

**Bonneville Power Administration
Fish and Wildlife Program FY99 Proposal**

Section 1. General administrative

Assessment of Captive Broodstock Technology

Bonneville project number, if an ongoing project 9305600

Business name of agency, institution or organization requesting funding
National Marine Fisheries Service, NOAA, DOC

Business acronym (if appropriate) NMFS

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List one subcontractor per row; to add more rows, press Alt-Insert from within this table

NPPC Program Measure Number(s) which this project addresses.

Measure 7.4D.1 in the NPPC F & W Program,

NMFS Biological Opinion Number(s) which this project addresses.

Other planning document references.

If the project type is "Watershed" (see Section 2), reference any demonstrable support from affected agencies, tribes, local watershed groups, and public and/or private landowners, and cite available documentation.

Task 4.1.c in the NMFS Proposed Snake River Recovery Plan

Subbasin.

Short description.

Improve effectiveness and assess risks of captive broodstock programs as a tool for recovery of depleted salmon stocks

Section 2. Key words

<u>Mark</u>	<u>Programmatic Categories</u>	<u>Mark</u>	<u>Activities</u>	<u>Mark</u>	<u>Project Types</u>
<input type="checkbox"/> *	Anadromous fish	<input type="checkbox"/>	Construction	<input type="checkbox"/>	Watershed
<input type="checkbox"/>	Resident fish	<input type="checkbox"/>	O & M	<input type="checkbox"/> *	Biodiversity/genetics
<input type="checkbox"/>	Wildlife	<input type="checkbox"/>	Production	<input type="checkbox"/>	Population dynamics
<input type="checkbox"/>	Oceans/estuaries	<input checked="" type="checkbox"/>	Research	<input type="checkbox"/>	Ecosystems
<input type="checkbox"/>	Climate	<input type="checkbox"/> *	Monitoring/eval.	<input type="checkbox"/>	Flow/survival
<input type="checkbox"/>	Other	<input type="checkbox"/>	Resource mgmt	<input type="checkbox"/>	Fish disease
<input type="checkbox"/>	Planning/admin.	<input type="checkbox"/> *	Supplementation	<input type="checkbox"/>	Enforcement
		<input type="checkbox"/>	enhancement/restoration	<input type="checkbox"/>	Wildlife habitat en-
		<input type="checkbox"/>	Acquisitions		

Other keywords.

genetics, fish nutrition, salmon reproduction, fish health

Section 3. Relationships to other Bonneville

Project #	Project title/description	Nature of relationship
9202200	Wild smolt physiology/behavior	Collaborate by evaluating reproduction of experimental group of spring chinook salmon.

Section 4. Objectives, tasks and

Objectives and tasks

Obj. 1,2,3	Objective	Task a,b,c	Task
1	Determine the effects of rearing husbandry on life cycle phenotypes, body coloration, and gamete quality of Pacific salmon.	a	Determine effects of growth rates and body fat levels on immunocompetence, smoltification, and age of maturity in male spring chinook salmon.
1		a	Determine effects of constant versus variable dietary protein and energy intake on growth, body conformation, natural

			spawning success, spawning behavior, and reproductive performance of chinook salmon.
		b	Determine effect of dietary treatment on survival of adults to spawning and age at maturity of chinook salmon.
		c	Determine effects of dietary treatments on the quality of gametes and percentage survival of fertilized eggs to the beginning of exogenous feeding
		d	Determine effects of dietary treatments on external coloration, body conformation, and behavior of juveniles and adult fish.
2	Develop techniques to improve fish health	a	Develop antibacterial therapy to reduce mortality due to BKD in chinook and sockeye salmon.
		b	Test the effectiveness of azithromycin as a prophylactic therapeutant against BKD in chinook salmon.
		c	Develop techniques for using live-food as a delivery system for drugs to treat bacterial disease in first feeding fry.
		d	Test effects of growth and nutrition on immune function in spring chinook salmon.
3	Determine homing imprinting timing of captive salmon	a	Determine the timing of olfactory imprinting by juvenile sockeye.
		b	Determine the timing of olfactory imprinting by juvenile chinook salmon.
4	Describe spring chinook salmon reproductive behavioral ecology	a	Investigate potential kin-based assortative mating patterns.
		b	Apply DNA microsatellite analysis to determine

			reproductive success.
5	Determine critical genetic factors in captive broodstock	a	Evaluate inbreeding depression
		b	Evaluate population differentiation and outbreeding depression

Objective schedules and costs

Objective #	Start date mm/yyyy	End date mm/yyyy	Cost %
1	12/1998	12/2000	10%
2	12/1998	12/2000	15%
3	12/1998	12/2000	10%
4	12/1998	12/2003	10%
5	12/1998	12/2003	10%
6	12/1998	12/2003	10%
7	12/1998	12/2003	15%
8	12/1998	05/2008	20%

Schedule constraints.

None anticipated

Completion date.

2008

Section 5. Budget

FY99 budget by line item

Item	Note	FY99
Personnel		\$260,600
Fringe benefits		\$102,416
Supplies, materials, non-expendable property		\$78,322
Operations & maintenance		
Capital acquisitions or improvements (e.g. land, buildings, major equip.)		\$3,200
PIT tags	# of tags:	
Travel		\$13,600
Indirect costs		\$140,224
Subcontracts		\$575,500

Other		\$26,138
TOTAL		1,200,000

Outyear costs

Outyear costs	FY2000	FY01	FY02	FY03
Total budget	\$1,200,000	\$1,200,000	\$1,200,000	\$1,200,000
O&M as % of total				

Section 6. Abstract

In response to Task 4.1.c in the NMFS Proposed Recovery Plan and to Measure 7.4D.1 in the NPPC F & W Program, this research project develops information needed to overcome some of the problems that limit the yield of viable offspring from Pacific salmon stocks reared in captivity and assesses some of the genetic consequences of captive broodstock programs. While basic fish husbandry techniques are well established and widely used for rearing juvenile salmonids from gametes collected from returning adults and domesticated stocks of salmonids in the commercial aquaculture industry, numerous problems have persisted when rearing wild stocks of Pacific salmon in captivity throughout the life cycle. These problems include poor survival of adults to spawning, poor quality gametes, and abnormal seasonal timing of spawning. The success of captive broodstock programs for stock restoration purposes is largely dependent on producing large numbers of offspring that do not differ substantially from the founder stock in genetics, behavior, appearance, or physiology. Solutions to the problems encountered by broodstock programs are needed to maximize the effectiveness of these programs as rehabilitative tools. In addition, the reproductive success of captive reared fish must be evaluated to determine if release of captive reared adults is a viable strategy. The overall goal of this project is to develop diets, rearing regimes, hatchery practices, and drug therapies that improve survival of adults to spawning, gamete quality, and viability of offspring and that can be applied to captive broodstock programs for depressed stocks of Pacific salmon. Results from this research will be published in peer-reviewed journals, annual reports and scientific meetings.

Section 7. Project description

a. Technical and/or scientific background.

One of the current barriers to restoration of many depleted stocks of Pacific salmon (*Oncorhynchus* spp.) in the Columbia River Basin is the availability of suitable numbers of juveniles for supplementation. The Northwest Power Planning Council's Columbia River Basin Fish and Wildlife Program was recently amended to include development and implementation of captive broodstock technology to aid recovery of salmon stocks (Phase II; Measure VI.B.6.A.2). 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.

Like salmon hatchery programs, however, captive broodstock programs are not without problems and risks to natural salmon populations. Captive broodstock can sustain high mortality, which may increase a population's risk of extinction if the captive component is a substantial fraction of this population. Rearing systems must be designed and operated to minimize the risk of loss due to disease, poor reproductive performance of adult fish, and poor survival of offspring once released into the native habitat. Additional risks include genetic change imposed on a population by a captive broodstock program, genetic interaction between captive and natural fish in the wild, and ecological impacts of releases of captive fish on natural populations.

Although captive broodstock technology has been widely applied to other vertebrates, its application to restoration of depleted stocks of Pacific salmon is in its infancy. Recent experience with captive broodstock programs has indicated that substantial problems exist with: 1) poor survival of fish to spawning, 2) inappropriate timing of sexual maturation, and 3) poor quality gametes. In addition, adequate therapies are not available for treating fish once disease, particularly bacterial kidney disease, occurs, and the reproductive success of captively reared adult fish and the survival of offspring from captively reared fish when released into the wild is unknown. We are conducting and propose to continue a multi-faceted research program designed to overcome these problems by establishing rearing regimes, diets, and environmental conditions that lead to appropriate body coloration, size, and timing of development through the life cycle, and high survival rates of broodstock and their offspring. Our literature review also indicated that little research has been done that relates directly to the genetic consequences of captive broodstock programs for Pacific salmon. We propose three primary quantitative genetic experiments to characterize some important potential effects of captive culture on the genetic constitution of the cultured population. The experiments require access to one or more large source populations and much of the rearing capacity of a single facility for up to four fish generations.

The project has three primary goals: 1) to develop standard, efficient hatchery practices for rearing captive Pacific salmon broodstock that will yield the greatest number of high-quality offspring (those that are as similar to the founder stock as possible), 2) to determine the genetic consequences of captive broodstock programs for natural salmon populations, and 3) to evaluate the reproductive success of captively-reared adult fish compared to wild fish and the viability of captively-reared offspring upon release. The work proposal has been divided into five major research elements: 1) effects of diet and growth on age of maturity, smoltification, body coloration, and gamete quality; 2) fish health; 3) olfactory imprinting in sockeye and spring chinook salmon; 4) reproductive ecology of captively-reared adult salmon; and 5) research on quantitative genetic consequences of captive broodstock programs for Pacific salmon populations.

Element 1- Effects of Growth and Diet on Age of Maturity, Smoltification, Body Coloration, and Gamete Quality in Chinook and Sockeye Salmon

One critical problem for captive rearing of chinook salmon is loss of fish due to early

sexual maturation of males. In many salmonid species, males may mature early relative to females, with the incidence varying among species, stocks, and rearing conditions for cultured fish. The chinook salmon has a high degree of plasticity in its life cycle compared to other Pacific salmon species. Early, or precocious, male maturation can occur at several stages of the life cycle. Jacking rates as high as 90% have been observed (Hard et al., 1985), although most chinook stocks exhibit rates around 5-15% (Heath 1992). In a captive broodstock program, it is undesirable to produce mature males at a time when females of the same stock are not mature. Although milt from early maturing males can be cryopreserved, the technique is not yet sufficiently reliable to obtain consistently high quality sperm. In addition, selective mortality of precocious males could reduce the effective breeding population size (N_e) of a captive broodstock. Thus, there is a critical need to develop methods to minimize precocious male maturation in captive broodstock programs for endangered fish species.

The time of sexual maturation is controlled by genetic, abiotic (e.g., photoperiod, temperature, salinity) and biotic (e.g., diet, growth rate, energy stores) factors. The relative importance of these factors and how they interact are poorly understood. Because genetic selection should be minimized in a captive broodstock program for depleted stocks, rearing strategies which minimize expression of the trait should be developed. Research to date, primarily from Atlantic salmon, indicates that growth rate, size and levels of stored energy at specific times of year, or critical periods of the life cycle are important factors affecting the incidence of precocious maturation. It may be possible to minimize the incidence of precocious male maturation through alteration in rearing conditions, growth rates, and diet. However, before methods that minimize the rate of precocious male maturation can be developed, research is necessary to determine how stored energy levels (body fat content), growth rates, or rates of energy deposition at critical developmental stages either permit or prevent the onset of maturation in chinook salmon.

We are attempting to develop diets and growth regimes that sustain somatic growth and provide sufficient stored energy for appropriate life-cycle transitions, development of gametes in adult fish, and achieve target size for adult fish. To develop a diet and growth regime that minimizes early maturation of male spring chinook salmon, we are engaged in two key areas of investigation. In initial studies we manipulated body fat levels through diet and found a significant positive correlation of percent of males maturing at 2-years of age with body fat levels. Second, we are investigating whether reduced growth during the autumn/winter period can reduce the number of males maturing at one or two years of age. One of the problems with previous research in this area is that effects of size, growth rate, and rate of energy deposition could not be distinguished. Therefore we conducted a study varying ration and fat levels and found that size or growth rate was the primary factor affecting the rate of maturation of 2-year old fish, and fat level was a secondary factor (Shearer et al. 1997; Silverstein et al. 1997). Results from this study are consistent with previous studies in Atlantic salmon which indicated that severe ration restriction can reduce the incidence of early male maturity. However, a protocol which utilizes severe ration restriction may have undesirable effects on smoltification, maturation of females in subsequent years, and ability of managers to achieve target sizes

for adults. Therefore, the aim of the proposed experiment is to more precisely determine how rapidly male chinook salmon can be grown from first feeding to two years of age without increasing the incidence of maturation. Since growth rate has been shown to affect both smoltification and immunocompetence we will also examine the effects of growth on these factors.

The natural diet of chinook salmon during the post-juvenile marine phase of its life cycle basically consists of herring, anchovy, other small fish, and squid. When this diet is expressed in terms of proximate composition; the protein, lipid, and ash (bones) fractions constitute more than 95% of the diet. If a feed were formulated to mimic the natural diet of chinook salmon in the ocean, it would be approximately 50% protein, 32% lipid, and 10% ash (Higgs et al., 1995). Feeds used to rear post-juvenile chinook salmon in captivity, e.g., net-pen farming, have a proximate composition of 45% protein, 18-22% lipid, and 12% ash, with the remaining constituents being crude fiber (~2%) and nitrogen-free extract (soluble carbohydrates) as the remainder. Farmed chinook salmon fed diets containing higher levels of lipid throughout the seasons accumulate excessive amounts of body fat, mainly during the period of declining day length. In contrast, the natural diet of chinook salmon during the early phases of marine life history, e.g., copepods, decapods, amphipods, and euphausiids, contains much higher dietary lipid levels than pelleted feeds. The protein and lipid levels of each average 52% and 37% for copepods, 73% and 12% for decapods, 47% and 24% for amphipods, and 54% and 22% for euphausiids, respectively (Higgs et al., 1995). Squid contain 78% protein and 13% lipid, while gastropods average 44% protein and 22% lipid. Despite this high lipid diet, wild post-juvenile chinook salmon do not accumulate excessive levels of body fat, most likely a consequence of activity level and caloric intake.

The natural diets of juvenile salmonids in freshwater consist mainly of aquatic and terrestrial insects. Many published studies have documented the natural diet of juvenile salmonids, both in terms of prey and in terms of nutrient intake, beginning with Embury and Gordon (1924) and most recently reviewed in depth by Higgs et al. (1995). These studies conclusively show that the proximate composition of natural diets of juvenile salmonids is approximately 45% protein and 15-17% lipid. Although the natural diet of juvenile chinook salmon in freshwater consists of insects having lipid levels ranging from 2-39%, on a dry weight basis, the main four groups of insects consumed by juvenile chinook average 53% protein and 16.5% lipid, similar to the composition of freshwater copepods and small fishes. Like wild post-juveniles, juvenile chinook are distinctly different from hatchery-reared chinook in body fat deposits, particularly in the amount of fat in visceral stores. These differences are greater among stream-type juvenile chinook than among ocean-type.

The body conformation of chinook salmon reared in captive broodstock programs differs from that of wild fish. The main difference in body conformation is length to girth ratio, with captively-reared fish having a lower length to girth ratio, meaning that they tend to be shorter and fatter. This difference is presumably related to dietary energy intake and differences between wild and captive fish in activity level. Dietary energy intake is similar between wild and captively-reared fish, when expressed in terms of proximate

composition, or percentage of the diet as protein and lipid. However, dietary energy intake is a function of the proximate composition of the diet as well as feed intake. Wild fish consume less food during certain periods of the year than do captive-reared fish. Rarely are chinook salmon with full stomachs captured in the fisheries, except in nearshore areas where fish are feeding heavily during the months before entering freshwater to spawn. Recent BPA-funded studies with chinook salmon (W. W. Dickhoff 1997) have identified annual variations in levels of the metabolic hormones IGF-1 and growth hormone which provides a metabolic rationale for numerous observations made for over 40 years concerning the higher level of whole body lipid in hatchery-reared fish compared to wild salmon. During periods of declining day length, circulating levels of these hormones are low and protein synthesis rates in the body are reduced. Fish convert both dietary protein and lipid into stored body fat during these periods. In contrast, when day lengths increase, body metabolism patterns change, with increases in protein synthesis rates and lipolysis, resulting in lower percentage whole body fat levels. When feeding levels are adjusted to correspond with this pattern of anabolism and catabolism, survival to hatchery return increases. With respect to changing dietary requirements as fish grow and develop, it is well known that the scope for growth of Pacific salmon decreases with fish size (Brett, 1979). Thus, the protein needs of salmon, expressed as a percentage of dietary metabolic energy, decrease with fish size. McCallum (1985) reported that juvenile chinook salmon required 1.3-1.6 g protein per kg fish per day at the maintenance level, e.g. no growth. The amount of protein intake to support weight gain varies with fish size and dietary energy intake but growth increases linearly in juvenile salmonids consuming between 6 and 18 g protein per kg fish per day (Fairgrieve, 1992). To develop meaningful recommendations for dietary protein and lipid levels for captive-reared chinook salmon, one must consider the daily dietary protein intake and the protein intake relative to total dietary energy level rather than total dietary lipid level.

We therefore intend to investigate the effects of varying daily protein intake and daily energy intake, expressed as g digestible protein (and kcals) per kg fish per day, on protein and energy accretion in juvenile and post-juvenile chinook salmon to mimic seasonal differences in food availability in the marine environment on growth, fish conformation, year of maturity, and reproductive performance, including spawning behavior.

Element 2- Fish Health

To reduce mortality due to disease in captive broodstocks there are two strategies: employ hatchery practices, environmental conditions, and feeding regimes which maintain good fish health and minimize disease transmission, and utilize effective drug therapies once disease outbreaks occur. In 1987, the Pacific Northwest Fish Health Protection Committee ranked bacterial kidney disease (BKD) as the major deterrent to the successful culture of salmonids in the Pacific Northwest. We are conducting research in three areas in an attempt to reduce mortality due to disease in captive broodstock. First, we are testing new drug therapies for treating BKD. Second we are testing live food such as *Artemia*, as a mode of administration for antibiotics to first feeding fry. And third, we are investigating the effects of nutrition and rearing temperature on immune function. In previous funded periods, we developed a range of standard assays to assess both humoral and cellular-mediated immunity. We now propose to use these assays to assess

immunocompetence in experimental fish.

Erythromycin has been the primary antibiotic used by fish culturists in attempts to prevent and control BKD in salmonids; however, this drug has not been an effective chemotherapeutant against this disease. Azithromycin is a new macrolide antibiotic that concentrates in polymorphonuclear leukocytes, macrophages, and fibrocytes. These cellular elements have been reported to sequester and protect *Renibacterium salmoninarum*, the causative bacterium of BKD.

Most antibiotics, including erythromycin, do not penetrate tissues well, but after administration orally or parenterally, they are bound to serum proteins and remain in extra-cellular spaces. Azithromycin is rapidly absorbed in tissues and is widely distributed at higher concentrations in cells than in plasma or in serum. Numerous studies report greatly increased intracellular uptake and superior antibacterial activity of azithromycin over erythromycin in vitro and in in vivo studies with animals other than fish. Researchers reporting in the Journal of Antimicrobial Agents and Chemotherapy (1994) measured the intracellular activity of azithromycin and erythromycin against several enteric pathogens that had been phagocytosed by neutrophils. Azithromycin was effective in reducing the intracellular viabilities of nearly all strains tested; however, erythromycin was found to be generally not as effective as azithromycin. The authors purported that the concentration of azithromycin in neutrophils may be particularly useful in treating infections caused by pathogens that invade intracellularly into host tissues. Other work in 1994 compared the intracellular activity of azithromycin and erythromycin against an intracellular protozoan parasite, *Toxoplasma gondii*, and reported superior performance of azithromycin. Azithromycin accumulated readily and remained inside macrophages infected with the protozoan, interfering with growth of the parasite. There are no publications or current studies using azithromycin in fish to our knowledge.

In 1996 and 1997, we compared the efficacy of azithromycin to erythromycin in reducing mortality due to BKD in sockeye salmon. We initiated a challenge of uninfected sockeye salmon by cohabitation with spring chinook salmon undergoing a BKD epizootic. All control fish subsequently died exhibiting severe kidney lesions and other signs typically associated with clinical BKD; however, the non-cohabitated, sockeye salmon, from the stocking population, remained healthy (< 0.1% mortality). Mortality in salmon fed erythromycin was 92.25% when the experiment was terminated. Mortality in fish fed azithromycin was 42.75% at termination (214 days post-infection; 30 weeks). Although our cohabitation challenge resulted in a severe BKD epizootic, oral treatment with azithromycin was clearly superior to treatment with erythromycin. We reared all surviving fish and found no detrimental effect of the azithromycin treatment on gamete viability. This new drug therapy is showing tremendous promise for reducing mortality due to BKD in endangered fish. We are proposing to continue testing azithromycin to ensure that the drug has no detrimental effect on gamete quality or offspring viability.

We are also investigating the use of live-foods, such as Artemia, for delivery of drug therapies to first-feeding fry. During previous funded periods, studies were conducted to determine the dynamics of erythromycin uptake in adult Artemia. Erythromycin was

encapsulated in liposomes, and added to Artemia rearing water. Effects of water concentration and time of exposure by the Artemia were determined by sampling the Artemia and measuring their erythromycin concentration (and its active metabolites) using a microbiological assay. Depuration rates were determined in sockeye salmon fry using the microbiological assay to measure erythromycin in the fry samples. Efficiency of erythromycin uptake by first feeding sockeye salmon was determined, again using the microbiological assay. In addition enrichment methods for producing adult Artemia with a high concentration of erythromycin were refined. Two new task areas are proposed which will follow-up on our success with using adult Artemia as a delivery system for first feeding salmonids. Studies will investigate (1) two additional antibiotics and (2) the effects of processing on bioencapsulated Artemia.

Two other antibiotic compounds, azithromycin and Romet -30, used alone or in combination with erythromycin, may have potential to treat BKD and other fish diseases in first feeding salmonids when bioencapsulated into adult Artemia. Methods analogous to those used with erythromycin to produce Artemia with high levels of these compounds will be investigated. Once workable methods have been developed, bioencapsulated azithromycin, Romet-30 and/or combinations will be fed to first-feeding salmonids to determine if this method can be used to deliver these compounds to fish tissues.

The benefit of using a bioencapsulated drug delivery system is that the organisms are readily consumed by first feeding salmonids. The drawback is that given current methods developed by this task, Artemia holding and the bioencapsulation process needs to be done on-site to be effective. This requires additional expertise and infrastructure at hatcheries. We will address these drawbacks by investigating the palatability of frozen and freeze-dried Artemia-bioencapsulated erythromycin to first feeding salmon. Such processing may greatly facilitate using this delivery system, as the bioencapsulation process could be done centrally and external to the fish rearing, with the processed product (frozen or freeze-dried bioencapsulated antibiotic) shipped to the hatchery and fed in a more-or-less conventional manner.

While it is important to have drug therapies available to reduce mortality due to disease, efforts should also be directed to prevent disease outbreaks through prudent hatchery practices and rearing conditions that promote fish health. In previous funded periods, we investigated the effects of rearing temperature on immunocompetence. Analysis of results from this study will be completed during FY98. In addition to environmental conditions, dietary factors are believed to play an important role in preventing or reducing the severity of disease in cultured fishes. There is evidence that many of the specific and nonspecific host defenses of salmonids and other fishes are effected by specific dietary components (Reviewed by Landolt 1989; Blazer 1991, 1992; Waagbo 1994). Their effects on disease resistance, however, are not completely understood. Until recently, this was largely the result of our lack of knowledge about a fish's immune system, and consequently, how it modulates in response to dietary modifications. Recent advances in the characterization of the immune functions of fish and the development of new methods for their study will allow us to gain a clearer understanding of the role of nutrition in fish health and disease resistance.

Element 3- Olfactory Imprinting in Sockeye and Chinook Salmon

Pacific salmon are well known for their ability to learn (or imprint) to odors associated with their natal stream as juveniles and then later use these retained odor memories to guide the final phases of their homestream migration. The imprinting process is critical for successful completion of the spawning migration, and salmon which do not experience their natal water during appropriate juvenile stages are more likely to stray to non-natal sites. Straying by captively reared salmon may jeopardize efforts to enhance endangered populations by either lowering the effective number of spawning adults in a captively reared target population or via competition and interbreeding of hatchery salmon with endangered wild populations.

The vast majority of imprinting research has utilized hatchery-reared coho salmon as model organisms because of their relatively simple life history compared to other salmonid species. For hatchery-reared coho salmon, the parr-smolt transformation is a critical period for olfactory imprinting. However, the freshwater rearing patterns of other salmonid species (e.g. chinook and sockeye) are much more complex and temporally plastic, and for these species the critical periods for imprinting are not known. To determine the critical period(s) for imprinting for sockeye and chinook salmon, we exposed juvenile salmon to known odorants at key developmental stages and will subsequently test whether these fish develop long-term odor memories of these odorants. Results of these experiments will be important to determine when critical periods of imprinting are for offspring from captively reared fish that are destined for release into natal rivers or lakes.

Element 4- Reproductive Ecology of Captively Reared Spring Chinook Salmon

Captive broodstocks are generally initiated to stem the further decline of severely depressed populations, and therefore are often initiated from a low number of individuals. In captive broodstock programs with known pedigrees, mating of closely related individuals (i.e., inbreeding) can be avoided (or at least quantified) during artificial spawning. However, in the case of a captive rearing and adult release strategy, such as that proposed for gene preservation of three stocks of endangered spring chinook salmon (*O. tshawytscha*) in the Snake River Basin, Idaho, mating among the broodstock will occur naturally in ancestral streams without knowledge of individual reproductive success, overall mating patterns, or levels of inbreeding. The probability of inbreeding among adults released to spawn naturally may be estimated using established methods, but these estimates rely on assumptions of mating patterns (e.g., random mating) within the population (Hard and Hershberger 1995).

Currently, there is no information regarding mating patterns of chinook salmon, and very little information on the general reproductive ecology of this species. Moreover, the reproductive behavior of captively reared chinook salmon and their ability to naturally reproduce has never been studied. Such information would be very useful to managers

involved in implementing adult release strategies both for determining numbers, sizes, and ages of fish to release, and for monitoring the success of stocked captive reared salmon. Therefore, in 1997 and 1998 we plan to investigate the reproductive behavior, adult-to-fry reproductive success, and kin-based assortative mating patterns of captive reared spring chinook salmon.

Anadromous salmonids may possess naturally adapted mechanisms that favor non-random (i.e., assortative) mating among families in naturally spawning populations. Evidence exists that several salmonid species can discriminate between kin and non-kin. For example, juvenile Atlantic salmon (*Salmo salar*) and rainbow trout (*O. mykiss*) prefer to inhabit areas in close proximity to kin and distance themselves from non-kin, and aggression within kin groups is less than within non-kin groups (Brown and Brown 1992, 1993). Sibling recognition has also been suggested one possible mechanism causing asymmetrical distribution and growth patterns among sympatric full-sib families of juvenile coho salmon (*O. kisutch*, Quinn et al. 1994). Kin recognition among naturally reproducing salmon has not been studied, but if present, may influence mating patterns and potential for inbreeding, and therefore, impact strategies for captive broodstock programs.

In general, Pacific salmon males compete for access to spawning females and the opportunity to fertilize eggs. The outcomes of these competitive (i.e., aggressive) contests are partially determined by overall body size and certain morphological characters such as body depth and kype development (Schroder 1981, Foote 1990, Fleming and Gross 1992, 1993, 1994, Quinn and Foote 1994). For females to be reproductively successful, a female must acquire a territory, construct a series of nests (redd), and defend her developing eggs from mechanical shock caused by neighboring females (Schroder 1982, Fleming and Gross 1993). In addition, females exhibit mate choice selection based on certain male characteristics (e.g., body size), although female control over whom they spawn with is limited by dominance hierarchies established through male-male competition (Quinn and Foote 1994). Whether kin recognition influences the overall mating patterns of Pacific salmon is unknown. Theoretically, kin recognition could affect female mate choice and male competition. For example, females may demonstrate a preference for non-kin over kin in their choice of mates as a naturally adapted mechanism to reduce inbreeding. Male salmon may demonstrate differential kin-based levels of aggression when competing for access to females. For males greater acceptance of kin and greater aggression towards non-kin during competition for mates could increase the inclusive fitness of dominant males. Alternatively, adult salmon may not possess kin recognition capabilities, or if they do, this may not be important to their overall mating patterns.

Element 5 - Research on Quantitative Genetic Consequences of Captive Broodstock Programs for Pacific Salmon Populations

Our research on the quantitative genetic consequences of captive broodstock programs is focused on three issues. These issues relate to three genetic risks of artificial propagation (Busack and Currens 1995): directional genetic change, loss of genetic variability within

a population, and loss of population distinctiveness. Domestication selection, or adaptation to a protective culture environment, can produce directional genetic change during this process (Doyle 1983, Kohane and Parsons 1988). Inbreeding depression, or the reduction in fitness due to low heterozygosity or to unmasking of deleterious recessives, can result from the loss of genetic variability or from nonrandom mating within a population, and can further reduce this variability through the loss of genotypes from either genetic drift or selection (Gall 1987, Hard and Hershberger 1995, Su et al. 1996). Outbreeding depression, which is a reduction in fitness due to loss of local adaptation or to the breakup of coadapted gene complexes, can result from interbreeding between distinct populations, and can further reduce this distinctiveness through the production of reproductively successful crossbred offspring (Lynch 1991, Hard and Hershberger 1995).

These processes relate to three aspects of a captive broodstock program. First, domestication selection can result from variability in mortality and reproduction of cultured individuals that reflects differences between natural and protective culture environments. Second, inbreeding depression can result from the loss of genetic variability or a change in mating opportunity in a founder population or during the culture process. Third, outbreeding depression can result when cultured individuals or their offspring are released to the wild to interbreed with wild individuals, if these groups are genetically distinct enough from each other.

The consequences of these processes in Pacific salmon are, for the most part, unknown. Domestication has been difficult to evaluate, but some experiments indicate that hatchery culture has consequences in nature (Maynard et al. 1995). Inbreeding depression in salmonids has been characterized only for freshwater *Oncorhynchus mykiss* (Kincaid 1976b, Kincaid 1976a, Kincaid 1983, Su et al. 1996), where substantial reductions in growth and survival were observed in response to inbreeding. Outbreeding depression has been evaluated empirically only for pink salmon (*O. gorbuscha*), with somewhat inconclusive results (Gharrett and Smoker 1991).

Experiments corresponding to two of the three objectives, domestication and outbreeding depression, have not yet been initiated. We are conducting an experiment on inbreeding and inbreeding depression, and propose to continue this experiment during FY99. Although the results from this study do not yet address directly the consequences of inbreeding and inbreeding depression in a hatchery population of fall chinook, they characterize levels of phenotypic and quantitative genetic variability in this population that indicate opportunities for aspects of its life history to change in response to different culture environments and levels of genetic variability.

b. Proposal objectives.

Element 1- Effects of Diet and Growth on Age of Maturity, Smoltification, Body Coloration, and Gamete Quality in Chinook and Sockeye Salmon

Objective 1.1 Determine effects of growth rates and body fat levels on smoltification, gamete quality, age of maturity, and immunocompetence in spring chinook salmon.

Objective 1.2. Determine effects of constant versus variable dietary protein and energy intake on growth, body conformation, natural spawning success, spawning behavior, and reproductive performance of chinook salmon.

Objective 1.2, Task 1. Determine effect of dietary treatment (dietary protein and energy intake levels) on growth, body conformation, proximate composition, and gonadal development .

Objective 1.2, Task 2. Determine effect of dietary treatment on survival of adults to spawning and age at maturity of chinook salmon.

Objective 1.2, Task 3. Determine effects of dietary treatments on the quality of gametes and percentage survival of fertilized eggs to the beginning of exogenous feeding.

Objective 1.2, Task 4. Determine effects of dietary treatments external coloration, body conformation, and behavior of juveniles and adult fish.

Element 2- Fish Health

Objective 2. 1 Develop antibacterial therapy to reduce mortality due to BKD in chinook and sockeye salmon.

Objective 2.2 Further develop techniques for using live-food mediated drug delivery.

Objective 2.3. Test effects of growth and nutrition on immune function in spring chinook salmon.

Element 3- Olfactory Imprinting in Sockeye and Chinook Salmon

Objective 3.1. Determine the timing of olfactory imprinting by juvenile sockeye.

Objective 3.2. Determine the timing of olfactory imprinting by juvenile chinook salmon.

Element 4- Reproductive Ecology of Captively-Reared Spring Chinook Salmon

Objective 4.1. Obtain adult spring chinook salmon for mating experiments.

Objective 4.2., Task 1. Investigate potential kin-based assortative mating patterns.

Objective 4.2, Task 2. Apply DNA microsatellite analysis to determine reproductive success.

Objective 4.3. Describe spring chinook salmon reproductive behavioral ecology.

Element 5 - Research on Quantitative Genetic Consequences of Captive Broodstock Programs for Pacific Salmon Populations

Objective 5.1. Evaluate Inbreeding and Inbreeding Depression

Objective 5.2. Evaluate Population Differentiation and Outbreeding Depression

c. Rationale and significance to Regional Programs.

One of the current barriers to restoration of many depleted stocks of Pacific salmon (*Oncorhynchus spp.*) in the Columbia River Basin is the availability of suitable numbers of individuals for release back into their habitat. The Northwest Power Planning Council's Columbia River Basin Fish and Wildlife Program was recently amended to include development and implementation of captive broodstock technology to aid recovery of salmon stocks (Phase II; Measure VI.B.6.A.2). Captive broodstock programs are a form of artificial propagation that are intended to give listed stock a "jump start" on the road to recovery. Although captive broodstock programs for listed salmon are technologically well enough to be started, they are not without problems and risks to natural salmon populations. Captive broodstock programs can sustain high mortality, which may increase a population's risk of extinction if the captive component is a substantial fraction of this population. Rearing systems must be refined and operated to minimize the risk of loss due to disease, poor reproductive performance of adult fish, and poor survival of offspring once released into the native habitat. Additional risks include genetic change imposed on a population by a captive broodstock program, genetic interaction between captive and natural fish in the wild, and ecological impacts of releases of captive fish on natural populations. The proposed project on technology assessment addresses some established problems and actively cooperates with on-going captive broodstock programs to address new problems as they arise.

d. Project history

- summary of major results achieved - past costs (see attached spreadsheet)

This project was initiated in 1993. During the first year an extensive literature review was conducted to assess the current status of captive broodstock technology and identify critical research needs. A multidisciplinary and multiagency research team was assembled to address identified research problems as they have emerged from ongoing captive broodstock programs. Research was initiated in FY 95. We are presently in our third year of research. Many of the ongoing experiments require rearing of fish for one or more generations; thus the major results of several studies initiated in FY95 will not be forthcoming until FY98 and FY99. Major results are summarized below.

Literature review:

Flagg, T.A., and C.V.W. Mahnken (eds.). 1995. An assessment of the status of captive broodstock technology for Pacific salmon. Final report to the Bonneville Power Administration, Contract DE-AI79-93BP55064, 285 p. plus appendices (Available from Bonneville Power Administration, Public Information Center - CKPS-1, P.O. Box 3621, Portland, OR 97208).

Major Results:

1. Completed studies of effects of rearing sockeye salmon in fresh water or seawater on reproductive performance. Sockeye salmon broodstock reared in fresh water throughout the life cycle had higher survival than those reared for a period in seawater, but the timing of spawning, age of maturity and quality of gametes did not differ. However, body size of both sexes and egg size in seawater-reared fish were slightly smaller.
2. Completed studies on induction of ovulation and spermiation in sockeye salmon using gonadotropin-releasing hormone analog. Timing of spawning in mature sockeye salmon males and females can be controlled (advanced and synchronized) with gonadotropin-releasing hormone analog without impairing gamete quality. This technology is now available to hatchery managers to prevent loss of gametes due to prespawning mortality and by asynchronously spawning male and female broodstock.
3. Completed studies on the effects of high and low growth rates and varying levels of dietary fat on maturation of spring chinook salmon males. Both growth rate and body fat levels can affect the number of male spring chinook salmon maturing at 2 years of age, with growth rate being the predominant factor. The number of early maturing males was lowest when fish were reared on reduced ration and low fat/high protein diets. These results suggest that early maturation of male chinook salmon may be reduced by reducing growth rate.
4. Completed initial tests of azithromycin as a therapeutant for bacterial kidney disease in sockeye salmon . Azithromycin was more effective than erythromycin in reducing mortality due to bacterial kidney disease. No apparent negative effect of the drug was found on fertilizability of eggs produced from broodstock treated with azithromycin.
5. Completed studies of the reproductive success of wild versus captively reared coho salmon. Wild coho salmon spawned with captively reared salmon, but wild males dominated captively reared males resulting in fewer offspring from captively reared males. There was no significant difference between the wild and hatchery-reared fish in the quality of gametes or survival of offspring. However, fry from wild females had more orange fin coloration than those from captively reared females.
6. Completed initial study on the effects of feeding carotenoid-supplemented diets on reproductive performance of sockeye salmon broodstock. Carotenoid supplementation of broodstock diets did not alter gamete quality or the percentage of maturing fish.
7. Completed initial studies of the effects of live foods on growth and behavior of first-feeding fry. Fry fed commercial diets ate live foods as well as those fed live food from the time of first feeding. These results suggest behavioral imprinting on live food is not necessary for fry prior to release.
8. Completed development of standardized assays for cellular- and humoral-mediated immunity in sockeye salmon. Initial tests of immunocompetence of fish reared on either 8 or 12 C water indicated that no major differences in immune function could be detected.
9. Completed first 3 years of a study on the effects of rearing temperature (8 or 12 C) on growth, development, smoltification, maturation, and immune function of sockeye salmon. No major differences in immune function could be detected between the two groups. However, fish reared on 12 C were larger than those reared on 8 C. None of the fish reared on 8C matured at 3 years of age, compared to approximately 30% of those reared on 12C.

10. Completed initial phase of inbreeding experiment (Objective 5.5).
11. Successfully incorporated erythromycin into Artemia as a vehicle to treat first-feeding fry.

e. Methods.

Element 1- Effects of Diet and Growth on Age of Maturity, Smoltification, Body Coloration, and Gamete Quality in Chinook and Sockeye Salmon

Objective 1.1 Determine effects of growth rates and body fat levels on smoltification, gamete quality, age of maturity, and immunocompetence in spring chinook salmon. The proposed project will rear six groups (in duplicate, 400 fish per tank) of Sol Duc River spring chinook (1997 brood) from start feeding to age 2+ on graded rations (100= satiation, 90, 80 70 60 and 50% of satiation) of a high protein (55%), low fat (8%) feed (Table 1).

Table 1. Provisional composition of experimental diets (based on estimated proximate composition of feed ingredients).

Ingredient	(g/kg)
Anchovy meal	600
High protein fish meal	120
Fish oil	60
Wheat gluten	170
Vitamin premix	30
Vitamin C	10
Trace element premix	10

Each tank will initially contain 400 fish. Fish will be reared at the NWFSC Montlake aquaculture facility. Temperatures will be allowed to fluctuate seasonally but will not exceed 13 C in the summer or go below 5 C in the winter. Artificial light will be used to duplicate the natural photoperiod. The ration fed to the replicates in each treatment will be adjusted slightly, if necessary, so that the mean fish weights between replicates remains similar throughout the experiment. Fish in each tank will be batch weighed and counted monthly to determine the mean fish weight in each tank. Twenty fish will be individually weighed and measured monthly to determine condition factor. Samples for proximate analysis and visual examination of testes will be collected every other month (10 fish/tank) when fish are not available from the immunocompetence or smoltification sampling. Smoltification status (gill K/Na-ATPase) will be determined in Oct-Dec 1998 and Mar-May and Oct-Dec 1999. Six fish from each tank will be examined biweekly. Immunocompetence will be assessed by Northwest Biological Research Center (USGS) personnel using methods described previously (see Objective 2.2). Sixty fish from each tank will be challenged at three points in the study (Table 2). In June the incidence of male maturation will also be assessed by measuring gonadotropin (GTH-I). All fish sacrificed for proximate analysis, immunocompetence or smoltification will be sexed and

testes will be weighed to determine maturation status.

The final sampling will take place in October of the fishes second year. At this time all fish will be sexed and the incidence of male maturation will be assessed by determining their gonadosomatic index.

Table 2. Sampling plan each year and month.

1997			1998					1999															
N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O
1	2	3																					
								I	I	I						I	I	I	I	I		I	
			P	P	P		P		P		P	P		P		P		P	P		P		P
										S	S	S				S	S	S					

- 1 Obtain eggs
- 2 First feeding
- 3 Distribute fish in to tank sand begin variable ration treatments
- 4 Final sampling and terminate experiment
- I= immunocompetence challenge
- P= proximate composition determination
- S= smoltification status determination

The effect of ration on body composition will be determined using allometric analysis of body weight vs total body fat. The effect of growth rate on smoltification and immunocompetence will be determined by regression analyses. The relationship between growth rate and the incidence of sexual maturation (%) will be examined using regression analyses after arcsin transformation of percentage data.

Objective 1.2. Determine effects of constant versus variable dietary protein and energy intake on growth, body conformation, natural spawning success, spawning behavior, and reproductive performance of chinook salmon.

Chinook salmon of the Coeur d'Alene Wolf Creek Lodge strain (1996 brood year) will be obtained as juveniles from the Idaho Department of Fish and Game Sandpoint Hatchery and transferred to the fish culture laboratory of the Aquaculture Research Institute, University of Idaho, Moscow, Idaho. The fish will be divided into four dietary treatment groups, with two replicate groups receiving each dietary treatment. Juvenile fish from two dietary treatments will be evaluated with respect to behavior, coloration, and fitness in connection with juvenile re-introduction strategies. Fish will be continued on these dietary treatment groups to adult maturation. These fish and fish from the other two dietary treatment groups will spawn in Fall 1999 or 2000, after exposure to the various dietary treatments for 2 to 3 years. The fish will be bulk-weighed, measured for length, and counted quarterly, and two fish from each replicate tank will be removed quarterly for determination of gonadosomatic index (GSI) and proximate composition. At maturation,

males and females will be identified, and fish from each dietary treatment group will be split into two groups, one to continue in tanks until spawning, and the other to be placed in an observation channel (artificial stream) for behavioral studies to assess their ability to compete with wild maturing chinook salmon and successfully spawn. The wild maturing chinook salmon will be the cohorts of the captively reared fish, captured as they return to the Idaho Department of Fish and Game Sandpoint Hatchery. Thus, this study will provide an opportunity to produce fish of differing size, conformation, and external coloration and test their reproductive success in an experimental setting similar to that in the wild. This portion of our proposed research will be conducted in concert with similar studies at the Manchester Marine Experimental and designed to complement on-going behavioral research at that facility. Fish that remain in tanks will be spawned in a factorial design so that eggs from each female are fertilized with milt from three males from each dietary treatment, and vice versa. A total of 20-25 males and females from each dietary treatment group will be spawned, and survival of offspring from each cross to swim-up will be enumerated. Specific objectives are described below.

Objective 1.2, Task 1. Determine effect of dietary treatment (dietary protein and energy intake levels) on growth, body conformation, proximate composition, and gonadal development .

The fish will be reared in indoor tanks supplied with temperature-controlled, recycled freshwater (9-11 C) at the fish rearing laboratory of the Aquaculture Research Institute, University of Idaho, Moscow, Idaho. Daylength will be controlled by timers to follow the natural photoperiod of the area. The fish will be weighed and counted at the start (October 1997) and monthly until spawning. Three dietary treatments will be employed: (1) constant diet, constant feeding level to provide 9 g protein per kg fish per day; (2) constant diet, variable feeding level to provide 6-12 g protein per kg fish per day with proportional dietary energy intake; and (3) variable diet adjusted to provide 6-12 g protein per kg fish per day at a constant dietary energy intake of 75 kcal/kg fish/day. The feed formulation for the experimental diets is shown in Table 3. The variable dietary treatments will have the lowest protein intake during late fall and early winter months, and the highest protein intake during spring and early summer months. The constant diet, constant feeding level (treatment one) will be fed at 2% of the biomass in each tank per day, adjusted weekly for predicted growth and adjusted monthly after the fish are weighed and counted. Feed consumption will be recorded daily to permit calculation of feed efficiency ratios. Ten to fifteen fish from each dietary treatment group (two per replicate tank) will be removed and sacrificed quarterly to determine gonadal development, calculate gonadosomatic indices (GSIs), and measure proximate composition. The proximate composition data will be used to calculate protein and energy retention at various stages of growth and maturation in the fish.

Objective 1.2, Task 2. Determine effect of dietary treatment on survival of adults to spawning and age at maturity of chinook salmon.

During fall 1999, fish from each tank will be examined for external signs of sexual maturation. These signs include development of secondary sex characteristics, such as hooked nose and reddening of skin in males, and actual signs of nearness to spawning, such as loose eggs in the body of females and running milt in males. The effects of diet on survival to spawning will be measured by simply counting the numbers

of maturing and non-maturing fish, and expressing the information on a percentage basis. Objective 1.2, Task 3. Determine effects of dietary treatments on the quality of gametes and percentage survival of fertilized eggs to the beginning of exogenous feeding

The eggs from each female fish will be divided into 6 lots of approximately 500 eggs each at spawning. Five of the egg lots will be combined with the milt from five different males from the same dietary treatment group. The sixth group will be preserved for later physical and chemical analyses. Fertilized egg lots will be incubated in Heath incubation trays divided into compartments so that each fertilized egg lot remains distinct. The egg lots will be subjected to an initial egg pick and to an egg pick at the eyed stage, after shocking. Numbers of viable eggs will be recorded at each stage and at hatching. The number of fish surviving to the first-feeding stage will be determined. The collected data will be tabulated to determine if differences exist between dietary treatment groups in offspring viability. The sixth group of eggs will be examined for physical attributes, including egg diameter, egg weight, and composition. The specific chemical analyses to be undertaken will be decided after the data from egg viability are analyzed. Fry will be combined by dietary treatment group for feeding and subsequent use in other studies.

Objective 1.2, Task 4. Determine effects of dietary treatments on external coloration, body conformation, and behavior of juveniles and adult fish.

One of the four dietary treatments used in this objective will involve a conventional salmon diet (diet one; Objective 3.1, Task 1) supplemented or not supplemented with an array of carotenoid-rich commercial products prepared from Pacific krill (*Euphausia pacifica*). These products are only recently available and early anecdotal evidence suggests that the inclusion of these products in salmonid feeds greatly influences external coloration. We will feed two diets containing several new krill-based products to juvenile chinook salmon for 6 months prior to the fish reaching appropriate sizes for re-introduction/release as part of restoration strategies involving juvenile salmon. The fish will be subjected to a computer based image analysis assessment of coloration, fin condition, and body conformation, with cohorts fed an unenhanced diet being used as controls. These fish will then be subject to behavioral testing to assess various behaviors associated with post-release success, e.g. foraging behavior, predator avoidance, aggressive behavior, in a study designed by and conducted in collaboration with Dr. Barry Berejikian, NMFS.

Table 3. Composition of experimental diets for chinook salmon captive broodstock studies (Objective 1.2).

INGREDIENT	DIETS 1 & 2 (%)	DIET 3 (%)
Anchovy meal	54.00	54.00
Blood meal	4.00	4.00
Wheat gluten	4.00	4.00
Wheat midds	16.00	12.50 to 19.50
Fish oil	13.00	6.00 to 13.00
Vitamin C	0.30	0.30

Choline	0.50	0.50
TM salt	0.10	0.10
Vitamin premix	3.00	3.00
Krill hydrolysate	5.00	5.00
Astaxanthin	0.10	0.10

Calculated proximate composition of diets 1 & 2 = 48% crude protein (45% digestible protein) and 17.5% crude lipid, expressed on an "as-is" basis (7% moisture). Diet 3 varies to keep energy intake constant while varying daily protein intake (g protein/kg fish/day).

Element 2- Fish Health

Objective 2. 1 Develop antibacterial therapy to reduce mortality due to BKD in chinook and sockeye salmon.

Approximately 1,000 fall chinook salmon smolts will be transferred to tanks supplied with pathogen-free seawater at the Manchester Marine Experimental Station. After fish are acclimated to seawater, they will be PIT tagged and randomly distributed among nine tanks. Feed prepared with azithromycin, erythromycin, and a non-medicated control will be fed to 3 replicates of 100 chinook for each of the 3 treatments for 14 days. One week after medication, all fish from all treatments will be pooled into one tank of freshwater and challenged with a 24h exposure to *Renibacterium salmoninarum*. After the 24h challenge, all fish will be returned to pathogen-free seawater. The chinook will be monitored for survival and mortality from BKD. All mortalities during this period will be necropsied for gross signs of clinical BKD, and kidney tissues will be examined by fluorescence antibody analysis for *R. salmoninarum* levels. Clinical and pharmacological studies in animals other than salmon and results of our preliminary trials suggest that azithromycin may be even more effective as a prophylactic therapy for BKD in chinook salmon.

Objective 2.2 Further develop techniques for using live-food mediated drug delivery.

Two new areas are proposed for FY99 which will follow-up on our success with using adult Artemia as a delivery system for first feeding salmonids. Studies will investigate two additional antibiotics and the effects of processing on bioencapsulated Artemia. The antibiotic compounds, azithromycin and Romet -30, used alone or in combination with erythromycin, may have potential to treat BKD and other fish diseases in first feeding salmonids when bioencapsulated into adult Artemia. Methods analogous to those used with erythromycin to produce Artemia with high levels of these compounds will be investigated. Once workable methods have been developed, bioencapsulated azithromycin, Romet-30 and/or combinations will be fed to first feeding salmonids to determine if this method can be used to deliver these compounds to fish tissues.

The benefit of using a bioencapsulated drug delivery system is that the organisms are readily consumed by first feeding salmonids. The drawback is that given current methods developed by this task, Artemia holding and the bioencapsulation process needs

to be done on-site to be effective. This requires additional expertise and infrastructure at hatcheries. We will address these drawbacks by investigating the palatability of frozen and freeze-dried Artemia-bioencapsulated erythromycin to first feeding salmon. Such processing may greatly facilitate using this delivery system, as the bioencapsulation process could be done centrally and external to the fish rearing, with the processed product (frozen or freeze-dried bioencapsulated antibiotic) shipped to the hatchery and fed in a more-or-less conventional manner.

Objective 2.3. Effects of growth and nutrition on immune function in spring chinook salmon.

During 1998 and 1999, the immunological competence of fish reared on different rations (Objective 1.1) will be assessed once by measuring selected factors related to specific and nonspecific host defense mechanisms. The results from these measurements will provide relative estimates of the general immune status of fish from each of the groups, and if the rate of growth may be effecting their disease resistance. Fish for this experiment will be reared as described in Objective 1.1.

We will measure specific immune response to the p57 protein of *Renibacterium salmoninarum*. using a recombinant p57 protein produced in an *E. coli* expression system and used to vaccinate fish from each feed group. At specific intervals following vaccination, fish from each vaccinate group will be bled and their specific humoral response to the p57 protein will be measured with the IgM-ELISA. The ability of fish from each feed group to produce a humoral response to the recombinant p57 protein will be compared during summer 1998 (July-September), and early spring (March-May) and early summer (May -July) 1999. For a vaccination, 50 fish will be removed from each of the two replicate tanks in a temperature group. Each 50-fish subgroup from each temperature group will be injected intraperitoneally with 200 µL per fish of a single concentration of the recombinant p57 protein in Freund's incomplete adjuvant and placed in a separate tank. Following the vaccinations, each subgroup will be maintained under the same rearing conditions as the experimental feed groups. The levels of specific serum immunoglobulin to the p57 protein will be measured in unvaccinated fish, and in fish sacrificed at regular intervals after vaccination.

The nonspecific immune functions of fish from each group will be measured during September 1998 and June-July 1999; for each evaluation, subgroups of 30 fish will be randomly chosen from each feed group. Briefly, a total of 15 fish will be tested from each replicate tank of a given feed group; five fish will be removed from each tank by repeated dip-netting every other day for 5 days. Fish will be anesthetized with tricaine methanesulfonate (MS-222), then the weight and fork length will be determined to calculate the condition factor. The condition factor will be calculated according to the method described by Piper et al. (1982). Condition factor values will be corrected for the use of metric measurements by the formula $C = 36.14K$, where C and K are condition factors based on English and metric units, respectively.

A blood sample will be taken by caudal vein puncture for the hematocrit, leukocrit, and plasma protein determinations. Duplicate smears of whole blood will be made for each fish, then the remainder of each sample was allowed to clot overnight at 4 C and centrifuged at 5,000 g for 20 min. The serum will be divided into two vials and stored at -80 C.

On each sample date, a five-fish pool of anterior kidney tissue will be prepared from each subgroup. The anterior kidney will be aseptically removed and a small portion from the center of the sample used to make several kidney imprints on each of two glass microscope slides. The tissue imprints will be air-dried, then fixed in absolute methanol for 5 minutes for differential cell counts. The other slide will be stored at 4 C for the myeloperoxidase assay. Leukocytes will be purified from the remaining tissue in each pool by gradient centrifugation for use in the phagocytosis and NBT assays.

Objective 3.1. Determine the timing of olfactory imprinting by juvenile sockeye. Experiments to determine the critical period(s) for olfactory imprinting by sockeye salmon were initiated in fall 1994. After emergence from their natal gravel, sockeye salmon immediately migrate to a lake, where they remain for 1 or 2 years before smolting and migrating to sea. To determine the relative importance of odorant exposure during these key developmental periods, Lake Wenatchee sockeye salmon were exposed to specific odorants as alevins (the period just prior to emergence from the natal gravel) (January-February 1994) or as smolts (April-May 1996). Odorant-exposed and control fish were marked and are currently being reared communally at the NWFSC. In 1996 nonmaturing fish were tested for olfactory sensitivity to exposure odorants; however, responses to odorants were highly variable. It is not known whether fish must undergo sexual maturity to regain odorant memory; therefore, during September 1997, maturing male fish will be tested for their olfactory sensitivity and behavioral responses to exposure odorants. Olfactory sensitivity to exposure odorants will be performed using electroolfactograms in collaboration with Dr. Peter Sorenson, University of Minnesota. During 1998 analysis of samples and data, and generation of reports will continue.

Objective 3.2. Determine the timing of olfactory imprinting by juvenile chinook salmon. Experiments to determine the critical period(s) for olfactory imprinting by spring chinook salmon were initiated in fall 1996. Typically, spring chinook salmon remain in fresh water for 1 year, smolt and migrate to the ocean during their second spring. However, there appears to be considerable plasticity in the seasonal timing of smolting in spring chinook salmon. For example, high growth rates during the first year can lead to smoltification in the fall. To determine whether spring chinook salmon imprint during the autumn and/or spring prior to migration to the sea, we are conducting the following experiment. Quilcene spring chinook salmon will be reared at the NWFSC. Fish that were reared on high growth rates (high ration) smolted in fall 1996 and spring 1997. Subgroups of fish from the high ration group were exposed to specific odorants during these periods. The critical periods for olfactory imprinting will be determined in fall 1997 and 1998 by measuring the olfactory sensitivity and behavioral responses of maturing male fish to exposure odorants. Olfactory sensitivity to exposure odorants will be performed using electroolfactograms in collaboration with Dr. Peter Sorenson, University of Minnesota. During 1998 analysis of samples and data and generation of reports will continue.

Element 4- Reproductive Ecology of Captively-Reared Spring Chinook Salmon

The proposed experiments will be conducted in an experimental stream channel that includes an underwater observation chamber that has been constructed at the NMFS Manchester Marine Experimental Station. The channel measures approximately 6 m

wide by 40 m long, has a 3% grade, and can be divided to create as many as sixteen 3-m wide by 5-m long isolated sections for replication purposes. Well water (10 C) will be supplied at 120 L/min, and the water will be recirculated with three submersible pumps, each delivering approximately 1800 L/min. Clean gravel (3- to 6-cm diameter) will be added to the channel to a depth of approximately 50 cm, and water depth will be maintained at 30 to 40 cm.

An underwater viewing chamber, located at the upstream end of the channel, has been fitted with a glass window (4 m long by 0.75 m high). A 5-m long by 3-m wide area of the channel in front of the window can be sectioned off to contain fish for experimental manipulation. Effluent from the stream channel will pass through an ozone depuration system to remove all pathogens from the water prior to discharge.

Objective 4.1. Obtain adult spring chinook salmon for mating experiments.

The following experiments will be conducted with adult captively reared chinook salmon collected as eyed eggs from spring chinook salmon nests in the Dungeness River (near Sequim, WA). The fish have been cultured in freshwater by the Washington Department of Fish and Wildlife (WDFW) at the Hurd Creek Hatchery, Sequim, WA. Fish from separate, individual nests (i.e., families) have been uniquely marked so the members of each family can be identified. Currently, age classes 1 through 5 are being cultured at the hatchery. The number of mature males and females from each of the various families will not be known until there are visible signs of maturity and sexual dimorphism. The sex and family composition of maturing fish will be monitored through the summer by the WDFW personnel. Hence, the number of males and females available from each family will be known in August 1997, at which time the details of the experimental design for this study will be finalized.

At the time of collection, the fish will be anesthetized, weighed, and morphometric characters likely to affect reproductive performance (e.g., fork length, kype length, girth, body depth, caudal peduncle height) will be measured for all fish. An individually numbered, 2.5-cm Peterson disc tag will be attached to each fish slightly posterior and ventral to the dorsal fin. All fish will be loaded into an oxygenated transport tank and trucked (approximately 1.5 h) to the Manchester Marine Experimental Station where they will be held temporarily in 2.5 m diameter tanks.

Objective 4.2., Task 1. Investigate potential kin-based assortative mating patterns.

We propose to conduct an assortative mating experiment with adult captively reared Dungeness River spring chinook salmon. We anticipate obtaining eight males and eight females from each of two full-sib families to test the general null hypothesis that mating between the two families is random. Females will be chosen from each family such that the mean body weight and range in body weight do not differ between the two families. Males will also be size matched between the families to eliminate potential size-confounding effects on male competition or female mate choice based on male size. Once the sexually mature fish from both families have been placed into the spawning channel, aggressive and courtship behaviors of all fish associated individual sexually active females will be recorded. Focal sampling observations on these spawning aggregations will begin as early in the nest construction process as possible, and will continue until approximately 30 min after spawning. Observed behaviors will be recorded on audio tapes with an emphasis towards determining the number and locations

of nests constructed by each female, mating combinations, male dominance hierarchies, the presence of satellite males during spawning, and female intersexual aggression (an indicator of female mate choice). The number of actual matings occurring within and between families will be determined and analyzed to assess the presence or absence of kin-based assortative mating.

Objective 4.2, Task 2. Apply DNA microsatellite analysis to determine reproductive success.

Deoxyribonucleic acid (DNA) microsatellite techniques (Park et al. 1994, Whitmore 1994, Herbinger et al. 1995) have been successfully applied to directly determine the contribution of individual spawners to the F1 fry population in our recent reproductive success studies with coho salmon (B. Berejikian and L. Park, NMFS, unpublished data). We propose to attempt to use these techniques to determine the reproductive success of naturally spawning fish from the assortative mating experiment described above (Objective 4.2., Task 1). A small portion of the caudal fin of each adult will be removed and preserved in ethanol. After all chinook salmon in the channel have spawned, the channel will be covered with netting to exclude avian and terrestrial predators. Once all the fry have emerged from the gravel, the entire fry population will be removed by a combination of seining and electroshocking, and the fry will be preserved in 100% ethanol for analysis and assignment to the single pair mating that produced them using DNA microsatellite analyses. The results of the DNA analyses will be compared with the observed mating patterns to clarify the importance of kin relationships in the overall mating patterns of captively reared spring chinook salmon. It may, however, be difficult to discriminate among fry produced from the closely related adults in the above experiment depending upon the degree of variation between the two families, which will not be known until several microsatellite loci from the two families have been screened for variation.

Objective 4.3. Describe spring chinook salmon reproductive behavioral ecology. Although the published literature contains some gross information on the size and distribution of chinook salmon redds (Burner 1951, Chapman et al. 1986), and residence time on the spawning grounds (Nielson and Geen 1981), virtually no information exists on basic chinook salmon reproductive characteristics, such as male and female reproductive lifespan, egg deposition through time and space, inter- and intra-sexual aggression, courtship behavior, or the effects of body size and morphometric characteristics on competitive ability and reproductive success.

Audio taped recordings of chinook salmon spawning behavior in the stream channel and video taped observations from the underwater viewing chamber taken during the assortative mating experiment (Objective 4.2., Task 1) will provide previously unreported descriptions of aggressive and courtship behaviors, nest and redd construction, nest guarding activity and temporal factors such as time to onset of spawning, duration of sexual activity (i.e., first to last spawning event), post-spawning lifespan, and nest-guarding duration.

If the appropriate numbers of fish are not available to conduct a valid assortative mating experiment (i.e., there are not enough males and females from each of two separate

families; see Objective 4.1), we will shift the emphasis from the kin-based assortative mating study (Objective 4.2, Task 1) to expand our investigation of chinook salmon reproductive ecology. Thus, a sample of 16 males and 16 females would be selected from the captive broodstock population to represent, as closely as possible, the size (standard length) range of the population. Fish handling, transportation, morphological measurements and tagging protocols would be the same as described in Objective 4.2. The sexually mature fish will then be simultaneously placed into the undivided stream channel. In addition to the observations outlined above for this objective, we will evaluate the effects of body size and morphometric variables (independent of body size) on various surrogate measures of reproductive success, and direct measures of reproductive success using DNA microsatellite analyses.

The proportion of eggs retained by individual females indicates their ability to compete for nest sites, construct nests and deposit eggs, and may be an important determinant of female reproductive success. We will dissect and enumerate the number of eggs remaining in females that have spawned and died. The ratio of unspawned eggs to total fecundity (estimated from fecundity to body size relationships obtained from artificially spawned females at the Hurd Creek Hatchery) will be estimated to examine individual variability in the proportion of eggs deposited. The number of nests constructed by individual females will also be monitored as another indicator of female competitive ability. The ability of males to participate in spawning events as the courting (dominant) male or as a satellite (subdominant) male strongly determines success in fertilizing eggs, and consequently, reproductive success. Thus, for females, we will examine statistical relationships of both percent egg deposition and number of nests constructed to body size and other morphological variables, and for males we will examine statistical relationships between male body size and morphology and the frequency with which individual males spawned as either a courting or satellite male. DNA microsatellite techniques will also be applied to these investigations to directly determine individual adult-to-fry reproductive success for both sexes, and the results will be compared with behavioral analyses.

The work proposed will provide an initial test of the suitability of the newly constructed stream channel for chinook salmon spawning, provide information for potential modifications, and help determine parameters for its use in future spawning studies. Based on our previous studies comparing captively reared and wild coho salmon, we anticipate that body coloration and morphology will play important roles in the reproductive interactions among captively reared chinook salmon and between captively reared and wild chinook salmon. As research in other disciplines within the Captive Broodstock Research Project continue to focus on improving husbandry practices, we will have the opportunity to evaluate the effects of various fish quality parameters (e.g., coloration and morphology) on reproductive success of captively reared salmon. In succeeding years we foresee continuing research in these areas with chinook salmon to provide critical information for captive rearing and adult release programs aimed at the recovery of endangered salmon populations in the Columbia River Basin.

Element 5 - Research on Quantitative Genetic Consequences of Captive Broodstock

Programs for Pacific Salmon Populations

Objective 5.1. Evaluate Inbreeding and Inbreeding Depression

Fall 1997 marked the third anniversary of the start of this experiment on the consequences of inbreeding in chinook salmon. The objective of the experiment is to determine the effects of controlled inbreeding on survival, development, age structure, and other aspects of the life history of chinook salmon (see below). In April 1995, 257,093 subyearling 1994-brood fall (ocean-type) chinook salmon were released from Grovers Creek Hatchery into northwestern Puget Sound, Washington. An estimated 3,000-4,000 3-year-old adults representing 96 full-sib and 30 half-sib families returned to the release site in September and October 1997. These adults include the first females to return from the initial releases. In addition, approximately 1,700 fish representing these families have been cultured in marine net-pens at the NMFS Manchester Marine Experimental Station since their transfer there in June 1995. The Manchester station is approximately 80 km southeast of Grovers Creek Hatchery in southwestern Puget Sound. These fish were individually tagged in June 1995 with passive integrated transponder (PIT) tags and averaged approximately 1 kg in weight in June 1997 and are growing rapidly. .

We plan to establish three first-generation inbred lines from these fish: a control line composed of single-pair matings made at random, an inbred line formed from matings of half-siblings, and an inbred line formed from matings of full-siblings. Matings among half-siblings are expected to yield an inbreeding coefficient of 12.5% in the first generation of inbreeding; matings among full-siblings are expected to yield an inbreeding coefficient of 25% (Hard 1995). We plan to construct corresponding matings with four- and five-year olds in 1998 and 1999, respectively. If so, this design will require that the evaluation of inbreeding treat age as a fixed factor in the analysis.

Due to budgetary constraints anticipated for fiscal year 1998, we eliminated the anadromous element of the experiment. This will have two major consequences: 1) it will eliminate the possibility of contrasting oceanic and captive environmental effects (and any genotype x environment interactions) on response to inbreeding, and 2) it dramatically increases the possibility that catastrophic mortality in netpen-reared fish will compromise our ability to evaluate inbreeding consequences. It does, however, retain the potential to evaluate inbreeding depression in captively reared fish, which is the primary goal of our work.

Under this proposal, we intend to use the currently captive 1994-brood adults (approximately 1,700 in June 1997) to establish the three experimental lines. (We either will not collect gametes from 1994-brood adults returning to Grovers Creek Hatchery or will likely destroy any offspring at the hatching stage, after we can evaluate any effects of a single generation of inbreeding on embryonic survival and development.) As in the parental generation, all of the offspring in each of these lines will be marked, preferably with PIT tags. These fish will be transferred to marine net-pens at the Manchester Marine Experimental Station for culture to adulthood. If sufficient fish and disease treatment facilities are available, a subset of these fish (approximately 15-20 fish per family from each of the three experimental lines) will be transferred to the National Biological Survey

laboratory in Seattle to conduct an experiment to determine the genetic basis of resistance to bacterial kidney disease.

We have collected and will analyze the following data for adults maturing in 1997: survival, body length and weight, morphometry (via digitized photographs), and for females, egg size and fecundity. We will continue to collect size and growth information on captively reared 1994-brood individuals at approximately half-yearly intervals, and will begin collecting this information on 1997-brood inbred and control offspring in summer 1998. If sufficient fish are available, we plan to analyze data obtained on morphometry of approximately 20 1997-brood offspring sampled from each full-sib family in each experimental group to determine first-generation effects of inbreeding, if any, on morphology. We will continue to remove and decode coded-wire tags from fish sampled as part of the vibriosis challenge experiment conducted in 1995. Collectively, these data will provide a comparison of first-generation inbreeding effects on early life-history traits and, through examination of the control population, an assessment of relative environmental influences in consecutive generations on these traits.

Objective 5.2. Evaluate Population Differentiation and Outbreeding Depression

An outbreeding depression experiment was initiated in October 1997 with southeastern Alaska coho salmon. In 1996 we contracted the Juneau Center, School of Fisheries and Ocean Sciences, University of Alaska Fairbanks to conduct the project with NMFS funds.

This experiment will be conducted with three populations. It was initiated in fiscal 1997 entirely with NMFS funds.

The experiment we propose involves three southeastern Alaska populations of coho salmon. Three populations are the minimum necessary to examine outbreeding depression in relation to degree of genetic divergence. We have proposed the experiment with coho salmon because 1) the almost complete lack of variable age structure in southern populations of this species considerably simplifies the mating design; 2) coho salmon have higher rates of smolt-to-adult survival than do most other species of Pacific salmon in the region; 3) an experiment spanning two salmon generations will require fewer years to complete than one involving most other species of Pacific salmon (e.g., an experiment with coho salmon would require 6 years, or about 4 years less than a comparable experiment involving chinook salmon); and 4) conservation and artificial propagation of coho salmon under the Endangered Species Act has recently become a prominent scientific and political issue. The coho salmon populations were chosen based on their perceived or measured genetic relationship as well as the ease with which they can be sampled in the wild. The populations are a hatchery and wild population from the Juneau, Alaska area, a hatchery population from the Sitka, Alaska area, and possibly a wild population from the Yakutat, Alaska area.

The basic plan is to create two generations of crosses between the three populations. Because we are also interested in determining the genetic mechanisms underlying observed outbreeding depression, if possible we will establish reciprocal first- and second-generation cross derivatives, an approach that maximizes the ability to quantify nonadditive genetic components of phenotypic divergence. The experiment involves comparing observed values of mean and variance in phenotypic characters for these

derivatives to the values predicted by different models of gene expression. Tests of the adequacy of this and other models to explain observed phenotypic patterns are made with a modified chi-square test (Hard et al. 1992; Hard et al. 1993; Lynch and Walsh In press). Theory predicts that outbreeding depression is likely if the evolutionary differentiation of populations has resulted from interactions among loci, and this design should permit evaluation of additive and additive-dominance models that exclude these interactions. In addition, the tests of variances may permit estimation of the number of leading factors that underlie differentiation in the trait, if an additive genetic model is sufficient to explain the phenotypic pattern of variances (Lande 1981; Hard et al. 1992).

Different characters may show different responses to outbreeding depression, so it is important to evaluate these characters independently. Characters of particular interest in this experiment are those that affect performance in the hatchery and in the wild, and may be correlates or components of fitness. Many of these traits are apt to be under quantitative genetic control, and they include: stage-specific survival rates, gamete viability, development rate, body morphometry and meristic variation, fecundity, egg size, age at maturity (precocity), homing, run and spawn timing, disease resistance, and possibly some behaviors.

All offspring will be marked with cross-specific coded-wire tags within 6 months of first feeding. All offspring will be released at a size and time that is thought to maximize their marine survival, and all offspring will be cultured under similar conditions. The fish in all lines will be cultured simultaneously by recreating the first-generation groups to avoid the confounding effects of environmental variation between generations on phenotypic expression. That is, the parental and first-generation hybrid offspring will be regenerated in the second generation.

Rearing all fish at a central hatchery/release site considerably simplifies recovery of adults and the setting up of second-generation offspring. This approach precludes any comparison among site environments and entails an assumption that no genotype-environment interaction is affecting phenotypic variation, but the experiment's complexity will not permit analysis of this issue. Recovery of adults throughout the return will require decoding of coded-wire tags before spawning to distribute gametes appropriately among the lines to be created in the second generation. If feasible, all first-generation fish should be marked with a visible mark or PIT tag, as this would simplify considerably the spawning procedures used to establish the second-generation groups. The analysis of phenotypic variation within and among the groups is based on well-established quantitative-genetic methods (Cavalli 1952, Mather and Jinks 1982). This analysis will provide a means of determining whether outbreeding depression can be expected among relatively closely related populations of Pacific salmon, such as wild fish and hatchery fish that are derived from them. The results of the analyses of phenotypic means and variances will help to identify the genetic mechanisms for the divergence of these populations and any outbreeding depression observed upon their crossbreeding. Results will be interpreted generally as follows. Reduced means and increased variances in fitness correlates in crossbred offspring relative to parental values is compelling evidence for outbreeding depression. Epistatic genetic variance correlated with these

results (reductions in mean, increases in variance) implies substantial adaptive divergence of parental genomes and suggests that the outbreeding depression results from genomic incompatibilities. The relationship between these results and independent measures of population divergence will shed light on the geographic patterns of adaptive population structure in coho salmon.

Outbreeding depression in Pacific salmon has been identified as a primary research need that has immediate management relevance (NMFS 1995a,b). Stock transfers and straying of hatchery fish have raised concerns about effects of gene flow on population structure, but previous attempts to substantiate these concerns have addressed either only the loss of population structure as measured by molecular markers or have noted reduced survival or performance in first-generation hybrids between populations such as natural and hatchery crosses. Neither of these approaches has determined where the loss of fitness due to outbreeding depression is realized, shown how this depression is related to the degree of population differentiation, or identified the genetic mechanism responsible for the observed depression. The research described here is designed to tackle all three of these issues.

f. Facilities and equipment.

Office space, hatchery, and laboratory facilities at the following locations are being utilized to conduct the proposed and ongoing research: Northwest Fisheries Science Center (NWFSC), Seattle, WA, NMFS Manchester Marine Field Station, Manchester, WA; USGS, Northwest Biological Science Center, Seattle, WA; School of Fisheries, University of Washington; University of Idaho Hagerman Fish Culture Experiment Station. Laboratories have all necessary equipment for large scale analysis of samples for endocrine, genetic, nutrition and fish pathology studies. The fish holding facilities are adequate for all proposed studies. Construction of unique facilities for behavioral observation of adult salmon spawning and juvenile fish have been completed at the Manchester Marine Field Station.

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Section 8. Relationships to other projects

This project has a close relationship with ongoing captive broodstock programs in Idaho, Oregon and Washington (Projects 9107200, 9700100, 9204000, 9606700, 9604400). Personnel from the captive broodstock research program communicate results to those involved in rearing captive broodstock, and in turn we focus our research on key problems encountered by those rearing captive broodstock.

Section 9. Key personnel

Penny Swanson, Ph.D., Project Investigator, Fish Reproductive Physiology

Duties: Project administration, oversee and conduct research in reproductive physiology of salmonid fish especially related to growth and reproduction.

Expertise: reproductive physiology of fish, particularly endocrine control of reproduction, pituitary hormone biochemistry

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Education

University of Washington Seattle, WA	B.A., Summa Cum Laude	1978	Zoology
University of Wisconsin and Madison, WI	M.S.	1980	Endocrinology Reproductive Physiology
University of Washington Seattle, WA	Ph.D.	1986	Zoology

Employment

1992-present	Physiologist, National Marine Fisheries Service, Seattle. Aff. Asst. Professor, School of Fisheries, Univ. Washington, Seattle		
1990-1992	Physiologist, (50 %) National Marine Fisheries Service, Seattle. Fish Biologist IV (50%), School of Fisheries, University of Washington, Seattle.		
1987-1990	Post-docotoral Research Associate, School of Fisheries, University of Washington, Seattle. Supervisor: Dr. W. W. Dickhoff		
1986-1987	Research Fellow, Japanese Society for Promotion of Science, School of Fisheries Sciences, Kitasato University, Sanriku, Japan. Supervisor: Dr. H. Kawauchi		
1986	Teaching Assistant, Dept. of Zoology, University of Washington, Seattle. Course: Reproductive Endocrinology of Vertebrates		
1985-1986	Research Assistant, Dept. of Zoology, University of Washington, Seattle. Advisor: Dr. A. Gorbman		
1984-1985	NIH Trainee, Molecular and Cellular Biology Training Program, University of Washington, Seattle. Advisor: Dr. A. Gorbman		
1982-1984	Teaching Assistant, Dept. of Zoology, University of Washington, Seattle.		

Courses: Comparative Anatomy; Comparative Physiology
1980-1982 Research Specialist, Aquaculture Research Laboratory, University of Wisconsin, Madison. Supervisor: Dr. T. Kayes
1978-1980 NIH Trainee, Endocrinology and Reproductive Physiology Program, University of Wisconsin, Madison. Advisors: Dr. R. Bremel and Dr. J. Gorski

Swanson, P., Suzuki, K., Kawauchi, H. and Dickhoff, W. W. (1991). Isolation and characterization of coho salmon gonadotropins: GTH I and GTH II. *Biol. Reprod.* 44:29-38.

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EDUCATION

1979, B.S. Biology, Oregon State University
1984, M.S. Fisheries, University of Alaska
1991, Ph.D. Ecology and Evolutionary Biology, University of Oregon

EMPLOYMENT HISTORY

1992-present Fishery Research Biologist, U.S. Department of Commerce,
NMFS, Northwest Fisheries Science Center, Seattle, Washington
1986-91 Graduate Fellow, Department of Biology, University of Oregon, Eugene
1982-86 Fishery Research Biologist, U.S. Department of Commerce,
NMFS, Auke Bay Laboratory
1977-82 Fishery Technician, U.S. Department of Commerce, NMFS, Auke
Bay Laboratory
1975-76 Research Assistant, Institute of Marine Science, University of
Alaska, Fairbanks, Alaska, and CEPEX (Controlled Ecosystem
Pollution EXperiment) Project, Patricia Bay Lab., Sidney, B.C., Canada

REPRESENTATIVE PUBLICATIONS

Berejikian, B. A., E. P. Tezak, S. L. Schroder, C. M. Knudsen, and J. J. Hard. In press. Reproductive behavioral interactions between wild and captively reared coho salmon (*Oncorhynchus kisutch*). *ICES Journal of Marine Science*.
Hard, J. J., R. G. Kope, and W. S. Grant. In press. Review of the status of pink salmon from Washington, Oregon, and California. In D. MacDonald, C. Steward, and J. Williams (editors), *Sustainable Fisheries Management*, Ann Arbor Press, Ann Arbor, MI.
Hard, J. J. 1995. A quantitative genetic perspective on the conservation of intraspecific diversity. In J. L. Nielsen (editor), *Evolution and the aquatic ecosystem: defining unique units in population conservation*, p. 304-326. *American Fisheries Society Symposium 17*, Bethesda, Maryland.
Hard, J. J. 1995. Genetic monitoring of life-history characters in salmon supplementation: problems and opportunities. In R. G. Piper and H. L. Schramm (editors), *Uses and effects of cultured fishes in aquatic ecosystems*, p. 212-225. *American Fisheries Society Symposium 15*, Bethesda, Maryland.
Hard, J. J., R. P. Jones, Jr., M. R. Delarm, and R. S. Waples. 1992. Pacific salmon and artificial propagation under the Endangered Species Act. U.S. Department of Commerce, NOAA Technical Memorandum NMFS-NWFSC-2, 56 p.

Lee Harrell, DVM, Project Investigator, Fish Pathology

Address:

National Marine Fisheries Service

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Education:

B.S. (Animal Husbandry). College of Agriculture, University of Florida, Gainesville, FL. 1960.

D.V.M. (Veterinary Medicine). School of Veterinary Medicine, Auburn University, Auburn, AL. 1964.

M.S. (Fisheries Biology). School of Fisheries, University of Washington, Seattle, WA. 1973.

Expertise:

Practice of fish medicine and parasitology. Prevention and therapy of infectious and parasitic diseases of marine and freshwater fishes.

Publications:

Harrell, L. W., and T. L. Deardorff. 1990. Human nanophyctiasis: transmission by handling naturally infected coho salmon (*Oncorhynchus kisutch*). *J. Inf. Dis.* 161(1):146-148.

Flagg, T. A. and L. W. Harrell. 1990. Use of water-to-water transfers to maximize survival of salmonids stocked directly into seawater. *Prog. Fish Cult.* 52:127-129.

Harrell, L. W. and T. M. Scott. 1985. *Kudoa thyrsitis* (Gilchrist) (Myxosporea, Multivalvulidae) in Atlantic salmon *Salmo salar*. *J. Fish Disease* 8:324-332.

L. W. Harrell, R. A. Elston, T. M. Scott, and M. T. Wilkinson. 1986. A significant new systemic disease of net-pen reared chinook salmon *Oncorhynchus tshawytscha* brood stock. *Aquaculture* 55:249-262.

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.

Karl Shearer, Ph.D., Project Investigator, Fish Nutrition

Education:

University of Washington, 1971, B. S., Fisheries Science

University of Washington, 1986, M. S., Fisheries Science

University of Bergen, 1991, Ph. D., Fisheries Biology

Employment:

1980-present Research Biologist, NMFS, Seattle, WA.

1978-1980 Research Biologist, DØmsea Farms, Bremerton, WA.

1978 Fisheries Biologist III, WA State Game Dept., Olympia, WA.

1973-1978 Biologist-In-Charge, Inland Fish. Res. Stn., NSW, Australia.

1972-1973 Research Fisheries Biologist, NSW, Australia.

1971 Biologist, Washington Water Power, Spokane, WA.

Research Interests:

Utilization of plant proteins in salmonid diets, pigmentation of salmonids, appetite regulation, bioenergetics and salmonid life cycle, dietary nutrient requirements.

Selected Publications:

Shearer, K. D., Åsgård, T., Andorsdóttir, G. and Aas, G. H. (1993). Whole body proximate and elemental composition of Atlantic salmon (*Salmo salar*) during the life cycle. *J. Fish. Biol.*, 44: 785-797.

Shearer, K. D. (1994) Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. *Aquaculture*. 119: 63-88.

Shearer, K. D. (1995) The use of factorial modeling to determine the dietary requirements for essential elements in fish. *Aquaculture*. 133: 57-72.

Shearer, K. D., Silverstein, J. T. and Plisetskaya, E. M. (1997) The role of adiposity in food intake control of juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Comp. Biochem. Physiol.*, 118A: 1209-1215.

Shearer, K. D., Silverstein, J. T. and Dickhoff, W. W. (1997) Manipulation of growth and adiposity of juvenile chinook salmon. *Aquaculture*. 157: 311-323.

Barry Berejikian, Ph.D, Project Investigator- Fish Behavior
National Marine Fisheries Service

Education

California Polytechnic State University, San Luis Obispo, Bachelor of Science, 1990
University of Washington, Master of Science, 1992
University of Washington, Ph.D., 1995

Expertise

Dr. Berejikian has been conducting scientific research on the effects of artificial culture on the behavior of salmonids since 1992. Beginning in 1995, Dr. Berejikian has lead a multi-year, multi-disciplinary, cooperative research effort between NMFS and the Washington Department of Fish and Wildlife to evaluate the relative reproductive success of captively-reared and wild salmon. He has annually conducted intense monitoring of chinook and coho salmon reproductive ecology to complete reproductive behavior experiments. This work has already produced one refereed journal article, with two other manuscripts currently under internal review. Dr. Berejikian has also worked closely with NMFS geneticists to develop and apply new DNA "fingerprinting techniques" to directly assess the reproductive success of spawning captively reared and wild salmon. The information generated from these studies has, and will continue to help guide development of captive broodstock strategies for use in recovery of salmon in the Snake River and Columbia River basins.

Publications

Berejikian, B. A. 1995. The effects of hatchery and wild ancestry and experience on the relative ability of steelhead trout fry (*Oncorhynchus mykiss*) to avoid a benthic predator. *Can. J. Fish. Aquat. Sci.* 52:2476-2482.

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Berejikian, B. A., E. P. Tezak, S. L. Schroder, C. M. Knudsen, and J. J. Hard. In press. Reproductive behavioral interactions between spawning wild and captively reared coho salmon (*Oncorhynchus kisutch*). *ICES Journal of Marine Science*.

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Education:

BA Biology. University of Colorado at Boulder, 1980.

MS Animal Science. University of California at Davis, 1987.

MS International Agricultural Development. Univ. California at Davis, 1987.

Ph D Fisheries. University of Washington, Seattle, 1995.

Employment:

Research Fishery Biologist, National Marine Fisheries Service, Resource Enhancement and Utilization Technology Division, Northwest Fisheries Science Center, Seattle, WA 98112, March 1994- Present

Visiting Scientist, Institute of Marine Research, Austevoll Aquaculture Research Station, N-5392 Storebø, Norway, March 1995 - July 1995.

Teaching Assistant, School of Fisheries, University of Washington, Seattle, WA 98195, March 1994 - June 1994.

Research Associate, School of Fisheries, University of Washington, Seattle, WA 98195, November 1989 - March 1994.

Scientist/Consultant, Aquaresearch Ltd., North Hatley, Québec, Canada, June 1988 - July 1989.

Chief Scientist for Haiti Project, Caribbean Marine Research Center, Cap Haitien, Haiti, March 1987 - May 1988.

Aquaculture Projects Coordinator, University of California at Davis, Sustainable Agriculture Program, April 1985 - March 1987.

Research Assistant, University of California at Davis, Department of Animal Science, January 1985 - March 1987.

Peace Corps Volunteer, Talibon District Fisheries Office, Talibon, Bohol, Philippines, June 1980 - July 1982.

Expertise: Developmental biology, nutrition and engineering related to fish. Primary expertise is in the development of the digestive system in fish; larval and broodstock nutrition; and live and microparticulate feeds for first-feeding fish.

Selected Publications

Ehrlich, K. F., M. C. Cantin, M. B. Rust and B. Grant. 1989. Growth and survival of larval and post larval smallmouth bass fed a commercially prepared dry feed and/or *Artemia* nauplii. *Journal of the World Aquaculture Society*, Vol 20:1, pp. 1-6.

Rust, M. B., Hardy, R. W., and Stickney, R. R., 1993. A new method for force-feeding larval fish. *Aquaculture*. 116: 341-352.

Rust, M. B. 1995. Quantitative aspects of nutrient assimilation in six species of fish larvae. Ph. D. Dissertation, University of Washington, Seattle, 150 p.

Scott, T. M., and M. B. Rust. 1996. A computer automated cold water recirculating system for aquaculture research. In: G. S. Libey and M. B. Timons (eds) *Successes and Failures in Commercial Recirculation Aquaculture*. Northeast Regional Agricultural Engineering Service, NRAES-98, pp 562-574.

Rust, M. B., and F. T. Barrows. Submitted. An image analysis approach to determine microparticulate feed acceptability with larval fish. *Proceedings of the symposium on*

Marine Finfish and Shellfish Aquaculture, Marine Stock Enhancement, and Open Ocean Engineering and the 26th UJNR aquaculture panel meeting, September 16-18, 1997.

Section 10. Information/technology transfer

Information generated by this project will be communicated by annual reports, publication in peer-reviewed scientific journals, presentations at workshops and scientific conferences, and regular communication with Technical Oversight Committees for the ongoing captive broodstock programs.