior growth and survival rates. As the offspring of fish from the broodstock program outperformed all other hatchery fish, the program expanded and became a major part of my responsibilities.

Selected Publications:

Flagg, T. A., W. C. McAuley, D. A. Frost, M. R. Wastel, W. T. Fairgrieve and C. V. W. Mahnken. 2001. Redfish lake sockeye salmon captive broodstock rearing and research, 1995-2000. Report to Bonneville Power Administration, Contract DE-AI79 92BP41841, 66 p.

Flagg, T. A., W. C. McAuley, M. R. Wastel, D. A. Frost, C. V. W. Mahnken, and J. C. Gislason. 1998. Redfish Lake Sockeye Salmon Captive Broodstock Program, NMFS. Proceedings of the 48th Annual Northwest Fish Culture Conference, Gleneden Beach, Oregon, p. 127-135.

Flagg, T. A., W. C. McAuley, M. R. Wastel, D. A. Frost, and C. V. W. Mahnken. 1996. Redfish Lake sockeye salmon captive broodstock rearing and research, 1994. Report to Bonneville Power Administration, Contract DE-AI79 92BP41841, 98 p.

Hymer, J., R. Pettit, M. Wastel, P. Hahn, and K. Hatch. 1992. Stock Summary reports for Columbia River Anadromous Salmonids, Volume 3. BPA, Division of Fish and Wildlife, Project No. 88-108.

CURRICULUM VITAE--Lee W. Harrell

Current Employer: Northwest Fisheries Science Center, National Marine Fisheries Service

Education:

- B.S., Animal Husbandry, University of Florida, Gainesville, FL, 1960.
- D.V.M., Veterinary Medicine, Auburn University, Auburn, AL, 1964.
- M.S., Fisheries Biology, University of Washington, Seattle, WA, 1973.

Professional Certifications:

- Certified Fish Pathologist (AFS) #35;
- Washington State Veterinary practice license # 653
- Florida State Veterinary license # 857.

Professional Experience:

Position/Title	Organization	City	State	Dates
Fisheries Research Biologist,	NMFS	Manchester	WA	73-present.

Current Responsibilities: Manager of Fish Health projects, Manchester Research Station. Duties include fish disease diagnosis and treatment; conducting research on freshwater and marine diseases of salmonids; and involvement in broodstock restoration methods. Pathologist representative, Pacific Northwest Fish Health Protection Committee. Consulting Veterinarian, Washington State Department of Fisheries and Wildlife.

Expertise: Dr. Harrell has expertise in all areas of fish veterinary fish medicine. He has conducted research with all phases of salmonid fish health and disease diagnosis and treatment; development of freshwater and seawater net-pen aquaculture husbandry; development of captive broodstock techniques for Atlantic and Pacific salmon.

Selected Publications:

Harrell, Lee W. 1995. Fish health aspects of broodstock restoration, pp. 5-1 - 5-14. *In* T. A. Flagg and C. V. W. Mahnken (eds.), An assessment of captive broodstock technology for Pacific salmon. Report to Bonneville Power Administration, Contract DE-AI79 93BP55064.

R. A. Elston, M. L. Kent, and L. W. Harrell. 1987. An intranuclear microsporidium associated with acute anemia in the chinook salmon, *Oncorhynchus tshawytscha*. J. Protozool. 34(3):274 277.

Harrell, L. W., T. A. Flagg, and F. W. Waknitz. 1987. Snake River Fall Chinook Salmon Broodstock Program, 1981-1986. Final report to Bonneville Power Administration. 24 p.

R. A. Elston, L. W. Harrell, and M. T. Wilkinson. 1986. Isolation and in vitro characteristics of chinook salmon *Oncorhynchus tshawytscha* rosette agent. Aquaculture 56:1-21.

Harrell, L. W., C. V. W. Mahnken, T. A. Flagg, E. F. Prentice, F. W. Waknitz, J. L. Mighell, and A. J. Novotny. 1984. Status of the NMFS/USFWS Atlantic salmon brood-stock program (Summer 1984). Annual report of research (to NMFS/NER). 16 p.

F. Project Description, Purpose, and Significance

1. Justification of the objective(s): motivation, history, goals, etc. and how the wild populations of the species will benefit from the proposed activities.

The Redfish Lake captive broodstock program is a safety net program producing fish for restoration of anadromous sockeye salmon runs to the Snake River Basin. In December 1991, NMFS listed Snake River sockeye salmon (*Oncorhynchus nerka*) as endangered under ESA (Waples et al. 1991). Snake River sockeye salmon are a prime example of a species on the threshold of extinction. The last known remnants of this stock return to Redfish Lake, Idaho. In 1991 and 1992 combined, only five adult anadromous sockeye salmon returned to Redfish Lake. On the basis of these critically low population numbers, NMFS, in cooperation with IDFG, the Bonneville Power Administration (BPA), the Shoshone-Bannock Tribe, and others, implemented a captive broodstock project as an emergency measure to save Redfish Lake sockeye salmon from extinction (Flagg 1993; Johnson 1993; Spaulding 1993; Flagg and McAuley 1994; Flagg et al. 1994; Kline 1994; Teuscher et al. 1994; Flagg et al. 1995; Johnson and Pravecek 1995, 1996; Kline and Younk 1995; Teuscher et al. 1995; Flagg et al. 1996; Teuscher and Taki 1996; Kline and Lamansky 1997; Pravecek and Johnson 1997; Taki and Mikkelsen 1997; Flagg et al. 1998; Flagg et al. 2001; Frost et al. 2002). The Redfish Lake project is intended as an emergency gene rescue program that can be used to produce large numbers of juvenile fish for restoring anadromous sockeye salmon runs to the Snake River.

The ESA mandates that listed population segments be restored to self sustaining populations in their natural ecosystems. It recognizes that conservation of listed species may be facilitated by artificial means, such as captive broodstock programs, while factors impeding population recovery are rectified (Hard et al. 1992). Frequently, restoration of severely depleted populations is hindered by lack of suitable numbers of juveniles for effective supplementation (i.e., release of hatchery-propagated fish to increase natural production), even if factors impeding recovery can be corrected (Flagg et al. 1995a). For restoration of these populations to occur in a timely fashion, the full reproductive potential of Pacific salmon must be harnessed in the short-term to produce large numbers of juveniles. Often the only reasonable avenue to build populations quickly enough to avoid extinction is through captive broodstock technology (Flagg and Mahnken 1995).

Captive propagation of animals to maximize their survival and reproductive potential has won acceptance in endangered species restoration (Gipps 1991, Johnson and Jensen 1991, DeBlieu 1993, Olney et al. 1994, Flagg and Mahnken 1995). These efforts range from establishment of free-roaming breeding colonies on localized preserves to full-term captive rearing (Gipps 1991, Johnson and Jensen 1991, DeBlieu 1993, Olney et al. 1994, Flagg et al. 1995b). Full-term rearing of captive broodstocks maximizes potential production of juveniles for enhancement. The relatively short generation time of Pacific salmon and their potential to produce large numbers of offspring make them suitable for captive broodstock rearing. Survival advantages offered through protective culture can be significant. Theoretically, survival of fish reared in protective captive broodstock culture can exceed wild

survival by 100-to-1,000 fold (Flagg et al. 1995b). The substantial survival advantage for captive-reared fish provides potential to produce large numbers of juveniles to amplify the natural population during the second generation.

The Redfish Lake captive broodstock project has been underway since 1992. The NMFS captive broodstocks are complementary to those reared by IDFG and are intended to reduce the risk of catastrophic loss of this valuable gene pool. The sources of NMFS captive broodstocks are wild juvenile and adult fish captured, held, and spawned by IDFG between 1992 and 1998. During this period, only 16 sockeye salmon adults (0 to 8 individuals per year) returned to Redfish Lake. IDFG divided the gametes from these fish between the NMFS and IDFG captive broodstock facilities. NMFS has maintained F_1 lineages for the 1991-, 1993-, 1994-, and 1996-broods produced from returning female anadromous fish (only males returned in 1992 and 1998, no fish returned in 1995 and 1997). In addition, a few fish from each of these broods have been kept full-term through F_2, and now F_3, generations to establish safety nets that can be used in poor survival years. Pre-spawning adults, eyed eggs, and juveniles are returned to Idaho to aid in recovery efforts.

The NMFS portion of the captive broodstock program has produced more than 840,000 eyed eggs that have been returned to Idaho for use in recovery programs (Table 1). In addition, NMFS has returned 181 prespawning adults to Idaho for direct release into Stanley Basin lakes. The overall production from the NMFS program equates to a direct amplification of about 165 times the total seed stock received from Idaho. Importantly, these amplification numbers are thousands of times the numbers that would have been produced had the 16 wild fish returning in the decade of the 1990s been left to reproduce naturally. The current relatively high juvenile survival in protective culture should result in combined IDFG and NMFS production of up to 100-200,000 eggs yearly.

Table 1. History of NMFS eyed egg production from Redfish lake sockeye salmon captive broodstocks.

Year	Number of eyed eggs
1994	48,000
1995	0
1996	412,000
1997	168,100
1998	47,500
1999	65,400
2000	96,700
2001	90,222

The release (by IDFG) of captive broodstock progeny produced by the NMFS and IDFG captive broodstock programs back into Snake River Basin lakes began in 1994 with the fall release of 14,119 yearling presmolts. In 1995, almost 95,000 juveniles were released back into Stanley Basin lakes. The first planting of eyed eggs (105,000) occurred in 1996 along with the release of about 14,000 juvenile-to-smolt stage fish. The number of eyed eggs (105,767) and juveniles (256,411) released into the lakes peaked in 1997. In 1998, over

140,000 juveniles and 81,000 smolts were released to restore anadromous salmon runs to the Snake River Basin. In 1999, about 20,000 eyed eggs and 50,000 juvenile-to-smolt stage fish were released. In 2000, more than 50,000 eyed eggs and 72,000 juveniles were released. In 2001 over 103,000 juveniles were released. In addition to these egg and juvenile plants, the captive broodstock programs have generated releases of up to 120 prespawning adults per year into the lakes.

The Redfish Lake captive broodstock project has reached its goal of building the captive population as a safety net to maintain the gene pool. The program is now focusing on producing significant numbers of captive broodstock progeny that can be used in release efforts designed to restore anadromous sockeye salmon runs to the Snake River Basin. These restoration efforts have returned 7 anadromous adults in 1999, 257 in 2000, 26 adults in 2001, and 23 adults in 2002 to Snake River Basin lakes. The vast majority of these returning adults were produced by the release of smolts from the NMFS program that were reared at Oregon Department of Fish and Wildlife (ODFW) Bonneville Hatchery. These returns clearly demonstrate that the captive broodstock project is succeeding both as a safety net and as a tool to restore anadromous salmon runs. It is virtually certain that without the boost provided by these captive broodstocks, Redfish Lake sockeye salmon would have become extinct.

2. Statement of whether the proposed project or program responds directly or indirectly to a recommendation or requirement of a Federal agency (include citations if applicable).

The project directly responds to several Federal agency recommendations and requirements. First, Redfish Lake sockeye salmon are listed as endangered under the United States Endangered Species Act (ESA) of 1973 which states:

> "All Federal departments and agencies shall seek to conserve endangered species and threatened species and shall utilize their authorities in furtherance of the purposes of this Act" (Sec. 2(c)).

Furthermore, the ESA recognizes these conservation efforts may include forms of artificial propagation, such as captive broodstocking, in the statement:

> "The use of all methods and procedures which are necessary to bring any endangered species or threatened species to the point at which the measures provided pursuant to this Act are no longer necessary. Such methods and procedures include, but are not limited to, all activities associated with scientific resources management such as research, census, law enforcement, habitat acquisition and maintenance, propagation, live trapping, and transplantation" (Sec. 3(3)).

In addition to being a direct requirement of the NMFS under its ESA mandate, the Redfish Lake sockeye salmon captive broodstock program is a required reasonable and

prudent action (Item 177) in the NMFS 2000 FCRPS Biological Opinion. The implementation and refinement of captive broodstocks for the recovery of Snake River sockeye salmon has also been identified as priorities in the 1994 Northwest Power Planning Council's (NWPPC) Columbia Basin Fish and Wildlife Program (7.4A.1-3), are part of the overarching and regional objectives of the 2000 NWPPC Columbia Basin Fish and Wildlife Program, and are priorities described in the NMFS proposed Recovery Plan for Snake River salmon.

3. Statement of whether the proposed project or program has broader significance than the individual project's goals, or is part of a larger scale research management or restoration plan.

The NMFS Redfish Lake captive broodstock program responds to Agency goal to recover and delist Snake River sockeye salmon as described in the NMFS Proposed Recovery Plan for Snake River Salmon (Schmitten et al. 1995) as described under the plan's following biological objective:

"4.1 Biological objective: Conserve remaining Snake River Salmon gene pools through implementation of captive broodstock/supplementation/gene banking programs."

The program is part of joint restoration effort being conducted by the NMFS, the Bonneville Power Administration, the State of Idaho, and the Shoshone-Bannock tribes.

4. Description of relationships or similarities of the proposed activities to other proposed or ongoing projects and programs, and whether potential exists to cooperate and coordinate with other similar activities.

This NMFS captive broodstock project is part of a joint regional effort by the National Marine Fisheries Service, Idaho Department of Fish and Game, Shoshone-Bannock tribes, and Bonneville Power Administration to restore a healthy population of anadromous sockeye salmon to the Snake River. The project's success has been an outcome of the excellent cooperation occurring between the partner agencies. The Stanley Basin Sockeye Salmon Technical Oversight Committee (SBTOC) coordinates the activities of the various agencies involved in these restoration efforts.

5. Justification for using listed species and possible alternatives to using listed species.

The listed species was taken into captivity in 1991 to prevent its extinction. The captive breeding program now maintains a segment of the population in a captive breeding program as a safety net to ensure the population does not incur increased risk of extinction in successive year periods when no fish return from the sea. In addition, the captive culture program also provides large number of eggs, juvenile, and adult fish for release back into Stanley Basin lakes to aid in recovery. There is no viable other alternative to using the listed species in these gene banking and recovery efforts.

G. Project Methodology

1. Proposed duration of the project or program, including start and end dates.

The duration of the captive broodstock program will ultimately be determined by the recovery program the Technical Recovery Team (TRT) for Snake River Sockeye Salmon develops. In the interim, the project is following the recovery goals established by the earlier Recovery Plan (Schmitten et al. 1995) proposed for Snake River salmon that called for multiple generations of captive broodstocks to help maintain and enhance Redfish Lake sockeye salmon while recovery efforts are under way. The proposed plan provided the following interim delisting criteria for Redfish Lake sockeye salmon:

"For sockeye salmon, the numerical escapement goal is an eight-year (approximately two-generation) geometric mean of at least 1,000 natural spawners returning annually to Redfish Lake and 500 natural spawners in each of two other Snake River basin Lakes".

The NMFS captive culture program plans to continue its efforts until this interim delisting goal has been achieved or the TRT for Snake River sockeye salmon provides other guidance for captive broodstock program termination.

2. Procedures and techniques which will be used during the project.

a. Method(s) of capture and release:

N/A; permitting of capture of fish for captive broodstocks was, and will continue to be, covered under ESA Permit #1120 to IDFG.

b. Description of tags, tagging method, location, and duration:

Sockeye salmon in the captive broodstock program are individually identified with passive integrated transponders (PIT) tags. These tags enable fish culturist to track the lineage of individual fish to ensure that only appropriate genetic crosses are made when fish are spawned. In addition, the tag allows scientists to monitor the downstream migration success of juvenile fish released back into the natural environment. The PIT tag has been demonstrated to be safe for use in juvenile salmonids and to be very reliable over the life of Pacific salmon (Prentice et al. 1990a, b). The PIT tag is injected dorso-ventrally into the peritoneal cavity of the fish and rests near the pyloric caeca. A sterilized needle is used for each individual fish to minimize lateral transmission of pathogens. The PIT tag is scanned with an external radio-frequency scanner, which allows non-intrusive documentation of the individual tag code. In addition to maintaining genetic identity, PIT tags enable caretakers to monitor individual fish growth. Sockeye salmon in the captive broodstock program are usually PIT-tagged just after they turn a year old and will retain their tag for the remainder of their life.

Any juvenile sockeye salmon NMFS rears for post yearling release will be tagged with coded wire tags (CWT) to measure adult survival. CWTs are stainless steel wires that are injected into the nose cartilage of the fish following procedures described by Jefferts et al. (1963). These tags remain with the fish throughout its life

and are recovered from dead fish collected in spawning surveys or taken as bycatch in fisheries.

c. Description of type and dosages of any drugs to be used, purpose of use, and method of application:

The captive broodstock program has historically used MS-222 as an anesthetic and erythromycin, oxytetraycline, and azithromycin as antibiotics to fight bacterial infections, and formaldehyde to control fungal growth on developing embryos. In addition, eggs are routinely disinfected in iodophore to prevent the potential spread of viruses. If new diseases or better therapeutic approaches develop, additional drugs prescribed by the captive broodstock program veterinarian maybe administered. Dosage, purpose of use, and method of application for currently used drugs are as follows:

- Fish will be anesthetized by being immersed in a buffered 50-100 mg/liter or less solution of MS-222 (Tricane methanesulfonate) (Stoskopf 1993).
- A 1,668 mg/liter solution of formalin is dripped into the water supply of incubating eggs for 15 minutes to control fungus.
- Erythromycin will be administered orally by topcoating feed to produce a dose of 100 mg/kg of body-weight. Salmon will be fed this medicated feed for up to a 28 day period to control Bacterial Kidney Disease. When oral administration is not feasible, a sub-cutaneous erythromycin injection will be given to fish at a dose of 20 mg/kg of body weight (Scott 1993).
- Salmon will be fed azithromycin medicated feed at a dose of 30 mg/kg of body-weight for up to 14 days. In some circumstances, fish will be given a sub-cutaneous azithromycin injection at a dose of 40 mg/kg (L. Harrell, NMFS, personal communication).
- Salmon will be fed oxytetracycline medicated feed at a dose of 75 mg/kg of body weight for 10 days to control outbreaks of pathological Aeromaonad, Myxobacteria, etc. bacteria (Scott 1993).
- Fertilized eggs will be water hardened in a 10 ml/liter solution of idodophor for 20 minutes to kill virus particles on the egg surface and in the perivitelline space (McDaniel et al. 1994).

d. Temporary holding time prior to release of individuals and the manner in which they will be detained:

N/A; permitting of capture and temporary holding of fish for captive broodstocks was, and will continue to be, covered under ESA Permit #1120 to IDFG. The details for long term holding of captive broodstock are covered in a following section on transportation and holding.

e. Number and types of samples to be taken from each individual, including sampling protocol:

The captive broodstock programs focus on generating fish for recovery, rather than research, minimizing sampling. Captive brood program fish are routinely sampled each month to determine weight to the nearest gram and fork length to the nearest mm. This nonlethal sampling is required to monitor fish health, determine feed size, and adjust daily ration. The data obtained is also used to evaluate the efficacy of new fish culture methods being employed to improve captive broodstock quality. In all these sampling procedures, the protocol is to anesthetize the fish in MS 222, determine their fork length and weight, and then allow them to recover in their rearing tank.

Tissue samples are obtained from in-culture mortalities and spawners to test for the presence of common bacterial and viral pathogens. All these fish have a piece of kidney tissue removed, homogenized, heat fixed on a slide, and reacted with fixative antibody technique (FAT) to examine for the presence of bacterial kidney disease (BKD). In addition, kidney tissue, spleen and ovarian fluid are taken for Enzyme-Linked Immunosorbent Assay (ELISA) for quantative evaluation of BKD and cell culture evaluation of the presence of viral pathogens. American Fisheries Society (AFS) "Bluebook" procedures will be employed to isolate bacterial or viral pathogens and to identify parasite etiology. All examinations are conducted under the direction of an AFS board certified Fish Pathologist.

The NMFS captive broodstock program previously had reared juvenile fish for release as smolts into the Salmon River watershed at the ODFW Bonneville Hatchery. If such an activity occurs in the future, two sixty fish lethal subsamples will be taken from each rearing group for fish health certification standards mandated by transfer permits. This lethal sampling is needed to ensure cultured fish do not serve as a disease vectors that spread virulent pathogens to the rest of the listed population.

Fin tissue is removed from all captive broodstock spawners and sent to Dr. Matt Powell at the University of Idaho for genetic analysis. This information is utilized to examine the genetic structure of the population.

A total of 7,200 eggs and yolk sack fry maybe lethally sampled each year in research designed to improve the egg to fry survival of Redfish Lake sockeye salmon in the captive broodstock program. A surrogate population cannot be used to solve the problem as it is stock specific to Redfish Lake sockeye salmon captive broodstock.

3. Potential for injury or mortality to the species involved, and steps that will be taken to minimize adverse effects and to ensure that the species will be taken in a humane manner.

Trapping and handling of fish captured for Redfish Lake sockeye salmon captive broodstocks has been, and will continue to be, covered under ESA Permit #1120 issued to

IDFG. Therefore, mortality from these activities (although known to be low) is not addressed in this permit application.

Rearing of fish for NMFS Redfish Lake sockeye salmon captive broodstocks is covered under the current permit application. Fish will be reared in a humane manner in the best possible fish culture environment. Nevertheless, some mortality is expected during captive broodstock rearing. This adverse effect will be minimized by continuing research aimed at improving captive broodstock quality and increasing egg-to-adult survival as close to 100% as humanely possible. Mortality during tagging should be minimal (0.1% or less). Human error, equipment failure, and pathogens (such as dislodged standpipes in tanks or disrupted water supplies) also may cause fish death. Every safeguard needed to eliminate mortality by foreseeable events (back up power generation, alarm systems, emergency life support oxygen, etc.) and fish health concerns (sanitation, hygiene, vaccination, prophylactic treatments) will be taken.

The majority of mortality during culture will probably be caused by common fish pathogens (Flagg et al. 1995a). BKD is the disease with the most potential negative impact on Pacific salmon during captive broodstock rearing (Harrell 1995). It is impossible to accurately predict survival during rearing for NMFS Redfish Lake sockeye salmon captive broodstocks. However, worst and best cases may be approximated by examining the low (13%) survival to age-3 spawning for the 1991-brood obtained from wild spawners and the high (81%) survival to age-4 spawning for the 1993-broods obtained from wild spawners. Although the potential survival range as described above is wide, all Redfish Lake sockeye salmon captive broodstocks currently maintained by NMFS are healthy, and we anticipate that they will continue to exhibit high survival during culture.

Maintaining diseased or deformed eggs, juveniles or adults in the captive broodstock program can have debilitating effects. To minimize the risks of disease transmission, good fish husbandry techniques such as sampling for disease, culling nonproductive or moribund fish, and culling diseased eggs will be followed. Previously established protocols for the management of disease are:

- Fish husbandry protocols must follow standard fish culture practices (for a general overview of methods see Leitritz and Lewis 1976, Piper et al. 1982, FRED 1983, Rinne et al. 1986, McDaniel et al. 1994, Schreck et al. 1995, Pennell and Barton 1996, Wedemeyer 2001), Integrated Hatchery Operation Team, NMFS Interim Standards for the Use of Captive Propagation, and similar guidelines approved by the SBSTOC to ensure high quality rearing conditions.

- Diseased, moribund, or non-productive fish and gametes should be removed from the captive population and disposed of following AFS Fish Health Blue Book and Pacific Northwest Fish Health Protection Committee guidelines to ensure overall health of rearing groups. This culling is necessary to prevent the spread of contagious diseases to remainder of the population.

- Gametes, embryos or fish may be sampled as necessary to detect diseases and to monitor fertilization and development of embryos. This lethal sampling is necessary to improve the reproductive success of fish in the captive broodstock program.

- Diseased eggs and fish that are culled must be disposed of per the permit requirements for the disposal of ESA-listed Snake River sockeye salmon if there is not a research, educational, or public outreach purpose identified.

- The annual report for the permit shall summarize the number, life stage, and condition of all fish or eggs culled or disposed of from the Snake River sockeye salmon captive broodstock program.

To ensure high quality eggs, juveniles, and adults in the captive broodstock program, NMFS will follow the above protocols and SBSTOC approved guidelines for fish sampling, culling diseased fish and maintaining healthy populations.

H. Description and Estimates of Take:

1. ***A list of each species and/or population and/or Evolutionarily Significant Unit (ESU) to be taken including the common and scientific name. Include specific population or subpopulation groups if appropriate.***

   N/A; permitting of capture of fish for captive broodstocks was, and will continue to be, covered under ESA Permit #1120 to IDFG.

2. ***Sampling schedule, including location and dates if available.***

   N/A; permitting of capture of fish for captive broodstocks was, and will continue to be, covered under ESA Permit #1120 to IDFG.

3. ***Description of recent status and trends of each species and/or population and/or ESU to be taken, relative to the location(s) or area(s) of taking.***

   N/A; permitting of capture of fish for captive broodstocks was, and will continue to be, covered under ESA Permit #1120 to IDFG.

4. ***Description and/or completed summary table of estimated take per annual period, for your activities at each discrete location and/or for each project.***

   N/A; permitting of capture of fish for captive broodstocks was, and will continue to be, covered under ESA Permit #1120 to IDFG.

5. ***Estimates of potential annual mortalities by take category, including a justification. You should specify the life stage of the potential mortalities, sex if known, and whether naturally-produced (wild) or artificially-propagated (hatchery).***

N/A; permitting of capture of fish for captive broodstocks was, and will continue to be, covered under ESA Permit #1120 to IDFG.

6. Provide details on how all take estimates, including mortalities, were derived.

N/A; permitting of capture of fish for captive broodstocks was, and will continue to be, covered under ESA Permit #1120 to IDFG.

I. Transportation and Holding

1. Transportation of a Listed Species

a. Mode of transportation and name of transport company, if applicable:

- Shipment of eggs from NMFS Redfish Lake sockeye salmon captive broodstocks to Idaho for use in recovery efforts at Redfish Lake will normally be by a common air carrier that provides service from Seattle to Boise. Horizon Air has a demonstrated track record of successfully shipping more than 40 egg lots from Seattle Washington to Boise, Idaho. Depending on the group(s) of eggs and their condition, transport containers of eggs may be hand carried or shipped by priority air cargo.

- Live fish, such as prespawning adults from NMFS facilities or juveniles being reared for smolt releases, will be transported in insulated tanks mounted on the bed of National Marine Fisheries Service, Oregon Department of Wildlife, United States Fish and Wildlife Service, or Idaho Department of Fish and Game trucks.

b. Length of time in transit for the transfer of the individual(s) from the capture site to the holding facility or to the target location:

N/A; permitting of capture of fish for captive broodstocks was, and will continue to be, covered under ESA Permit #1120 to IDFG. All NMFS captive broodstock transfers will be between captive culture facilities or to release locations.

c. Length of time in transit for any planned future move/transfer of the individual(s):

Truck transit times to Idaho average 10 hours from the Manchester Research Station to Eagle Fish Hatchery and 12 plus hours from Manchester Research Station to Redfish Lake, Idaho. Egg transfers between IDFG's Eagle Fish Hatchery and the Burley Creek Fish Hatchery average two hours flight time along with another three hours drive time from the hatcheries to the airports. Transit time between the Manchester Research Station and the Burley Creek Fish Hatchery are less than a half hour.

d. Qualifications of the common carrier or agent used for transportation of the individual(s):

NMFS personnel responsible for fish transfers all have college degrees with a major related to fish husbandry, have at least 10 years of fish culture and transport experience, and are well skilled in all phases of salmon culture.

e. Description of the pen, tank, container, cage, cradle, or other devices used, both to hold the individual(s) at the capture site and during transportation:

N/A; Trapping and transport of fish captured for Redfish Lake sockeye salmon captive broodstocks has been, and will continue to be, covered under ESA Permit #1120 to IDFG. Therefore, only the containers used for transport of fish already in the captive broodstock program is addressed in this permit application.

Egg transfer technology using specialized containers is commonly accepted as reliable for use with any common carrier. Eggs to be shipped to other facilities for outgrowing are placed into open mesh plastic tubes (27-cm long by 6-cm diameter) at approximately 2,700 eggs per tube. Each packed tube is wrapped in wet cheesecloth and placed in a small insulated shipping container. Ice is placed in a top layer of cheesecloth to keep the eggs cool and moist during shipment. This supplies the cool oxygenated environment necessary to support the eggs during transport.

Live fish are transported in a variety of insulated containers commercially manufactured for the transportation of live salmonids. These include 2,000 liter fiberglass tanks, 3,200 liter fiberglass tanks, 920 liter polyethylene tanks, 680 liter polyethylene tanks, 280 liter polyethylene tanks, and 90 liter polyethylene tanks. All transport tanks above 280 liters are equipped with ports that allow for water-to-water transfer of fish. Each tank has airstones that are used to refresh oxygen in the water maintaining the fish during transport. The tanks are all equipped with oxygen and temperature monitoring instruments.

f. Special care before, during and after transportation (e.g., use of oxygen, temperature control, anesthetics, antibiotics, etc.)

In general, the fish will be handled with extreme care and kept in water to the maximum extent possible during transport and processing procedures. Whenever possible, ESA-listed fish will be transferred with a sanctuary net or fish transfer tube that holds water to prevent the added stress of a waterless transfer. In no case will a fish be left out of water for more than 10 seconds. Prior to transport fish will be fasted for 48 hours to reduce metabolic demand and stress.

Transportation of juvenile or adult fish will emphasize fish health and safety. All transportation will occur in insulated containers and temperature will not normally be allowed to rise more than 2^{o}C. The transport containers will be supplied with a continuous oxygen supply that maintains dissolved oxygen at full saturation. The oxygen reservoir will contain at least twice the oxygen needed to make the entire trip. The containers will be loaded at no more than 0.06 kg/L (0.5 lb/gallon). Drivers will be equipped with cell phones and have backup personnel ready to respond in event of equipment failure.

2. Holding of a Listed Species

a. Dimensions of the pool(s) or other holding facilities and the number of individuals, by sex, age, and species to be held in each.

The NMFS Redfish Lake sockeye salmon captive broodstock project is being conducted at the NMFS Manchester Research Station located on Clam Bay, a small bay adjoining the central basin of Puget Sound. The station is located on nine hectares of land surplused from the U.S. Navy to NMFS in the late 1960s. The main building at the Manchester Research Station contains three laboratories, nine offices, and computer and conference rooms. Adjoining the main building is a disease diagnostic laboratory containing a pathology lab, a bioassay lab, and two offices. A land-based seawater captive broodstock rearing complex houses three offices, wet and dry labs, and 400 m^2 of floor space for fish rearing tanks in one building, and 1,280 m^2 in another.

A 700-m-long pipeline from the end of the pier supplies about 5,000 L/min (1,250 gpm) of pumped seawater to the Station's land-based facilities. Water is pumped via 50-hp centrifugal pumps. The system is outfitted with a back-up 50-hp pump in case of primary pump failure. An alarm system monitors the pumps and electrical supply and is tied into an automatic dialer system linked to pagers and home and office telephones. Redundant emergency generators are automatically serially activated in the event of a power failure.

The 400-m^2 seawater laboratory contains six 4.1-m, four 3.7-m, and six 1.8-m diameter circular fiberglass tanks. The 1,280-m^2 facility houses 20 6.1-m diameter circular fiberglass tanks. Portions of both buildings are used for the project.

Freshwater rearing is conducted at the Station's Burley Creek Hatchery satellite facility near Burley, WA (approximately 21 km from Manchester). The facility is leased by NMFS from Fish Pro Farms, Inc., Port Orchard, WA. This fresh water hatchery has been redesigned as a protective rearing facility for salmonid captive broodstocks. The facility includes a 613-m^2 building containing eleven 3.6-m and thirteen 1.5-m diameter tanks. A separate incubation room accommodates down-well incubators for isolated egg incubation.

From 1991 to 1996, NMFS annually obtained lots of about 300-1,000 fertilized eggs from anadromous adults returning to Redfish Lake from IDFG for the safety net captive broodstock program. During the early 1990s, additional lots of eggs from outmigrating smolts captured, reared, and spawned by IDFG were incorporated into the program. Currently, lots of about 300-500 eggs from select second and third generation captive broodstock lineages and from ocean returns of captive broodstock releases are incorporated into the program annually. NMFS currently rears an annual total of about 2,000 captive broodstock fish from all lineages and year classes.

The captive broodstock fish are reared 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, Rinne et al. 1986, McDaniel et al. 1994, Schreck et al. 1995, Pennell and Barton 1996, Wedemeyer 2001). All fish culture practices conform to the husbandry requirements detailed in ESA Section 10 Propagation Permit 1148 for NMFS's rearing of Idaho stocks of ESA-listed Snake River sockeye salmon. Generally, juvenile-to-adult rearing density in the tanks will be maintained at under 8 kg/m^3 (0.5 lb/ft^3) during most of the culture period; however, fish density may range to 15 kg/m^3 (1.0 lb/ft^3) at maturity. Loading densities in freshwater will range from 0.29 kg/Lpm (2.0 lb/gpm) to 0.84 kg/Lpm (7 lb/gpm). Seawater loading densities may be capped at 0.60 kg/Lpm (5 lb/gpm). These density and loading rate criteria, coupled with fish size, determine the number of fish per tank. In general, fish in a tank are all the same age because they are derived from a single broodyear. It has only recently become possible to tell the sex of immature fish. Generally, fish within a broodyear are randomly distributed to rearing tanks with the assumption the number of fish of each sex in the tank is representative of the sex ratio within the population.

All tanks used for sockeye captive broodstock rearing will be completely covered with a taut 2.5 x 2.5 cm or smaller mesh nylon netting to prevent fish from jumping out. The energy absorbing properties of the nylon mesh minimizes injuries that can occur to fish when they leap against it. In addition to the mesh, half of each tank will be covered with solid black fabric to provide a covered refuge area fish can move under when disturbed.

Center standpipes will be constructed to hold at least 15 cm of water depth in the tank when the external standpipe is pulled to lower tank water level. This will minimize the chance of fish being accidentally dewatered during tank draining or flushing.

A mild current (< 35 cm/sec) will be generated in the rearing tanks by their circular shape, center drain, and a subsurface water jet inlet. This current will provide a self cleaning action in the tank and a very slight exercise potential. At least once a week, bottom material that is not swept out of the tank by the current will be removed by vacuuming or brushing.

b. Water supply, amount, and quality, including controls on temperature and dissolved oxygen:

A major advantage of the Manchester Research Station is the 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 between 7-15°C and salinity ranges between 26-29 ppt. The high quality seawater environment, combined with a 250-m pier made available to the station by the EPA Region X Laboratory, make the Manchester Research Station an excellent site for experimentation and culture of a variety of finfish and shellfish.

The seawater supplied to the captive broodstock tanks at the Manchester Research Station is processed to prevent naturally occurring pathogens from entering the rearing tanks. Filtering consists of primary sand filters containing number 20-grade sand; this filters out all organic and inorganic material more than 20 microns in diameter. Water exiting the sand filters immediately enters a secondary cartridge filter system capable of filtering out all material more than 5 microns in diameter. The water then passes through a UV treatment system to inactivate remaining organic material. Sensors monitor water flow and pressure through the seawater filtration/sterilization system.

Before entering fish rearing tanks, the processed seawater is passed through packed column degassers to strip out any excess nitrogen and to boost dissolved oxygen levels. In addition, the tanks are directly supplied with oxygen to maintain life support in the event of an interruption in water flow. All 6.1m tanks are supplied with combinations of ambient and chilled water. The Station complies with Washington State Department of Fish and Wildlife quarantine certification standards by depurating all effluent from the captive broodstock rearing areas with ozone.

The freshwater hatchery near Burley Creek is supplied with about 2,000 L/min (500 gpm) of high-quality 10°C well water pumped from two separate wells. Well water is generally considered to be pathogen free. Before distribution to the tanks, the water is passed through packed column degassing towers that strip out any excess nitrogen and boost dissolved oxygen levels to 90% saturation. Water flow, and intruder alarms are monitored through a security system linked to pagers and home and office telephones. Effluent from the hatchery is depurated through a settling basin and UV-sterilization system. An emergency generator is automatically activated in the event of a power failure. In addition, all tanks can be supplied with emergency oxygen in the event of a water delivery system failure.

c. Amount and type of diet used for all individuals, and food storage:

Fish will be reared on a commercial (e.g., Biodiet) diet. Swimup fry will begin feeding on a semimoist starter "crumble". At about 1 g body weight, the fish will be fed a standard pellet semimoist grower ration. At about 100 g body weight, they may be converted to a dry diet. Daily ration will range from 0.4 to 5.6% body weight per day depending on estimated fish size and water temperature (Iwama 1996). Fish are fed at 5% of body-weight/day for the first 30 days and 3% of body-weight/day, or less, thereafter. The ration will be designed to grow the fish on the profile described in Figure 1. In order to ensure the fish are provided a healthy rearing environment, the feeding level will not be allowed to exceed 0.075 lbs feed/gpm/day. This maximum is set at about 50% the value known to be associated with pathological bacteria outbreaks in salmon culture tanks (Fowler 1989). Pellet size will be determined from a chart provided by the feed manufacture (e.g., Moore-Clark, Biodiet) that is based on current guidelines for commercial aquaculture and the guidance provided in Fowler (1989) and Wood (1979). If necessary, pellet size will be adjusted from the chart recommendation to ensure the smallest fish in the population can feed.

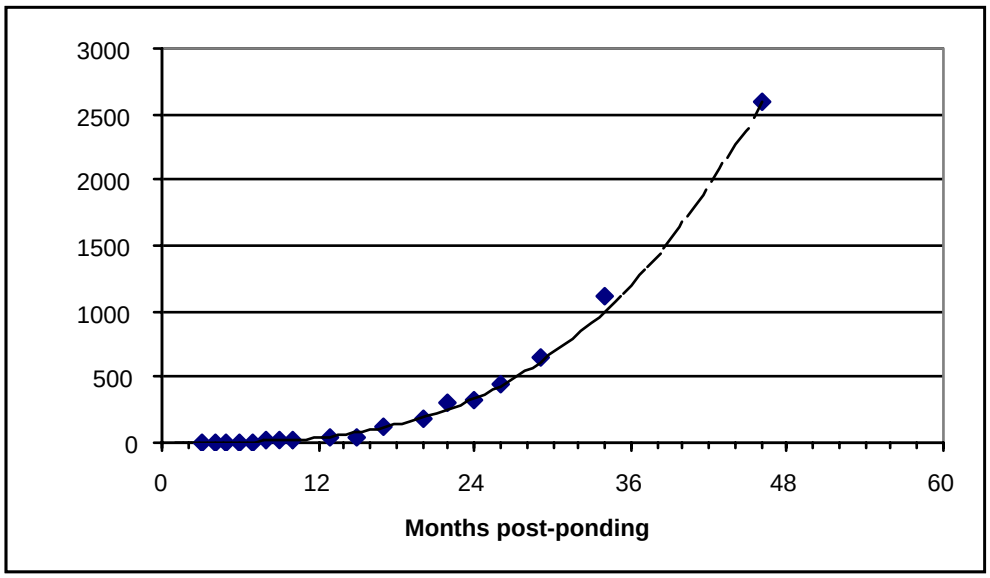

Figure 1. Growth rate projection for Redfish Lake sockeye salmon reared in 10°C freshwater at Burley Creek Hatchery. Growth profile based on historical data for sockeye reared in freshwater by NMFS.

Swimup fry will be started with hand feeding in 1.5-m diameter circular tanks. When the fish are about 10 g, they will be PIT tagged, and transferred to 3.6-m diameter circular tanks where their diet will be rationed by automated feeders (Allen or belt feeders). Each day, prior to loading the feeders, a small portion of the day's ration will be broadcast over the surface to observe the fish's feeding response. The belt feeders will be programmed to disperse feed continuously during daylight hours.

Moist feed is maintained frozen and the daily ration is thawed prior to feeding. Dry feed is maintained at room temperature. Food preparation areas will be cleared and sanitized daily. Feed is stored separately from fish carcasses. If the presence of pests is detected, appropriate measures will be taken to ensure sanitation and eliminate pests.

d. Sanitation practices used:

Fish tanks are inspected daily. Water flows in most tanks are maintained to prevent the settling out of fecal material and feed. Tanks with lower inflows will be cleaned a minimum of twice a week. At all facilities, rearing guidelines follow the same generalized procedures. The fish are reared using standard fish culture practices and FDA-approved therapeutics (for an overview of standard methods, see Leitritz and Lewis 1976). Fish are reared on pathogen-free water sources and fed a high quality commercially available ration.

e. General fish culture and spawning protocols:

The captive broodstock fish that are reared in seawater will be transferred to freshwater as soon as they show signs of maturation. Fish culturists will conduct maturation checks on fish in all groups suspected of having maturing adults. Maturation will be determined by changes in skin sheen, skin coloration, and body morphology. In the late spring of 2003, ultrasound scanning will be used to determine maturation status of fish. All fish determined to be maturing in seawater will be transferred to freshwater facilities as soon as possible. The goal will be to transfer fish as close as possible to the time that natural migrating sockeye salmon would enter freshwater.

Mature captive broodstock salmon will be anesthetized with tricaine methanesulfonate (MS-222) and checked for ripeness on a weekly basis during the spawning season, typically after October 1. Hormone implants (gonadotropin releasing hormone analog (GnRHa)) will be injected into the dorsal sinus of some unripe fish to expedite ovulation and spermiation to coordinate spawn timing between males and females (Swanson 1995). Females that are ready to spawn, as determined by egg expression, will be humanely killed and have their PIT tag code, length, and weight recorded. Females will first be bled by cutting the caudal peduncle to the depth of the caudal artery. This bleeding is a standard procedure that is done to limit the amount of blood accumulating with the eggs that might clog the eggs' micropyle. Females will be bled for 5-10 minutes and then abdominally incised with a sterile spawning knife. The free flowing eggs will then be manually stripped and collected in a plastic bag. The eggs from each female will then be divided into two lots and held on an insulating blanket placed over ice in a cooler until they can be fertilized. Males that are used for spawning will be live or dead spawned, depending on the need for their reuse on future spawning dates. In either case, milt will be expressed into Whirl-pak bags by

compressing the ventral surface. Milt quality and motility will then be checked with a microscope.

Mating strategies will be structured to maintain genetic diversity. These strategies may include random pairing, pairing in as many different combinations as possible, avoidance of pairing between close siblings, fertilization between different year classes and fertilization with cryopreserved sperm from other generations as suggested by Hard et al. (1992). Specific mating protocol matrices for individual year classes and lineages will be developed by geneticists in consultation with the SBSTOC.

Eggs will be fertilized following "dry method" procedures. Milt from one male will be pipetted into the plastic bag containing about half the eggs of one female. The milt will be gently worked into the eggs for a several seconds, well water will then be added to activate the sperm, and the eggs will be lightly agitated to distribute the activated milt. The bag will then be left undisturbed during the initial stages of the fertilization process. After about five minutes, the eggs will be water hardened in a 1:100 buffered iodophore solution for 20 minutes and placed in down-flow containers for isolated incubation. Beginning two days after fertilization, the eggs will be treated with a formalin drip into the water supply (1,668 ppm for 15 minutes) on alternating days for control of *Saprolegnia* spp. The eggs will be left undisturbed from the sensitive period at 48 hours after fertilization until they have reached the eye stage. When the eggs have eyed, they will be shocked and weighed. Dead or unfertilized eggs will be removed, counted, and weighed to determine fertilization rates.

Spawning adults will be analyzed for common bacterial and viral pathogens, such as bacterial kidney disease (BKD), infectious haematopoietic necrosis virus, and viral hemorrhagic septicemia. Tissue samples will be collected from the kidney, spleen, and pyloric caeca of each fish and ovarian fluid samples will be collected from each female and analyzed at a NWFSC Fish Health Laboratory. Results of fish health analysis of spawners will be used by NMFS and the SBSTOC to determine disposition of eggs and subsequent juveniles. IDFG has established protocol that 1) Eggs from parents having a BKD enzyme-linked immunosorbent assay (ELISA) optical density (OD) of 0.4 or less can be returned directly to Idaho for use in restoration efforts; 2) Eggs with high parental ELISA levels (OD > 0.4) should be culled (Paul Kline, IDFG, personnel communication).

Eggs to be shipped to Idaho will be placed into open mesh plastic tubes (27-cm long by 6-cm diameter) at approximately 2,700 eggs per tube. Each packed tube will be disinfected for 10 minutes in a buffered iodophore solution, wrapped in wet cheesecloth, and placed in a small shipping container. Ice will be placed in a top layer of cheesecloth to keep the eggs moist during shipment. Shipment to Boise, ID will be by a common air carrier flight of about 2 h. NMFS will receive eggs shipped in the same manner as above from IDFG spawning of Redfish Lake sockeye salmon. NMFS will also retain a safety net of eggs from spawning to be

used in either the captive broodstock or the prespawning adult release programs. NMFS will obtain all required permits for interstate transport of eggs and fish.

Fish health will be checked daily by observing feeding response, external condition, and behavior of fish in each tank as initial indicators of developing problems. In particular, fish culturists will observe for signs of lethargy, spiral swimming, side swimming, jumping, flashing, unusual respiratory activity, body surface abnormalities, and unusual coloration. Presence of any of these behaviors or conditions will immediately be reported to the fish health staff. The presence of moribund fish will be immediately reported to fish health staff for blood and parasite sampling. A fish pathologist will routinely monitor captive broodstock mortalities to try to determine cause of death. When a treatable pathogen is either detected or suspected, a NMFS veterinarian, in consultation with IDFG fish health staff, will prescribe appropriate prophylactic and therapeutic drugs to control the problem. All spawned fish will be analyzed for common bacterial and viral pathogens, e.g., bacterial kidney disease, infectious hematopoietic necrosis virus, etc. Select moralities may be appropriately preserved for pathology, genetic, and other analyses. After necropsy, specimens that are not vital to further analysis will be disposed of in a manner consistent with ESA permits.

NMFS will coordinate its captive broodstock rearing protocols and activities through the SBSTOC. NMFS will provide daily staffing throughout the workweek at both the Manchester and Burley Creek facilities for protective culture of these fish, with constant electronic security and facilities monitoring. Staffing during holidays may be covered by drop-in site inspections or electronic monitoring. The unique seawater and freshwater rearing facilities and staff expertise make the NMFS Manchester Research Station, with its Burley Creek Hatchery satellite, an ideal facility for conducting captive broodstock rearing and research projects.

3. Emergency contingencies

Daily staffing is provided for protective fish culture of Redfish Lake sockeye salmon throughout the normal workweek and weekends. Electronic security and facilities monitoring are continuous (see above, Section I.2.a). Automated backup life support oxygen is available in every fish rearing container at MRS (presently manually activated backup at Burley Creek) to help protect fish life during staff response in the event of a water flow stoppage.

The captive broodstock programs rearing experience has shown that occasionally groups of fish will have unacceptably high pathogen levels. When epidemic levels of pathogens are detected in eggs or juvenile fish being reared for release, a destroy or release decision must be made. IDFG mandates that if IHN is detected in a group of sockeye they will not be released back into Stanley Basin Lakes. If no alternative out of basin release location can be found that ensure adult returns will not serve as vectors that carry IHN to the healthy population, then it will be necessary to destroy these fish. Current IDFG guidelines allow eggs and fish from parents with ELISA values less than 0.2 to be

returned to Idaho for release in Stanley Basin Lakes. Fish from parents with ELISA values of 0.2-0.4 can be reared outside of Idaho, retested prior to release, and be released if the subsamples show no signs of BKD infection. However, when the presence of BKD is detected in these subsamples or parental values are greater than 0.4, the SBTOC will not permit the fish to be returned to Stanley Basin Lakes. As with IHN infections, if no alternate release location can be found that ensures these fish will not spread BKD to the healthy population, they will be destroyed. Similar decision trees for other types of virulent pathogens detected in captive broodstock population will be made as the need arises.

J. Cooperative breeding program.

The Redfish Lake sockeye salmon captive broodstock programs is a cooperative breeding program between NMFS, IDFG, BPA, the Shoshone-Bannock Tribes, and others involved in salmon recovery in the Pacific Northwest. The NWFSC has been acting, and will continue to act, to further cooperative breeding programs for this endangered species as well as to continue to maintain and contribute data to a breeding program, if such action is requested.

K. Previous or Concurrent Activities Involving Listed Species.

1. Identify all previous permits where you were the permit holder or primary investigator working with federally-listed species.

NMFS NWFSC captive broodstock fish propagation for endangered Redfish Lake sockeye salmon has historically been conducted under ESA Section 10 Propagation Permit #1148 issued by NMFS to IDFG.

In 1996, the NWFSC was issued ESA Permit #1005 for the NMFS portion of Redfish Lake sockeye salmon captive broodstock propagation. In 1998, this captive broodstock work was reauthorized under ESA Permit #1148 to the NMFS NWFSC REUT Division.

2. For the above permits, list all mortality events of listed species which have occurred in the last five years.

a. List the species, including scientific name and population where applicable.

Redfish Lake sockeye salmon, *Oncorhynchus nerka,* Snake River population (ESU).

b. Describe the number and cause of mortalities.

- NMFS received 310 smolts from sockeye salmon taken at Redfish Lake by IDFG in 1997. Survival of this group of fish during 29 months of culture in seawater was about 78%. Most mortalities were attributed to BKD. Approximately 10% (n = 28) fish were lost in July 2000 due to possible toxic buildup of ammonia due to inadequate water flow.

- NMFS received 296 eggs from sockeye salmon reared in captivity and spawned by IDFG in 1997. Survival during 36 months of rearing to spawning was about 71%. Most mortalities were attributed to etiology that could not be determined with current fish health diagnostic procedures.
- NMFS received 55 eggs from sockeye salmon from IDFG's captive broodstock spawning fertilized with milt from the lone returning wild male in 1998. NMFS retained another 127 eggs from a second generation safety-net captive broodstock for the 1987 wild spawning lineage group. Survival of these fish from ponding through spawning was about 68% for the IDFG group and 64% for the NMFS group. Most mortalities were attributed to early developmental problems.
- NMFS retained 403 eggs from a precocious female spawned in 1996. Survival from ponding to smolt was 95%. Survival from smolt to age-3 spawning was 78%. Most mortalities were attributed to BKD.
- NMFS received 9 groups of 594 eggs from sockeye salmon spawned by IDFG in 1999. Survival of these fish from ponding through 34 months of culture has been 77%. Most mortalities were attributed to etiology that could not be determined with current fish health diagnostic procedures.
- NMFS received 400 fry from sockeye salmon spawned by IDFG in 1999. Survival of these fish through 32 months of culture has been about 46%. Most mortalities were attributed to possible iodophore toxicity after moving fish to an inadequately flushed tank after disinfection.
- NMFS received ten groups of 389 eggs from sockeye salmon spawned by IDFG in 2000. Survival from ponding through 21 months of culture has been 93%. Most mortalities were attributed to early developmental problems. Most mortalities were attributed to etiology that could not be determined with current fish health diagnostic procedures.
- NMFS retained two groups of 538 eggs from sockeye salmon spawned in 2000. Survival of these fish from ponding through 21 months of culture has been about 87%, with some early developmental abnormalities occurring. Most mortalities were attributed to etiology that could not be determined with current fish health diagnostic procedures.
- NMFS received eleven groups of 435 eggs from sockeye salmon spawned by IDFG in 2001. Survival of these fish has been about 83%. Most mortalities were attributed to early developmental abnormalities.
- NMFS retained two groups of 500 eggs from sockeye salmon spawned in 2001. Survival of these fish has been about 80%. Most early mortalities were attributed to early developmental abnormalities.
- NMFS transported 17,761 eggs from sockeye salmon spawned in 1999 to ODFW Bonneville Fish Hatchery. About 16,000 alevins were ponded in February 2000. Survival of these fish from ponding through 15 months of

culture was 87%. Most mortalities were attributed to etiology that could not be determined with current fish health diagnostic procedures.

- NMFS transported approximately 39,200 eggs from sockeye salmon spawned in 2000 to ODFW Bonneville Fish Hatchery. These fish were combined with approximately 70,000 eggs from IDFG and 96,096 fish were ponded into tanks as swimup fry. In spring 2002, IHN virus was detected in the population, precluding their transfer back to Idaho. After more than 20,000 fish died of the infection, the remaining 40,596 fish, that were dying at epidemic levels were euthanized in June 2002 to prevent any survivors spreading IHN virus to the uninfected population in Redfish Lake.
- NMFS transported 90,222 eggs from sockeye salmon spawned in 2001 to ODFW Bonneville Fish Hatchery. Survival from ponding through eight months of culture was 88%. Most mortalities were attributed to etiology that could not be determined with current fish health diagnostic procedures. In August 2002, the remaining 75,428 fish were transferred and released into Stanley Basin Lakes. Equipment failure resulted in 2,195 fish dying during transfer.

c. Describe the measures that have been taken to diminish or eliminate such mortalities, and the effectiveness of those measures.

Standard hatchery procedures permit the use of disinfectants as a prudent practice to control the spread of disease. Iodophores have been known to be safe and effective for use in fish rearing containers. However, because of the mortality experienced in a just-disinfected container, new procedures require a minimum of 24-hours flushing time and/or complete drainage of the container before fish are introduced.

The use of iodophore disinfectant on shared equipment, unique equipment for each rearing container, and periodic prophylactic antibiotic treatments all have helped to reduce the number of clinical BKD outbreaks.

Bonneville Hatchery wells do not supply enough pathogen-free well water to maintain the Redfish Lake sockeye salmon during the brief periods of annual well maintenance. The water supply is switched to Tanner Creek water during these maintenance periods. The IHNV outbreak at Bonneville Fish Hatchery probably resulted from exposure of the fish to pathogens in Tanner Creek water. As a result, the SBSTOC has determined to terminate the smolt rearing program at Bonneville Fish Hatchery. A new smolt rearing program may be established at a facility to be determined by IDFG and the SBSTOC.

The loading density rates and flows to tanks have been set to eliminate the potential for ammonia toxicity and to ensure very good hygienic conditions.

All life support equipment on transport trucks will be inspected prior to fish hauling. Redundant monitoring and life support equipment will be carried as need to ensure fish are not lost due to in-transit equipment failure.

L. Certification:

I hereby certify that the foregoing information is complete, true and correct to the best of my knowledge and belief. I understand this information is submitted for the purpose of obtaining a permit under the Endangered Species Act of 1973 (ESA) and regulations promulgated thereunder, and that any false statement may subject me to the criminal penalties of 18 U.S.C. 1001, or to penalties under the ESA.

_____ _____

Signature **Date**
Name and Position Title

Attachment I

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Attachment II

Certification from a licensed veterinarian knowledgeable about the requested species, or from a recognized expert on the species that he/she has personally reviewed the criteria for transporting and maintaining the animal(s) and that in his/her opinion they are adequate to provide for the well-being of the animal. Include the name and phone number of this veterinarian, consulting expert, or equivalent who will be available during the proposed activities.

I hereby certify that I have personally reviewed the criteria for transporting and maintaining the animal(s) and it is my opinion they are adequate to provide for the well-being of the animal.

_____ _____

Signature **Date**

Dr. Lee Harrell
(360) 871-8307
Veterinarian license: 28301 VR00001695

Attachment IV

The current year workplan for Redfish Lake sockeye salmon captive broodstock rearing and research, Project 92-40, Contract De-AI79-92BP41841, funded by the Bonneville Power Administration.