

PART I - ADMINISTRATIVE

Section 1. General administrative information

Title of project	
Heritability of Disease Resistance and Immune Function in Chinook Salmon	
BPA project number	20106
Contract renewal date (mm/yyyy)	
Multiple actions? (indicate Yes or No)	
Business name of agency, institution or organization requesting funding	
U.S. Fish & Wildlife Service	
Business acronym (if appropriate)	USFWS
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NPPC Program Measure Number(s) which this project addresses	
Sections 4.2A, 7.1B, 7.2D.1, 7.2D.3, and 7.2D.4	
FWS/NMFS Biological Opinion Number(s) which this project addresses	
Other planning document references	
Short description	
Determine the heritabilities and genetic correlations of resistance to bacterial kidney disease (BKD) in spring chinook salmon, and evaluate whether broodstock culling based on ELISA can cause genetic changes in disease resistance and immune function.	
Target species	
Chinook salmon (spring-run/stream-type), <i>Oncorhynchus tshawytscha</i>	

Section 2. Sorting and evaluation

Subbasin
Systemwide

Evaluation Process Sort

CBFWA caucus	CBFWA eval. process	ISRP project type
X one or more caucus	If your project fits either of these processes, X one or	X one or more categories

		both	
X	Anadromous fish	Multi-year (milestone-based evaluation)	Watershed councils/model watersheds
	Resident Fish	Watershed project eval.	Information dissemination
	Wildlife		Operation & maintenance
			New construction
			X Research & monitoring
			X Implementation & mgmt
			Wildlife habitat acquisitions

Section 3. Relationships to other Bonneville projects

Umbrella / sub-proposal relationships. List umbrella project first.

Project #	Project title/description

Other dependent or critically-related projects

Project #	Project title/description	Nature of relationship
	None	

Section 4. Objectives, tasks and schedules

Past accomplishments

Year	Accomplishment	Met biological objectives?

Objectives and tasks

Obj 1,2,3	Objective	Task a,b,c	Task
1	Spawn spring chinook salmon (Carson National Fish Hatchery)	a	Screen 100 adult males for <i>R. salmoninarum</i> ELISA levels and

Obj 1,2,3	Objective	Task a,b,c	Task
	<p>adults in a nested design (1:6 male:female) to yield 120 full-sib (20 half-sib sire) families.</p> <p>[Note: Male parents will be selected to maximize the variance of their ELISA values; Female parents will be selected randomly to represent the observed distribution of ELISA values of the population.</p>	<p>b</p> <p>c</p> <p>d</p>	<p>temporarily store their milt at 4° C.</p> <p>Select milt from 24 males, based on their ELISA values, to fertilize 600-800 eggs from each of six, randomly selected females (144 females, 6 females/male).</p> <p>Transfer eyed eggs to the Abernathy SCTC, and retain 20 half-sib sire families after hatch for subsequent rearing and tests.</p> <p>Rear 120 full-sib families for one year. Purchase and set-up 120 30"-diameter x 30" deep circular (fiberglass) tanks.</p>
2	<p>Test H₀: The proportion of chinook salmon eggs containing detectable <i>R. salmoninarum</i> will not differ among groups of eggs from females with different levels of <i>R. salmoninarum</i> infection.</p>	<p>a</p> <p>b</p> <p>c</p>	<p>Randomly collect 50 eyed eggs from each full-sib family and store at -70°C.</p> <p>Separately test 30 eggs per family for the presence of <i>R. salmoninarum</i> by PCR amplification of DNA encoding the p57 protein of the bacterium.</p> <p>Perform contingency table statistical analyses and calculate nonparametric phenotypic correlations.</p>
3	<p>Test H₀: The ability of progeny to resist bacterial infections to <i>Vibrio anguillarum</i> does not differ among families and is not phenotypically correlated with, or compromised by, parental levels of <i>R. salmoninarum</i>.</p>	<p>a</p> <p>b</p> <p>c</p> <p>d</p>	<p>PIT-tag 72 fish (60 + 12 backups) from each full-sib family and transfer to the Western Fisheries Research Center (BRD-USGS, Seattle) for the challenge experiments.</p> <p>Develop challenge cultures of <i>V. anguillarum</i>.</p> <p>Challenge (a) 30 fish from each full-sib family with <i>V. anguillarum</i> and (b) 30 control fish from each family with uninnoculated growth media, and monitor mortalities.</p> <p>Estimate mean survival times of each family and calculate nonparametric phenotypic correlations.</p>
4	<p>Test H₀: Humoral immune responses to (a) the p57 protein of <i>R. salmoninarum</i> and (b) an unrelated foreign protein do not differ among families and are not</p>	<p>a</p> <p>b</p>	<p>PIT-tag 120 fish (100 + 20 backups) from each full-sib family and transfer to the Western Fisheries Research Center. Immunize 40 fish from each family with p57 (trial 1) and 40 fish from each</p>

Obj 1,2,3	Objective	Task a,b,c	Task
	correlated phenotypically with parental levels of <i>R. salmoninarum</i> .	c d	family with an unrelated foreign protein (trial 2). Use IgM-ELISA to measure, for each trial, levels of specific serum immunoglobulin to p57 or the foreign protein in vaccinated (40 fish/family) and unvaccinated (10 fish/family) fish. Compare immunocompetency of progeny, as measured by IgM-ELISA absorbance values, in a mixed model ANOVA and compare to parental levels of <i>R. salmoninarum</i> .
5	Determine heritabilities and genetic correlations of all traits evaluated under Objectives 2, 3, and 4.	a	Collate all the data generated by all experiments and use quantitative genetic methods to analyze those data statistically.
6	Prepare and submit final (first annual) report to BPA and scientific manuscripts for publication.		

Objective schedules and costs

Obj #	Start date mm/yyyy	End date mm/yyyy	Measureable biological objective(s)	Milestone	FY2000 Cost %
1	01/2000	12/2000	Production and rearing of 120 full-sib family groups	XX	26%
2	08/2000	12/2000	Tests for the presence of <i>R. salmoninarum</i> among 30 eyed eggs from each female parent.	XX	15%
3	03/2001	05/2001	<i>Vibrio</i> challenge expts.	XX	22%
4	05/2001	08/2001	Immunocompetency experiments	XX	32%
5	08/2001	10/2001	Quantitative genetic analyses	XX	4%
6	10/2001	12/2001	Prepare and submit final report	XX	1%
				Total	100%

Schedule constraints

Spawning of spring chinook salmon begins in August. Consequently, if the proposed project is approved for funding for FY2000, we would not be able to initiate the spawning of adults until August 2000. We also need to purchase and set-up rearing tanks for maintaining 120 progeny families until those fish are large enough to PIT tag. Purchasing and setting up these tanks will require 2-3 months of lead time, and we would need to order those tanks before the adult parents were spawned. On the other hand, if funds for FY2000 could be made available during FY1999 (e.g. by July 1, 1999), then we could begin the proposed project during the summer and fall (July-Dec) of 1999. The budget and time schedules presented in this proposal assume that funds would not be available until after October 1, 1999.

Completion date

2002 (Two-year project, including funding from both FY2000 and FY2001 programs). Only those studies and activities proposed for the first year are described here in this proposal.

Section 5. Budget

FY99 project budget (BPA obligated):	\$ N/A, new project.
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FY2000 budget by line item

Item	Note	% of total	FY2000 (\$)	
Personnel	Lead Laboratory Technician, GS-7	6.9	27,611	
	Laboratory Technician, GS-5	5.6	22,292	
	Biological Science Technician, GS-5	5.6	22,292	
Fringe benefits	Federal fringe benefit rate: 30%	5.4	21,659	
Supplies, materials, non-expendable property	<u>Expendable supplies</u>			
	Antigen immunoassays	2.6	10,400	
	Antibody immunoassays	3.4	13,500	
	FAT/MF-FAT reagents	0.4	1,400	
	Bacteriological culture	0.1	500	
	PCR reagents	4.5	18,000	
	Misc. lab supplies (tubes, pipets, etc)	0.5	2,000	
	Fish food	0.8	3,000	
	Fish rearing supplies	0.3	1,000	
	<u>Non-expendable property</u>			
	96-well PCR thermocycler	1.2	4,800	
	Microplate dispenser	1.9	7,500	
	Liquid nitrogen container	0.6	2,500	
	120 30"-diameter tanks (\$350 ea)	10.5	42,000	
	Lumber for tank frames and supports	0.3	1,000	
Operations & maintenance	Fish maintenance (water, electricity)	1.5	6,000	
	PIT-tagging, USFWS crew	7.0	28,000	
	Publications and photocopying	0.5	2,000	
Capital acquisitions or improvements (e.g. land, buildings, major equip.)				
NEPA costs				
Construction-related support				
PIT tags	# of tags: 200/family (\$2.90 ea)	17.4	69,600	
Travel	Car Rental	0.1	100	
	Airfare	0.1	200	
	Per diem (60 person-days, \$80/day)	1.2	4,800	
	Fish transportation (truck, oxygen)	0.5	2,000	
Indirect costs	22% (includes I/C on subcontracts)	18.0	71,932	
Subcontractor	Nat'l Marine Fish. Serv.	3.1	12,510	
Other				
TOTAL BPA REQUESTED BUDGET			398,596	

Cost sharing

Organization	Item or service provided	% total project cost (incl. BPA)	Amount (\$)
Total project cost (including BPA portion)			

Outyear costs

	FY2001	FY02	FY03	FY04
Total budget				

Section 6. References

Watershed	Reference
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PART II - NARRATIVE

Section 7. Abstract

Genetic effects of hatchery management practices have become a major concern in recent years. In particular, questions have been raised regarding the effects of “broodstock culling” to prevent outbreaks of bacterial kidney disease (BKD) in Columbia River hatcheries. The causative agent of BKD, *Renibacterium salmoninarum*, is transmitted vertically from female parent to progeny, and a common practice - particularly for spring chinook salmon - is to screen female parents for *R. salmoninarum* using an enzyme-linked immunoabsorbent assay (ELISA) and to segregate or destroy progeny of female parents with high ELISA values. This culling practice has proven to be a powerful tool for reducing significant losses due to BKD in spring chinook salmon during hatchery rearing and after release. However, this practice could also reduce the future fitness of these populations if disease resistance or immune function is heritable and correlated genetically with high ELISA values. Such data are currently absent but are needed to assess the possible genetic impacts of broodstock culling on long-term fitness. Information is also needed concerning the efficacy of vertical transmission of *R. salmoninarum* from female fish with various levels of infection. These data are critical for optimizing broodstock maintenance procedures, particularly where artificial propagation may be necessary to help recover ESA-listed populations. The proposed study will investigate vertical transmission of *R. salmoninarum* in spring chinook salmon, and estimate the heritabilities and genetic correlations of disease resistance indicators. Adult chinook salmon, with levels of *R. salmoninarum* varying throughout the range commonly observed in hatcheries, will be mated in a nested design (1:6 male:female) to yield 120 full-sib (20 half-sib) families. We will quantify levels of *R. salmoninarum* infection among the resulting progeny and evaluate their specific and nonspecific immune functions. Quantitative genetic methods will be used to estimate genetic correlations between level of *R. salmoninarum* infection

in parental fish and level of infection and immune response among progeny. The proposal addresses the following research needs of the 1994 Columbia River Basin Fish and Wildlife Program: Sections 4.2A, 7.2D.1, 7.2D.3, and 7.2D.4.

Section 8. Project description

a. Technical and/or scientific background

Bacterial kidney disease (BKD), caused by *Renibacterium salmoninarum*, is among the most detrimental bacterial diseases of Pacific salmon (PNFHPC 1994). This is a chronic, systemic disease that is transmitted both vertically (Bullock 1978; Evelyn et al. 1986) and horizontally (Mitchum and Sherman 1981; Bell et al. 1984). The disease is considered more severe in spring chinook salmon (*Oncorhynchus tshawytscha*) than in other salmonids (Bullock and Wolf 1986), and is difficult to control in Columbia River hatcheries (Raymond 1988); effective vaccines do not exist and antibiotic therapy is only partially effective (Elliott et al. 1989; Evenden et al. 1993). Oral chemotherapy of salmonids with erythromycin reduces mortality but does not eliminate infections from all treated fish (Wolf and Dunbar 1959; Austin 1985). Some evidence exists that juvenile anadromous salmonids infected with *R. salmoninarum*, while in fresh water, die of BKD upon entry into seawater (Banner et al. 1986; Elliott et al. 1995).

Most hatcheries in the Columbia River Basin have aggressive programs for monitoring and controlling BKD. A standard hatchery practice for spring chinook salmon is to screen female parents for *R. salmoninarum* using an enzyme-linked immunoabsorbent assay (ELISA), and to segregate or destroy progeny from female parents with high ELISA values (e.g. optical densities >1.0). This culling practice has proven to be a powerful tool for reducing BKD in salmon hatcheries (Pascho et al. 1991), and it may result in increased survival of hatchery smolts after their release (Pascho et al. 1993; Elliott et al. 1995).

Concerns have been raised regarding the potential genetic effects of broodstock culling on the future fitness and disease resistance of spring chinook salmon because resistance may be heritable and correlated genetically with high ELISA values. In general, genetic mechanisms of disease resistance in fishes are not well understood (Price 1995; Chevassus and Dorson 1990; Fjalestad et al. 1993), and only limited genetic research has been conducted to date on BKD resistance in salmonids (Suzumoto et al. 1977; Winter et al. 1980; Withler and Evelyn 1990; Beacham and Evelyn 1992a,b; preliminary results from our group, see below). Some evidence exists for a correlation between transferrin genotype and the ability of coho salmon to survive infection by *R. salmoninarum*, thus suggesting a major gene effect of transferrin or other linked loci (Suzumoto et al. 1977; Winter et al. 1980). Transferrin is an iron-binding protein that can limit the amount of this trace metal available to a pathogen, and has been associated with resistance of fish to other diseases (Sussman 1974). However, the correlation between transferrin genotype and BKD resistance appears to be population specific (Winter et al. 1980). Moreover, substantial variation in BKD resistance appears to exist among conspecific populations for several salmonid species (Winter et al. 1990; Fryer and Sanders 1981; Withler and Evelyn 1990; Beacham and Evelyn 1992a; Olivier et al. 1992).

Only limited information exists regarding genetic variation for BKD resistance within populations. Withler and Evelyn (1990) reported heritabilities (proportion of the total phenotypic variation among individuals due to genetic variation) of 0.53 ± 0.16 and 0.26 ± 0.10 for threshold survival and time to death, respectively, following intraperitoneal injection of *R. salmoninarum* in

coho salmon (*O. kisutch*). Similarly, Beacham and Evelyn (1992a) reported heritabilities of 0.26 and 0.38 for threshold mortality caused by BKD in two populations of chinook salmon, but the small number of families and large standard errors (0.33 and 0.25, respectively) limit the value of those results. A third population studied by Beacham and Evelyn (1992a) exhibited substantially lower mortality due to BKD relative to the first two populations (6.9% vs. 42.1 and 29.4%, respectively), and that third population exhibited virtually no heritability for survival (0.00 ± 0.05). These results suggests that chinook populations most susceptible to BKD may also show the highest heritabilities for this trait.

We recently conducted three preliminary studies (manuscripts in prep.) that helped us formulate the experimental design described in this proposal. Those studies are summarized below.

Heritability of infection by *R. salmoninarum* in spring chinook salmon - A sample (88 males and 103 females) of adult spring chinook salmon returning to the Carson National Fish Hatchery in 1994 were screened by ELISA for kidney levels of *R. salmoninarum*. Based on the distribution of ELISA absorbances (range = 0.052-3.211), adult males and females were grouped into three levels of infection: “high”, “low”, and “uninfected.” Twenty males were each mated to two females in four combinations of infection: high-high, high-low, low-high, low-low (5 males and 10 females per category), thus yielding 40 full- and 20 half-sib families. We used the statistical methods described below under “Methods” to estimate the heritability of infection by *R. salmoninarum* on both the threshold and continuous scales. In general, the incidence of infection among the tested progeny ($n = 7,945$) was low ($<10\%$) and poorly correlated with that of their parents. The estimated heritability of *R. salmoninarum* infection level averaged 0.061 ± 0.004 , suggesting that approximately 6% of the phenotypic variation in *R. salmoninarum* prevalence was due to genetic causes. On the continuous scale (Robertson 1950), we obtained a heritability estimate of 0.282, suggesting that approximately 28% of the variation in infection levels among *R. salmoninarum*-positive progeny was due to genetic causes.

Heritability of survival to *Vibrio anguillarum* in fall chinook salmon - We conducted a study in 1995 to evaluate the genetic components of resistance to vibriosis in fall chinook salmon (Grovers Creek Hatchery, Puget Sound). Thirty, 1994-brood subyearling fish were randomly removed from each of 86 full-sib families and challenged with *V. anguillarum*. We estimated the heritability of survival at two separate concentrations of the pathogen (10^6 cells/ml or 10^7 cells/ml; 15 fish/family/treatment). Mortalities were 8.2 and 16.1% at the lower and higher concentrations, respectively, and averaged only 12.2% overall. Treatment, replicate (2 replicates/treatment), sire, and dam effects on survival were each highly significant ($P < 0.0001$), but those modelled factors explained only 14.5% of the total phenotypic variation in survival. We estimated the heritability of survival on the threshold scale to be 0.014 and on the continuous scale to be 0.036, both not significantly different from zero ($P > 0.05$). These results are similar to those of Beacham and Evelyn (1992a, 1992b) who detected little or no genetic variation for vibriosis-induced mortality in juvenile chinook salmon. However, a retrospective power analysis of our study indicated that the power of the test for detecting survival differences among half-sib sire families was only about 12%. Increasing the bacterial dose or expanding the experimental design with more parental fish may have led to a greater ability to detect and quantify genetic variation in survival after infection.

Efficacy of broodstock segregation - Concurrent with our preliminary study of *R. salmoninarum* infection level described above, we also conducted a broodstock segregation study with spring chinook salmon at the Carson National Fish Hatchery in 1994 (BPA Contract Number

91AI20868, Project Number 91-22). The results of that study indicated that females with very high numbers of *R. salmoninarum* cells in the ovarian fluid ($>1 \times 10^8/\text{mL}$) would likely transmit the bacterium to progeny with subsequent development of clinical BKD in a significant proportion of those progeny (Elliott et al. 1997a). However, that study did not monitor progeny at the egg stage to determine the efficacy of vertical transmission of *R. salmoninarum* from female parents with intermediate levels of infection. Such data are crucial for controlling BKD in hatchery stocks, particularly those originating from depressed or ESA-listed populations, and for evaluating the relative risks (genetic vs. disease control) of broodstock culling.

Research needs - Previous genetic studies of disease resistance have focused on estimating the heritability of survival in challenge tests. This has been particularly true in previous studies of BKD resistance in salmonid fishes. Other studies have focused on the inheritance of disease indicators such as tissue levels of a pathogen or putative host responses to it. A critical element missing from such studies has been an evaluation of a potential link between such indicators of disease and the actual immunocompetence of the host, particularly in a quantitative genetic framework where genetic and non-genetic components of the observed phenotypic variation can be evaluated. Characterizing this potential link, or genetic correlation, is essential to (a) evaluating the practical utility of these indicators as measures of controlling infection, (b) using these indicators as indirect measures of phenotypic resistance or susceptibility of individual host fish, and (c) understanding the underlying immunogenetic mechanisms of disease resistance. This latter information is particularly important for determining whether the culling of progeny on the basis of their parental ELISA values can genetically change the susceptibility of a population to BKD in future generations.

b. Rationale and significance to Regional Programs

The potential genetic and biological effects of hatchery management practices on salmonid fishes have become major concerns in recent years (Hindar et al. 1991; Waples 1991; Campton 1995). Many past practices are known to have caused genetic changes in some populations, for example, changes resulting from selective breeding for early return time of adults and the simultaneous pooling of milt and eggs from multiple males and females (Leider et al. 1984; Simon et al. 1986; Waples and Teel 1990; Withler and Beacham 1994). The potential genetic effects of these past management practices are now widely recognized and have been largely discontinued (IHOT 1995), although other ill-advised practices do continue (e.g. the transfer of eyed eggs or fish among facilities, sometimes over distances spanning hundreds of river miles within the Columbia River Basin).

One common misconception, often conveyed by the popular press, is that hatchery fish routinely transfer diseases to “wild” or naturally-spawned fish. However, as stated earlier by Steward and Bjornn (1990), we too are unaware of a single documented case where Pacific salmon or steelhead of hatchery origin have infected wild fish under natural conditions, although such possibilities cannot be excluded. Virtually all pathogenic organisms known to cause diseases to salmon and steelhead in Columbia River hatcheries also occur naturally within the Basin. Indeed, *R. salmoninarum* (as detected by ELISA) appears, in general, to be significantly more prevalent among wild smolts of spring/summer chinook salmon than hatchery smolts (Congleton et al. 1995; Elliott et al. 1997b). For example, 95% versus 68% of wild and hatchery smolts, respectively, at Lower Granite Dam in 1995 had detectable levels of *R. salmoninarum* (Congleton et al. 1995). Aggressive measures associated with the control of BKD at Columbia River

hatcheries may have contributed to the lower prevalence of *R. salmoninarum* among hatchery smolts (Maule et al. 1996).

Many populations of spring chinook salmon are now federally listed as “threatened” under the Endangered Species Act (ESA). Naturally spawning populations in the Columbia River above Wells Dam and in the Snake River above Lower Granite Dam are especially imperiled (unpublished data; Washington Department of Fish and Wildlife, Idaho Department of Fish and Game, Oregon Department of Fish and Wildlife, Nez Perce Tribe). Artificial propagation via hatcheries and captive breeding/rearing techniques are being used increasingly to conserve or “salvage” those indigenous populations and their inherent genetic resources. Future recovery of those populations, and restoration or reintroduction of extirpated populations, will depend increasingly upon the ability of our hatchery technologies to propagate and preserve those irreplaceable genetic resources. This new conservation role of hatcheries in the Columbia River Basin is imposing - and will continue to impose - increasing demands on managers and technical personnel to control disease outbreaks and maintain populations without affecting the genetic constitution of those populations via artificial propagation or the hatchery environment. As described previously, broodstock segregation and culling have proven to be very effective management tools for controlling BKD in hatchery populations of spring chinook salmon. However, the long-term genetic consequences of those activities are unknown. Such information is clearly needed if conservation and recovery goals are to be met.

The quantitative genetic and immunological research proposed here would allow us to evaluate the relative value and genetic risks associated with broodstock culling to control BKD in Columbia River hatcheries. This will be accomplished by (a) robust estimation of the heritabilities of phenotypic indicators and the degree of immunocompetence (i.e., a more direct measure of susceptibility) and (b) estimation of the genetic and phenotypic correlations between those traits. The analysis would also aid in developing reliable hatchery protocols for (a) screening individual fish based on such phenotypic indicators of infection and (b) conserving genetic resources associated with disease resistance. The proposed research would also provide more information about the relative contributions of adult spring chinook salmon with known infection levels of *R. salmoninarum* to the persistence of BKD via vertical transmission. Such information is especially important for the maintenance and recovery of depressed ESA-listed stocks.

This proposal directly addresses specific research needs identified in the 1994 Fish and Wildlife Program. Section 4.2A deals specifically with the need to conduct research to “reduce scientific uncertainty and increase knowledge to achieve the salmon and steelhead goal and policies of this [fish and wildlife] program.” Section 7.1B deals with the need to “conserve genetic diversity”, a theme central to the motivation for this proposal. In addition, Section 7.2D of that program describes research required to improve propagation at existing facilities including instructions for Bonneville to: fund research, development and demonstration of improved husbandry practices at hatcheries, which will lead to increased production and improved fish survival to adulthood (Section 7.2D.1); fund research, development and testing of hatchery rearing operations and release strategies aimed at improving the efficiency of hatcheries and increasing survival of artificially propagated fish to adulthood (Section 7.2D.3); and fund development of programs and methods to improve fish health protection in hatchery facilities (Section 7.2D.4) that includes methods to prevent the spread of fish pathogens, improve breeding and rearing practices, and minimize impact of fish diseases on wild and cultured stocks.

c. Relationships to other projects

One of us (DEC) is currently working collaboratively with Dr. Jerri Bartholomew, Department of Microbiology, Oregon State University, and Dr. Gary Thorgaard, Department of Zoology, Washington State University on a genetic study of inheritance of resistance to the parasite *Ceratomyxa shasta* in rainbow trout. This work involves quantitative genetic analyses of F1 and backcross progeny resulting from the initial crossbreeding of *C. shasta* resistant and *C. shasta* susceptible strains of rainbow trout. Future work will involve the use of gene mapping techniques to potentially identify major genes (quantitative genetic loci) associated with resistance to the parasite (Young et al. 1998). This study of *C. shasta* resistance and the one proposed here for BKD both reflect the need to understand genetic mechanisms of disease resistance in salmonid fishes and the evolutionary implications of population-specific adaptations, particularly in the Columbia River Basin where pathogenic or parasitic organisms are known to have, or may have, mosaic geographic distributions.

d. Project history (Not applicable. The project proposed here is a new project.)

e. Proposal objectives

1. Spawn spring chinook salmon adults, returning to the Carson National Fish Hatchery (NFH), in a nested design (1 male to 6 females) to yield 120 full-sib (20 half-sib) families. Justification: Our relatively large experimental design is necessary for genetically evaluating traits with low heritabilities (preliminary studies described previously) and for detecting genetic correlations between them. In addition, traits related to BKD resistance and immune response may have very high maternal environmental components (i.e. the phenotypic covariance between female parents and their progeny may be due largely to maternal environmental effects and the vertical transmission of the bacterium), and this environmental covariance necessitates the mating of many females to individual males. A quantitative genetic study based on a classical breeding design provides the greatest power to detect the genetic underpinnings of disease resistance, particularly where measurements are available on both parents and offspring and immunological characters can be evaluated (see below). This genetically based experimental design is a critical aspect of our study because phenotypic correlations among characters with strong environmental covariances often bear little resemblance to the corresponding genetic correlations (Cheverud 1988; Falconer and MacKay 1996; Willis et al. 1991). Only if traits are correlated genetically (e.g. the prevalence of *R. salmoninarum* within parents and their genetic ability to resist infection) will natural or artificial selection for one trait result in a genetic response in the other trait. **Assumptions:** (a) Sufficient number of spring chinook adults will return to the Carson NFH during 1999 (or 2000) to allow us to collect milt and livers from 100 adult males. (b) Milt from the screened males not used in the experimental crosses (approximately 76 males) can be used to fertilize eggs used for regular hatchery production. (c) Removing approximately 600 eggs from each of 144 females for our experiments will not significantly affect the production requirements of the Carson NFH. (d) All necessary fish health and transportation permits will be issued by the Washington State Department of Fish and Wildlife and the U.S. Fish & Wildlife Service. (e) Egg or fry losses from females with high BKD-ELISA levels will not be 100% and will not exceed 65% in most families, thus leaving sufficient number of progeny within families to perform the experiments associated with Objectives 2-4. (f) *R. salmoninarum* is enzootic in the Carson NFH spring chinook population and will yield BKD-ELISA absorbances

between 0.09 and 3.00 (values >1.0 are considered “high”).

2. Test the null hypotheses: The proportion of chinook salmon eggs containing detectable *R. salmoninarum* will not differ among groups of eggs from females with different levels of *R. salmoninarum* infection. Justification: The clinical signs of BKD, and ultimately mortality, may result from a failure of the fish to respond immunologically to infection by *R. salmoninarum*. Such unresponsiveness could be caused by the vertically-transmitted presence of the bacterium within individual eggs during embryonic development of the fish’s immune system, and subsequent disruption of an effective immune response later in life. Those deleterious effects may also cause a more general immunosuppression that affects the ability of fish to respond to any pathogenic microorganism (see objective 3 below). Objective 2 will provide critical background data on the probability that *R. salmoninarum* occurs within individual eggs (as opposed to ovarian only) relative to the maternal levels of *R. salmoninarum* infection. These data will help us to determine if there is a threshold maternal infection level, above which eggs will need to be isolated or destroyed because they have a high probability of harboring the BKD bacterium. **Assumptions:** The number of *R. salmoninarum* cells within individual eggs necessary to potentially suppress the immune function of developing embryos is greater than the threshold level necessary for detecting the p57 protein gene of the bacterium by the polymerase chain reaction (PCR).

3. Test the null hypothesis: The ability of progeny to resist bacterial infections to *Vibrio anguillarum* is not correlated phenotypically with parental levels of *R. salmoninarum*. Justification: We need an independent assessment of disease resistance capability among our progeny families because some proportion of those families may be infected with *R. salmoninarum* due to vertical transmission from their female parents. Also, this aspect of the study will allow us to determine whether a genetic correlation exists between indicators of BKD infection and resistance to a second bacterial pathogen. **Assumptions:** (a) Sufficient number of progeny fish from each family group will be available for the *Vibrio* challenges. (b) Progeny fish from each family can be PIT-tagged prior to challenges. (c) Progeny fish will not be clinically suffering from BKD at the time of the *Vibrio* challenges.

4. Test the null hypotheses: Humoral immune responses to (a) p57, a secretory protein of *R. salmoninarum*, and (b) an unrelated “foreign” protein, are not correlated with parental levels of *R. salmoninarum*. Justification: The purpose of this experiment is to determine whether potential loss of disease resistance is related to the inability of the progeny fish to respond immunologically to only a protein of *R. salmoninarum* or to any foreign protein. If the presence of *R. salmoninarum* in the egg and developing embryo (via vertical transmission) results in the inability of the fish’s immune system to recognize p57 as a foreign antigenic protein, then the fish may not respond immunologically to a vaccination with p57 (see Methods below) or, ultimately, to a pathogenic infection by *R. salmoninarum*. A second, foreign protein will help us determine if the presence of *R. salmoninarum* during embryonic development results in immunosuppression on a much broader, nonspecific scale (i.e. lack of a humoral immune response to both proteins). **Assumptions:** (a) Same as 3a and 3b above. (b) A measurable humoral response will be produced by fish in families from females with very low, or undetectable, levels of *R. salmoninarum* infection.

5. Determine the heritabilities and genetic correlations among all traits evaluated under Objectives 2, 3, and 4. Justification: This is the primary motivation for performing the proposed work, as described in the “background” section. **Assumptions:** (a) Sufficient number of progeny within most full-sib families will survive from fertilization until the time the experimental trials are performed. (b) Unidentified or unexpected sources of mortality (e.g. viral or parasitic infections) will not cause excessive mortalities to entire families of fish prior to, or during, the experiments.

6. Submit final (first annual) to BPA and scientific manuscripts for publication.

f. Methods

Objective 1. Spawn spring chinook salmon adults, returning to the Carson National Fish Hatchery (NFH), in a nested design (1 male to 6 females) to yield 120 full-sib (20 half-sib) families. **Task 1a:** Approximately 100 adult males will be screened for infection levels of *R. salmoninarum*. During the first or second week of spawning, milt from 100 males will be exuded and sealed with oxygen in sterile, plastic bags containing a sperm extender with antibiotics (Dr. Gary Thorgaard, WSU, pers. comm.), and stored for approximately one week at 4°C. The kidney from each male will be tested by the BKD-ELISA II method to determine level of infection (Pascho et al. 1991). **Task 1b:** The milt from males will be selected on the basis of their ELISA values to maximize the phenotypic variance among sires and to ensure that males with the highest (>> 1.0) and lowest (<0.1) values will be included as sires. Over the next two spawning days, stored milt from each of the 24 selected males will be used to fertilize 600-800 eggs (counted volumetrically) from each of six, randomly selected females (6 females/male; 144 females total). The kidney and the ovarian fluid of each female parent will be tested for *R. salmoninarum* by ELISA. Ovarian fluids from each female will be tested by the MF-FAT method also (Pascho et al. 1991). Each full-sib egg lot will be incubated separately to the eyed egg stage at the Carson NFH. These 144 full-sib (24 half-sib) families will be transferred to the Abernathy Salmon Culture Technology Center (SCTC; Longview, WA) at the eyed egg stage of development and incubated in separate hatch trays until hatch and yolk absorption. **Task 1c:** Twenty half-sib sire families (goal: 120 full-sib families, each with ≥ 300 surviving fry) will be retained after hatch for subsequent rearing and tests. **Task 1d:** At the “swim-up” stage, 300 fry from each family will be transferred to separate 30"-diameter x 30"-deep circular (fiberglass) tanks, plumbed with well water, at the Abernathy SCTC. We will purchase and set-up these tanks (n=120) as soon as funding is approved so that they are in place at least 1-2 months prior to transfer. Subsamples of fish from each family (tank) will be removed periodically for the experiments described below (Objectives 2-4). At approximately 9-10 months post-fertilization, all remaining fish within each family will be PIT-tagged and moved to an outside raceway for subsequent rearing to smoltification and use for experiments funded during FY2001. No fish will be released from the Abernathy SCTC. Standard feeding and husbandry protocols for chinook salmon will be followed.

Objective 2. Test the null hypotheses: Percentages of “eyed” eggs infected with *R.*

salmoninarum from single females are (a) equal for all 120 families and (b) not correlated phenotypically with parental levels of *R. salmoninarum*. **Task 2a:** Approximately 50 eyed eggs will be removed from each full-sib family (at the time of transfer to the Abernathy SCTC) and stored at -70°C for later testing. **Task 2b:** Up to 30 eggs from each family group will be tested by the nested-polymerase chain reaction (nPCR) with primers designed to detect a diagnostic region of the gene encoding the p57 protein of *R. salmoninarum* (Chase and Pascho, in press). **Task 2c:** The proportions of *R. salmoninarum*-positive eggs among family groups will be compared by G-test, contingency table analyses (Sokal and Rohlf 1981). Assuming that each female parent produced 3,000 eggs and all subsamples obtained for fertilization and PCR were random, 30 eggs would be required to detect at least one positive egg at a 95% confidence level when 9.5% of the eggs are infected with *R. salmoninarum*. Phenotypic correlations between proportion of infected eggs and maternal ELISA values will be estimated and tested by Kendall's Tau nonparametric statistic. A strong, positive correlation would confirm earlier results regarding the likelihood of vertical transmission, and it would be consistent with the hypothesis that immunosuppression could be a significant factor leading to BKD among progeny. These data would also allow us to assess the relative risks of culling versus not culling eggs (families) of a particular threshold level of infection if a genetic correlation exists between parental levels of *R. salmoninarum* (based on ELISA) and disease resistance (Objective 3) or immune function (Objective 4) of the progeny,

Objective 3. Test the null hypothesis: The ability of progeny to resist bacterial infections to *Vibrio anguillarum* is not correlated phenotypically with parental levels of *R. salmoninarum*. **Task 3a:** Seventy-two fish (60+12 backups) will be randomly sampled from each full-sib family, given a PIT-tag, and transported to the Western Fisheries Research Center in Seattle during the spring (March-April) of 2001 after all fish have attained a minimum length (FL) of 70 mm. **Task 3b:** Challenge suspensions of *V. anguillarum* will be developed by standard methods. Briefly, isolate 775 of *V. anguillarum* will be grown in trypticase soy broth (TSB) supplemented with 3% (w/v) sodium chloride for 15-17 h at 17°C . The concentration of total *V. anguillarum* cells in the challenge suspension will be determined on the basis of absorbance at 650 nm and standard curves describing the correlation between absorbance and bacterial concentration. The concentration of viable bacteria in the challenge suspension will be determined by bacteriological culture on trypticase soy agar supplemented with 3% (w/v) sodium chloride. Ten-fold dilutions of the suspensions will be prepared with TSB, and the culture plates incubated at 17°C for 48-72 h. **Task 3c:** Control and waterborne bacterial challenges will be performed in separate 4,271 L tanks containing static, aerated water at 17°C , supplemented with 0.85% (w/v) sodium chloride. Fifteen fish from each full-sib family (1,800 fish total) will be placed in each of four tanks, with two tanks used as controls and two tanks used for the bacterial challenges (i.e. a total of 60 fish per full-sib family will be used for this experiment: 30 for controls and 30 for the challenges). Fish in the challenge tanks will initially be held in the bacterial suspension for 15 min at a minimal volume such that the loading density is about 0.5 lb/gallon (57 g/L) within each tank. On the basis of preliminary test challenges with *V. anguillarum* 775, we will select a bacterial concentration that causes approximately 50% mortality in 14 d at 17°C among fish from parents with undetectable levels of *R. salmoninarum*. Control fish will be exposed to uninoculated bacterial growth medium for 15 min. at the same loading density as the challenged fish. Following these initial challenges, water will be added to all tanks to reduce fish densities to approximately 0.05 lb/gallon (5.7 g/L). The fish will be maintained on standard feed rations at

17°C, and mortalities monitored daily until all death has ceased in all groups. Dead fish from control and challenge groups will be removed daily and the PIT-tag number recorded. Tissues from a subsample of the dead fish (10% of mortalities on two sample dates) will be tested by bacteriological culture to ensure that the cause of death was a *V. anguillarum* infection. **Task 3d:** The Kaplan-Meier estimator, a nonparametric survival function, will be used to estimate the mean survival time for each full-sib family. Sample sizes and experimental conditions are based on the criteria described by Amend (1981). Phenotypic correlations between mean survival times of families and maternal ELISA values will be estimated and tested by Kendall's Tau nonparametric statistic. An inverse correlation between survival times and maternal *R. salmoninarum* infection levels would be consistent with the hypothesis that the presence of *R. salmoninarum* (or metabolic products thereof) during embryonic development of chinook salmon reduces their general resistance to infection by pathogenic bacteria.

Objective 4. Test the null hypotheses: Humoral immune responses to (a) p57, a secretory protein of *R. salmoninarum*, and (b) an unrelated "foreign" protein, are not correlated with parental levels of *R. salmoninarum*. **Task 4a:** 120 fish (100 + 20 backups) will be randomly sampled from each full-sib family, given a PIT-tag, and transported from the Abernathy SCTC to the Western Fisheries Research Center (Seattle, WA) during the late spring (May-June) of 2001. **Task 4b:** Forty fish from each family will be immunized with a recombinant p57 protein of *R. salmoninarum*; an additional 40 fish from each family will be immunized with an unrelated, foreign protein (see justification under the list of objectives). Each fish will be injected intraperitoneally with a 200 µL solution containing p57 or the other protein (200 µg/µL) in Freund's incomplete adjuvant (Anderson 1992). An additional 10 fish from each family per immunization trial (20 fish per family total) will be sacrificed without being immunized, and blood taken for immunoglobulin assays (see Task 4c below). All vaccinated fish for each trial (40 fish/family/trial) will be transferred to a single 4,271 L tank, maintained in flowing Lake Washington water, and fed a commercial trout diet at a rate of approximately 5% of body weight each day for nine weeks (63 days). **Task 4c:** All vaccinated fish will be sacrificed and bled 63 days after vaccination. Levels of specific serum immunoglobulin to p57 or the foreign protein will be measured in all vaccinated (40 fish/family) and unvaccinated fish (10 fish/family). All fish will be scanned with a PIT-tag reader when blood samples are taken to identify each fish's family. Blood samples will be clotted overnight at 4°C, then centrifuged at 2,000 × g for 10 min to recover a serum sample. Individual sera will be stored at -70°C for later analysis by the IgM-ELISA (Pascho and Swanson 1995). **Task 4d:** The IgM-ELISA absorbance values for vaccinated and unvaccinated fish within and among each family will be compared by mixed model analyses of variance. Vaccination type will be modeled as a fixed effect, and sire and dam effects will be treated as random variables. In addition, ELISA values of the sires and dams will be treated as covariates in separate analyses and the resulting regression coefficients tested for statistical significance by ordinary least squares. Sample sizes associated with the experimental design are based on the criteria of Amend (1981).

Objective 5. Determine the heritabilities and genetic correlations among all traits evaluated under Objectives 2, 3, and 4. Well-established quantitative genetic methods will be used to estimate variance and covariance components for quantitative characters and apply these to both continuous and threshold characters (Falconer 1965, Falconer 1989, Lynch and Walsh 1998). Genetic components of variance and their derivatives (heritability and genetic correlation)

for continuous characters will be estimated by restricted maximum likelihood (REML) (Harville 1977, Patterson and Thompson 1971, Shaw 1987). Unlike least squares, REML provides unbiased estimates of genetic parameters, even when family structure is unbalanced and the resulting trait covariance matrices are sparse, as in our case (Henderson 1953, Searle 1971; Searle et al. 1992). We will also evaluate genetic parameters by regressing offspring values on the ELISA values of their sires, treating ELISA values of female parents as a covariate or adjustment factor (Thompson 1976; Hill and Thompson 1977).

A variety of methods have been proposed to analyze threshold characters within a quantitative genetic framework (Crittenden 1961, Falconer 1965, Robertson 1951, Robertson and Lerner 1949). For a hierarchical mating structure such as that proposed in this experiment, the genetic and environmental variance components of a threshold character can be estimated with nested anova and the heritability transformed to the continuous scale. We shall use the following equation to estimate heritability on the observed scale (Gianola 1982, Robertson and Lerner 1949):

$$h_o^2 = \frac{\frac{SSF}{p(1p)}(sI)}{r(ks + I)}$$

where SSF is the sum of squares among families, s is the number of families, r is the intraclass correlation (0.25 for half sibs), and $k = \frac{Sn_i - Sn_i^2}{Sn_i}$, where n_i is the size of family i . The standard error of this heritability estimate is approximately

$$SE(h_o^2) = \sqrt{\frac{2(sI)}{r^2(ks + I)^2}}$$

As shown by Robertson (1950), heritability expressed on the observed scale (h_o^2) will be converted to heritability on the underlying continuous scale (h_c^2) by the expression

$$h_c^2 = \frac{h_o^2 p(1p)}{z_p^2}$$

where p is the frequency of mortalities and z_p is the ordinate of the standard normal distribution corresponding to the threshold.

The genetic correlation between any of the traits can be estimated from the cross prevalence of these traits, by computing the sib correlations for these traits as follows (Lynch and Walsh 1998):

$$r_{xy} = \sqrt{\frac{b_{xy} b_{yx}}{b_{xx} b_{yy}}}$$

where r_{xy} is the genetic correlation between traits x and y , and b_{ij} are the sib correlations corresponding to the four possible correlations involving the two traits.

Objective 6. Submit final (first annual) report to BPA and scientific manuscripts for publication. All manuscripts and reports will be prepared electronically. Target journals for scientific publications are the *Canadian Journal of Fisheries and Aquatic Sciences*, *Journal of Aquatic Animal Health*, and *Journal of Heredity*.

g. Facilities and equipment

All progeny fish will be hatched and initially maintained at the Abernathy SCTC, U.S. Fish and Wildlife Service, Longview, WA. The Abernathy SCTC is a complete salmon hatchery with approximately 1200 Heath incubator trays, 95 4-ft circular tanks inside the hatchery building, and 12 outdoor raceways. A new well with a capacity of 2500 gpm recently came on line. A fish pathologist, Dr. Pete Taylor, works on site and is available for monitoring fish health.

Genetic studies requiring large numbers of progeny groups (families) have not been conducted previously at the Abernathy SCTC. Our study will require the maintenance of 120 families, each with approximately 300 fry or subyearlings, in separate tanks until the fish are large enough to PIT-tag and transfer to outside raceways or to the Western Fisheries Research Center for the experimental tests. Consequently, we will need to purchase 120 30"-diameter x 30"-deep fiberglass tanks, and the cost of these tanks is included with our budget requests for this proposal. If this proposal is funded, we will remove 30 of the 4'-circular tanks from the hatchery building and replace them with 120 of the smaller tanks, four 30" tanks in place of each 4' tank that is removed. The plumbing and drains are already in place; we would only need to construct 4x4/2x4 wood frames to support the tanks and then add some retrofitted plumbing to provide inflowing water to all four tanks in a cluster..

All experimental trials and immunological work will be performed at the the Western Fisheries Research Center (WFRC), Biological Resources Division of the U.S. Geological Survey, Seattle. The WFRC houses a state-of-the-art center for work on infectious diseases of fish that includes over 10,000 square feet of wet laboratory space for fish holding and experimental work. The wet laboratory is supplied with pathogen-free fresh water to 20 individual bays (each with temperature control from 4-25°C) containing a total of more than 300 tanks of various sizes. The laboratory effluent is treated with chlorine gas. Within the dry laboratory complex at the Center is a special Biosafety Level 3 laboratory containing restricted-access dry and wet laboratories for work with exotic fish pathogens. The WFRC also has more than 21,000 square feet of laboratory space for cell culture (including monoclonal antibody production), bacteriology, immunology, virology, histology, parasitology, and molecular biology. Also in the dry lab are a walk-in cold laboratory (4° to 20°C), walk in cold storage (4° and -20°C), fluorescence microscopy rooms, a common computer room and an animal care facility meeting NIH guidelines. The laboratory is equipped with ultracentrifuges, refrigerated centrifuges, refrigerated microfuges, PCR machines, an automated DNA sequencer, a peptide synthesizer, a DNA synthesizer, protein and nucleic acid electrophoresis equipment, spectrophotometers, a scintillation counter, chemical fume hoods, laminar flow hoods, ultra-cold freezers, research microscopes (some with advanced photomicrography capabilities), networked and stand-alone computers with both DNA and image analysis capabilities, and other large and small equipment items commonly found in microbiology

and molecular biology laboratories.

The quantitative genetic analyses will be performed by Dr. Jeff Hard, Northwest Fisheries Science Center (NFSC), NMFS, Seattle. Both UNIX workstations at the NFSC and mainframe computers at the University of Washington will be available for performing the statistical and iterative computations associated with analyzing the quantitative character data.

h. Budget

1. Personnel: The Lead Laboratory Technician (GS-7) and the Laboratory Technician (GS-5) will perform most of the routine laboratory assays (e.g. PCR reactions, ELISAs, *Vibrio* cultures, etc.) associated with Objectives 2, 3 and 4. These two technicians will be stationed at the Western Fisheries Research Center, BRD-USGS, Seattle, and will be under the direct supervision of two co-P.I.'s: D.G. Elliott and R.J. Pascho. The Biological Science Technician (GS-5) will be stationed at the Abernathy SCTC, USFWS, Longview, WA, and will perform all of the duties necessary for producing and maintaining the 120 full-sib family lots. This latter technician will be under the direct supervision of one co-P.I. (D.E. Campton) and the fish culture staff at Abernathy.

2. Supplies, materials, non-expendable property: Except for fish food and fish rearing supplies, all of the **expendable supplies** will be used for the laboratory work associated with Objectives 2, 3, and 4. The budgets for fish food and fish rearing supplies reflect our estimates of the costs for maintaining the progeny lots and experimental fish over a 12 month period. Justifications for the requested **non-expendable property** are described below.

96-well PCR thermocycler: The thermocycler will be used for completion of objective 2, which requires the testing of 3,600 individual eggs by the nested PCR for *R. salmoninarum*. A 96-well thermocycler is necessary to complete these analyses within the funding period. We estimate it would take 38 assay days after sample processing to complete testing. In contrast, the same analyses will take 105 assay days with a conventional 32-well thermocycler.

Microplate dispenser: This apparatus is used for dispensing reagents for the BKD- and IgM-ELISAs. A semi-automated dispenser is necessary to accurately test the 6,364 tissue and serum samples required for completion of Objectives 2, 3, and 4.

Liquid nitrogen container: This container will be used for cryogenic storage of serum and tissue samples. We will also use this container for cryogenic storage of milt collected at the Carson NFH if we are unable to screen all males or initiate our fertilizations within seven days of collection.

30"-diameter fiberglass tanks + supporting lumber: These tanks will be used to maintain each of our full-sib progeny lots at the Abernathy SCTC. Currently, the Abernathy SCTC has approximately 100 4'-diameter tanks, plumbed with well water, for maintaining experimental fish. We will replace approximately 30 of these latter tanks with 120 of the new tanks using existing plumbing. These 30" tanks are identical to ones recently installed at the new Livingstone Stone NFH (Sacramento River, Redding, CA) for maintaining pedigreed progeny lots

of the endangered Sacramento River winter-run chinook salmon. They are also on federal contract (Glassline Inc., Redding, Calif.). Another vendor will be used if tanks of similar specification can be obtained at a lower cost. This portion could also be reduced or eliminated if equivalent tanks could be donated by another agency.

3. Operations and maintenance: The **fish maintenance** costs are to cover the direct costs of electricity for lights and pumps, plumbing and building maintenance, diesel-powered backup generator, and all other forms of operational overhead incurred directly at the Abernathy SCTC. Also, approximately 25% of these funds will be used to provide direct overhead support at the Carson NFH until the eyed eggs are transferred to Abernathy. **PIT-tagging** will be performed primarily by the USFWS tagging crew in Vancouver, Washington. They have provided us with a written estimate for PIT-tagging 24,000 (200 fish/family x 120 families) subyearling (min. size = 70mm FL) chinook salmon. They estimate that it will take 85.5 man-days of their time to PIT-tag 24,000 subyearlings. The cost of tagging these fish, excluding the tags, breaks down as follows: \$19,600 for salaries, \$4,300 for travel, \$700 for vehicles, and \$3,000 for operations (total = 27,600, rounded up to \$28,000). **Publication and photocopying** charges are primarily to cover page charges and other miscellaneous costs of publishing the results of our work in peer-reviewed, scientific journals.

4. PIT tags: All of our experiments will require that fish from each family be treated equally as much as possible to minimize the environmental components of phenotypic variance among full- and half-sib family groups. Also, we need to ensure that approximately equal numbers of full-sibs from each family are included in each of our experimental trials. It is also extremely desirable that we are able to identify the families of origin of each fish at the time data are collected. Considering all of these factors collectively, we concluded that PIT tags would provide the greatest capability and efficiency for achieving our experimental objectives, as opposed, for example, to coded-wire tags in which family identifications could not be determined until sometime after the fish died. These immediate identifications are especially critical for the quantitative genetic analyses (Objective 5) because they cannot be completed until the family identifications of all experimental fish are determined. However, because our experimental fish will not be released, but instead will be retained exclusively for our experimental trials, we could use the older lower frequency tags if available for free or at a discounted rate. This would reduce the cost of the tags significantly. Also, we plan to dissect out the tags from our experimental fish so that they can be used in our smolt experiments that we will propose one year from now for FY2001.

5. Travel: Car rental and airfare are to cover direct transportation expenses of the WFRC (Western Fisheries Research Center) staff for travel between the Seattle and the Carson NFH (Carson, WA). **Per diem** expenses are for supporting the P.I.'s and/or their technical staff while they are working at the Carson NFH during spawning season, the WFRC staff when they are working at Abernathy, and for the Abernathy staff when they are working at the WFRC. **Fish transportation** is our rough estimate of the overall cost of transporting live fish from the Abernathy SCTC to the WFRC for Objectives 3 and 4. We are budgeting \$1000 to cover those transportation costs for each objective. This will require borrowing vehicles from other National Fish Hatcheries and reimbursing those hatcheries accordingly.

6. Indirect costs: The Portland (OR) Regional Office of the U.S Fish & Wildlife Service has established an indirect charge of 22% on all contracts and grants from the Bonneville Power Administration. These overhead charges are used exclusively by the Regional Office to cover administrative costs associated with administering purchase orders, advertising and hiring of personnel, etc. These charges do not cover the overhead costs of the field facilities, for example, at the Abernathy SCTC (see fish maintenance costs under budget item 3 above).

7. Subcontractor: A small proportion of the requested funds will be used for a subcontract to the Northwest Fisheries Science Center, National Marine Fisheries Service, Seattle. This subcontract is for four weeks of salary compensation to Dr. Jeff Hard (GS-13/02), co-P.I., for performing the quantitative genetic analyses associated with Objective 5 and for two weeks of salary support for a molecular biologist technician (GS-9/04) to collect and archive tissue samples for future DNA analyses, and to extract DNA from the adult fish spawned under Objective 1. The cost breakdown for this subcontract is itemized below:

Fishery Biologist, Research (Dr. Hard), 4 weeks	4,540
Molecular Biologist (GS-9), 2 weeks	1,400
Leave surcharge on salaries (18%)	1,200
Total Direct Labor (TDL):	
	\$7,140
Employer contribution of fringe (21.3% TDL)	\$1,550
Total Direct Cost:	
	\$8,690
GSA rents (9.3% TDL):	
	660
NOAA overhead (44.3% TDL):	
	3.160
Total Subcontract:	
	\$12,510

Section 9. Key personnel

1. **Donald E. Campton**, Fish Geneticist.

Abernathy Salmon Culture Technology Center, U.S. Fish & Wildlife Service,
1440 Abernathy Creek Road, Lonview, WA. 98632

Responsibilities: Will serve as Project Manager and co-Principal Investigator, and will assume primary responsibility for Objective 1, and will share co-responsibility with the other P.I.'s for Objective 6.

2. **Diane G. Elliott**, Fish Pathologist.

Western Fisheries Research Center, Biological Resources Division, U.S.
Geological Survey, 6505 N.E. 65th Street, Seattle, WA 98115.

Responsibilities: Will serve as co-Principal Investigator, and will share co-responsibility for Objectives 2, 3, and 4 with Ronald J. Pascho, and share co-responsibility with the other P.I.'s for Objective 6.

3. **Ronald J. Pascho**, Fish Pathologist.

Western Fisheries Research Center, Biological Resources Division, U.S.
Geological Survey, 6505 N.E. 65th Street, Seattle, WA 98115.

Responsibilities: Will serve as co-Principal Investigator, and will share co-responsibility for Objectives 2, 3, and 4 with Diane G. Elliott, and share co-responsibility with the other P.I.'s for Objective 6.

4. **Jeffrey J. Hard**, Quantitative Geneticist.

Northwest Fisheries Science Center, National Marine Fisheries Service,
2725 Montlake Boulevard. E., Seattle, WA 98195.

Responsibilities: Will serve as co-Principal Investigator, and will assume primary responsibility for Objective 5, and share co-responsibility with the other P.I.'s for Objective 6.

(1-page resumes follow)

DONALD E. CAMPTON

Title: Regional Fish Geneticist, GS-12

Current employer: U.S. Fish & Wildlife Service, Abernathy Salmon Culture Tech. Center

Current responsibilities: Address fish genetic issues and problems for the USFWS within a five state region

Education

Ph.D., Genetics, University of California, Davis, 1986.

M.S., Fisheries, University of Washington, Seattle, 1981.

B.S., Genetics, University of California, Berkeley, 1974.

Recent employment

Associate/Assistant Professor, Dept. of Fisheries & Aquatic Sciences, University of Florida, Gainesville, FL, 1986-1997.

Expertise

Population and quantitative genetics of fish: use of molecular methods for studying population structures; use of quantitative methods for studying genetic components of life history traits; applications to fisheries management, fish culture, and conservation.

Relevant publications

Campton, D.E. 1995. Genetic effects of hatcheries on wild populations of Pacific salmon and steelhead: What do we really know?, p. 337-353. *IN:* R.G. Piper and H.L. Schramm, Jr. (eds), *Uses and Effects of Cultured Fishes in Aquatic Ecosystems*, American Fisheries Society, Bethesda, Maryland.

Miracle, A.L., and D.E. Campton. 1995. Tandem repeat sequence variation and length heteroplasmy in the mitochondrial DNA D-loop of the threatened Gulf of Mexico sturgeon, *Acipenser oxyrhynchus desotoi*. *J. Heredity* 86:22-27.

Campton, D.E. 1992. Heritability of body size of green swordtails, *Xiphophorus helleri*. Sib analyses of males reared individually and in groups. *J. Heredity* 83:43-48.

Campton, D.E., F.W. Allendorf, R.J. Behnke, and F.M. Utter; M.W. Chilcote, S.A. Leider, and J.J. Loch. 1991. Reproductive success of hatchery and wild steelhead. *Trans. Am. Fish. Soc.* 120:816-827.

Campton, D.E. and G.A.E. Gall. 1988. Responses to selection for body size and age at sexual maturity in the mosquitofish, *Gambusia affinis*. *Aquaculture* 68:221-241.

Campton, D.E. 1987. Natural hybridization and introgression in fishes: methods of detection and genetic interpretations, p.161-192. *IN:* N. Ryman and F. Utter (eds.), *Population Genetics and Fishery Management*. Washington Sea Grant College Program and University of Washington Press, Seattle, Washington.

DIANE G. ELLIOTT

Title: Research Microbiologist, GS-13

Current employer: BRD, U.S. Geological Survey, Western Fisheries Research Center, Seattle.

Current responsibilities: Project leader and principal investigator for fish health research projects, particularly those involving bacteriological and histopathological procedures.

Education

Ph.D., School of Fisheries, University of Washington, 1985

M.S., School of Fisheries, University of Washington, 1976

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

Recent positions

Research Microbiologist, Project Leader, Western Fisheries Research Center, USGS (formerly NBS, USFWS), 1986-present

Affiliate Associate Professor, University of Washington, Seattle, 1995-present

Affiliate Assistant Professor, University of Washington, Seattle, 1986-1995

Professional certification

Certified Fish Pathologist, Fish Health Section/American Fisheries Society

Expertise

Epizootiology and pathogenic mechanisms of important fish pathogens, particularly bacterial diseases of salmonid fishes. Her research emphasizes the development of more rapid and sensitive methods for detecting bacterial pathogens in fish and in the environment, and the development of improved methods for controlling the diseases caused by these pathogens.

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