

F1-017



United States Department of the Interior

NATIONAL BIOLOGICAL SERVICE

Northwest Biological Science Center
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Seattle, Washington 98115
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24 July 1997

Ms. Kate Hansel
CALFED Bay-Delta Program
1416 Ninth Street, Suite 1155
Sacramento, CA 95814

Dear Ms. Hansel,

Enclosed please find 10 copies of each of three proposals from the Northwest Biological Science Center. The proposals have been prepared and are being submitted in accordance with guidelines given in the CALFED Bay-Delta Program 1997 Category III Request for Proposals.

Sincerely,

James R. Winton. Ph.D.
Acting Center Director

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I. Executive Summary

a. Project Title: **Immune Indicators for Monitoring and Assessing Fish Health in the Bay-Delta Ecosystem.**

Applicant Name: Ronald J. Pascho, U.S. Geological Survey, Biological Resources Division, Northwest Biological Science Center, 6505 N.E. 65th Street, Seattle, Washington 98115.

b. Project Description and Primary Objectives. The overall objective of this project is to provide biologists and managers in the CALFED Bay-Delta Program with critical information about the potential for environmental contaminants to impair the defense mechanisms of high priority salmonid species. The project will investigate the effects of both natural (disease) and anthropogenic (contaminant) stressors on the immune function and subsequent ability of salmonids to resist infection by *Vibrio anguillarum* or infectious hematopoietic necrosis virus (IHNV), two important pathogens of the priority species in the Sacramento and San Joaquin Rivers and in San Francisco Bay. The anticipated products of the study are: (1) A method for fishery managers to assess the risk of contaminant exposure on the health and survival of anadromous salmonids, (2) a quantitative assessment of the relationship between contaminant exposure, immune function, and the ability of salmonids to resist infection by pathogenic microorganisms, and (3) important information to assist in the recovery of endangered and threatened stocks of salmonids in the Sacramento and San Joaquin Rivers and the San Francisco Bay-Delta Region.

c. Tasks and Schedule. The proposed study will require 3 years to complete and is divided into two elements. In the first element, an *in vitro* assay for measuring immune suppression will be modified to screen anthropogenic stressors (contaminants) present in the Sacramento, San Joaquin and San Francisco Bay regions for their ability to impair leucocyte function of healthy salmonids. During the second year, we will conduct a series of laboratory disease challenges to investigate the effect of contaminant exposure on the ability of healthy fish to resist infection by IHNV and *V. anguillarum*. Anthropogenic stressors will include polycyclic aromatic hydrocarbons, polychlorinated biphenyls, heavy metals, and pesticides. To correlate results from the *in vitro* assay and laboratory studies with what is occurring in the environment, we will conduct two field studies in the Sacramento and San Joaquin River systems. The first will be a 3-year monitoring program in which we will collect juvenile chinook salmon from 24 locations and measure the levels of selected contaminants. The second study (during year 3), will test tissues from healthy juvenile salmon held at various locations in the watersheds to determine the rate of contaminant uptake and to assess if exposure to contaminants under natural conditions affects the ability of salmonids to respond to an immune stimulus. These field studies will provide the first comprehensive information on contaminant levels in anadromous salmonids from the study regions.

The second research element will be coordinated by staff of the California-Nevada Fish Health Center, Coleman National Fish Hatchery (USFWS) in conjunction with other USFWS biologists, and biologists from the California Dept of Fish and Game. We will test fall chinook salmon from various locations in each of 3 years to measure the prevalence and levels of common fish pathogens, and to measure energy reserves, smolt development, plasma protein levels, and organosomatic indices. Information gained from this research element will be important for understanding the relationship between fish health, disease resistance, and environmental anthropogenic stressors. The completion of

these research elements will provide solid information on the health status of salmonids in the Sacramento-San Joaquin and San Francisco Bay ecosystems.

- d. **Justification.** This research is critical to the understanding of factors affecting the health and survival of threatened and endangered salmonids in the Sacramento-San Joaquin and San Francisco Bay ecosystems. There is increasing evidence that anthropogenic contaminants can have serious negative effects on the ability of salmon to resist enzootic diseases. The *in vitro* assays and laboratory studies proposed represent standard and well-accepted methods for determining the immune status and disease resistance of fish. The contaminants selected will be those that are present at high levels in the system. The bacteria selected represent the most important pathogens of the priority salmonids in fresh water (*R. salmoninarum*) and in estuaries (*V. anguillarum*). *Renibacterium salmoninarum* is particularly important because of the high prevalence of this organism among salmonids in the Western United States, including the winter-run chinook salmon in the captive propagation program and the Bodega Marine Laboratory. Infectious hematopoietic necrosis virus contains to be a limiting factor in the propagation of chinook salmon at Coleman NFH on Battle Creek, a tributary of the Sacramento River.
- e. **Budget Costs and Third Party Impacts.** For the first year of the proposed study, \$453,534 is requested from CALFED. The USGS will provide \$92,750 of cost-sharing in the form of non-reimbursable salaries, and laboratory facilities for immunology, virology, bacteriology, and toxicology research. The completion of this study will have broad third party impacts because it will assist state and federal fisheries managers in meeting their goals for restoring listed or endangered stocks.
- f. **Applicant Qualifications.** This research will be conducted by a team with exceptional qualifications to complete the proposed work. The Northwest Biological Science Center has a 60 year history of work in infectious diseases of salmonids. The proposed study includes collaborators from the national Environmental and Contaminants Research Center of the USGS. The Coleman Laboratory is one of the National Fish Health Centers of the USFWS. Together, the principal investigators have authored more than 200 publications in this area.
- g. **Monitoring and Data Evaluation.** Standard statistical methods will be used analyze data from the field studies and laboratory investigations. All reports will be peer-reviewed under the guidelines of the strategic science plan for the Biological Resources Division (USGS) before submission to CALFED.
- h. **Local support and coordination.** This proposal includes the participation of Dr. Scott Foott from the U.S. Fish and Wildlife Service Fish Health Center at the Coleman National Fish Hatchery and Dr. Michael Saiki from our Dixon, California field station. Both these researchers have exceptional support from local agencies and will be invaluable in coordinating the sampling and field studies to be conducted in the Sacramento and San Joaquin Rivers and the San Francisco Bay-Delta Region.

Immune Indicators for Monitoring and Assessing Fish Health in the Bay-Delta Ecosystem

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Type of Organization: Federal Agency, tax exempt.

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Project Group: Group 3, Services.

III. Project Description

a. Project Description and Approach. The principle goal of the proposed study is to provide biologists and managers in the CALFED Bay-Delta Program with critical information about the potential for environmental contaminants to impair the defense mechanisms of priority salmonid species.

In the first research element, an *in vitro* method for measuring immune suppression will be modified to specifically screen anthropogenic stressors (contaminants) for their ability to impair the leucocyte functions of healthy salmonids. To correlate the results of the *in vitro* analyses with what may happen in the environment, we will conduct two field studies in the Sacramento River and San Joaquin River watersheds. The first will be a monitoring program in which we measure the levels of selected contaminants in juvenile chinook salmon at 24 locations. In the second study, tissues from healthy juvenile salmon held at various locations in the watersheds will be tested in the *in vitro* assay to determine if exposure to contaminants under natural conditions affected their ability to respond to an immune stimulus. This will provide the first information about contaminant levels in the study areas.

To examine the role of anthropogenic stressors in disease transmission among hatchery and wild salmonids, we propose a series of laboratory disease challenges to investigate the effect of contaminant exposure on the ability of healthy fish to resist infection by the freshwater fish pathogen; infectious hematopoietic necrosis virus, and the estuarine bacterium, *Vibrio anguillarum*. Anthropogenic stressors will include at least one polycyclic aromatic hydrocarbon, one polychlorinated biphenyl, one heavy metal, and one pesticide. This segment of the study will also investigate the consequences of healthy or diseased fish entering a polluted estuary and encountering a ubiquitous marine pathogen such as *V. anguillarum*.

The second research element will be coordinated by personnel at the California-Nevada Fish Health Center, Coleman National Fish Hatchery (USFWS) in conjunction with other USFWS biologists, and biologists from the California Dept of Fish and Game. We propose to test fall chinook salmon from various locations to measure the prevalence and levels of common fish pathogens, and to measure energy reserves, smolt development, plasma protein levels, and organosomatic indices. Information gained from this research element will be important for understanding the relationship between fish health, disease resistance, and environmental anthropogenic stressors. The completion of these research elements will provide solid information on the health status of salmonids in the Sacramento-San Joaquin and San Francisco Bay ecosystems.

Element 1. Determine the effects of pathogenic microorganisms and contaminant exposure on the immune function and disease resistance of anadromous salmonids.

Task 1.1. Monitor the prevalence and levels of contaminants in the tissues of juvenile salmonids migrating from the Sacramento River and San Joaquin River basins.

A systematic monitoring program will be implemented at up to 24 locations in the Sacramento and San Joaquin River basins, and the San Francisco Bay area (Table 1). Three composite samples (10 fish each) from each site will be analyzed for contaminants at the Environmental and Contaminants Research Center (USGS), Columbia, Missouri. Measurements will include selected metals, PAH metabolites, PCB congeners, and organochlorine pesticides that are most likely to occur in aquatic organisms within the study areas.

Task 1.2. Measure the effect of a single anthropogenic stressor on susceptibility of juvenile salmonids to *Vibrio anguillarum* and infectious hematopoietic necrosis virus.

Subtask 1.2.1. Determine the median lethal dose (LD50) for each anthropogenic stressor.

Subtask 1.2.2. Measure the effect of contaminant exposure on the ability of juvenile chinook salmon to resist infection by infectious hematopoietic necrosis virus.

Subtask 1.1.3. Measure the effect of contaminant exposure on the ability of juvenile chinook salmon to resist infection by *V. anguillarum*.

Task 1.3. Measure the effects of multiple stressors on the susceptibility of juvenile salmonids to *V. anguillarum*.

Subtask 1.3.1. Measure the cumulative effects of infection by *R. salmoninarum* and contaminant exposure on the susceptibility to infection by *V. anguillarum*.

Subtask 1.3.2. Measure the effect of infection by *R. salmoninarum* on the susceptibility to infection by *V. anguillarum*.

Task 1.4. Characterize the effects of anthropogenic stressors on the immune response of salmonids by *in vitro* methods.

Subtask 1.4.1. Monitor the immune functions of fish exposed to different anthropogenic stressors under laboratory conditions.

Subtask 1.4.2. Standardize an *in vitro* method to characterize the effects of anthropogenic stressors on the immune system of salmonids; *in vitro* immunization and monitoring of cultured spleen leucocytes.

Subtask 1.4.3. Screen various industrial, urban, and agricultural contaminants *in vitro* for immunosuppressive effects on the salmonid immune system.

Task 1.5. Compare the immune status of fish from groups held at various locations in the Sacramento River and San Joaquin River basins.

Subtask 1.5.1. Compare the relative *in vitro* immune responsiveness of spleen leucocytes from fish in the groups held at various locations.

Subtask 1.5.2. Measure the levels of contaminants in organs or tissues from fish exposed to contaminants.

Element 2. Monitor the health of juvenile salmon in the Sacramento River and San Joaquin River watershed regions.

Sample sites will coincide with selected sites among those proposed for Element 1, including the upper Sacramento River below the Red Bluff Diversion Dam, the lower Sacramento River at Knight's Landing, the San Francisco Bay delta at Chipps Island, and the lower San Joaquin River northwest of Stockton, CA. Sampling would begin during March and end prior to the hatchery releases during April. Up to 60 fall chinook salmon will be sampled at each site by rotary screw traps, seining or trawl; no more than 120 fish will be sampled from each river basin. No fish will be collected from stocks identified as endangered, listed, or in low abundance. Non-salmonid fish, such as cottids, cyprinids, catostomids, and tule perch, captured during sampling will also be tested for common fish pathogens.

Task 2.1. Measure the prevalence and levels of selected bacterial, viral, and protozoan pathogens in hatchery and wild fall chinook salmon.

Task 2.2. Measure the physiological status of hatchery and wild fall chinook salmon.

Task 2.3. Monitor the physiological and fish health status of hatchery fall chinook salmon at various points during their migration to the Pacific Ocean.

Subtask 2.3.1. Monitor the physiological and fish health status of hatchery fall chinook salmon before release, and during their migration to the Pacific Ocean.

Subtask 2.3.2. Monitor changes in the physiological and fish health status of hatchery fall chinook salmon held at various locations in the San Francisco Bay delta.

Table 1. Proposed data-collection sites to measure the levels of contaminants in resident juvenile salmonids. Up to 20 fish will be sampled at each site.

Sacramento River	Redding Anderson Jellys Ferry Road Red Bluff Colusa Meridian Knights Landing Sacramento Metro Airport at I-5 Port of Sacramento Freeport
San Joaquin River	Hills Ferry Road Durham Ferry State Rec Area Port of Stockton
San Francisco Bay	Honker Bay at Chipps Island Carquinez Strait at Martinez
Sacramento-San Joaquin Tributaries	Battle Creek at Jellys Ferry Road Butte Creek at Butte Slough Road Feather River at Drescher Road American River at Highway 160 Mokelumne River at I-5 Calaveras River at I-5 Stanislaus River at Caswell Park Tuolumne River at Shiloh Road Merced River at Hatfield Rec Area

b. Location of Project. The proposed study includes tasks that would be completed in the Sacramento River Watershed Region, the San Joaquin River Watershed Region, and the San Francisco Bay Region. Laboratory and field study locations are outlined below.

Element 1.

Task 1.1. Northwest Biological Science Center (USGS), Dixon Duty Station, Dixon, CA. Sample locations in the Sacramento and San Joaquin River basins, and the San Francisco Bay delta. Contaminant analyses at the Environmental and Contaminants Research Center (USGS), Columbia, MO.

Tasks 1.2, 1.3 & 1.4. Northwest Biological Science Center (USGS), Seattle, WA.

Task 1.5. Northwest Biological Science Center (USGS), Dixon Duty Station, Dixon, CA. Livebox locations in the Sacramento and San Joaquin River basins.

Element 2.

Tasks 2.1, 2.2, & 2.3. Coleman National Fish Hatchery (USFWS), Anderson, CA.

Task 2.3. Livebox locations in the Sacramento and San Joaquin River basins, and the San Francisco Bay delta.

c. Expected Benefits. The proposed study focuses primarily on the water quality stressor category. Data from this study will also be pertinent to the flows and other effects of water management category, and to the population management category. All priority habitats important for the migration and survival of fall-run chinook salmon, winter-run chinook salmon, spring-run chinook salmon, late-fall run chinook salmon, and steelhead trout are relevant to this study. The primary benefits of this study are a quantitative assessment of the spatial distribution of contaminants in the Sacramento and San Joaquin River basins, and methods to assess the effects of those contaminants on the immune system of priority salmonid species. Data from this project will also benefit the U.S. Fish and Wildlife Service Central Valley-San Francisco Bay Ecoregion Plan under section HP-5: Double the natural production of anadromous fish in the Central Valley, and section HP-7: Impacts of hatchery programs on natural anadromous fish populations.

d. Background and Biological Justification. Anadromous salmonids are susceptible to a variety of bacterial and viral pathogens (Rohovec et al. 1988). Whereas the effect of microorganisms on salmonids in natural rearing areas is difficult to measure, losses from disease among hatchery fish are both common and well-documented. The presence of a pathogen will not always result in disease and fish can remain asymptomatic carriers under favorable conditions (Thoesen 1994). However, stressors exceeding the limits of physiological tolerance will diminish a fish's ability to resist disease (reviewed by Barton and Iwama, 1991). In a review of stress and fish physiology, Wedemeyer *et al.* (1981) emphasized that disease is not the result of a single event, rather the consequence of an imbalance in the host-pathogen relationship. For fish, this relationship is especially sensitive to environmental factors. In the hatchery, these factors include practices such as handling, high rearing densities, and inattention to water temperature or water quality. Among wild fish, toxicants, contaminants, adverse temperatures and poor water quality have important effects on health.

Management of salmonids in the Western United States includes methods to reduce the prevalence and level of pathogens through improved hatchery practices. For example, bacterial kidney disease (BKD) is a widespread, chronic, and often devastating disease, caused by *Renibacterium salmoninarum*. Development of new detection methods has given managers new control measures, such as brood stock segregation (Pascho et al. 1991), that show promise for reducing transmission of the bacterium among fish in fresh water. Unfortunately, little is known about the effect of BKD on survival after fish enter the estuary or ocean. Whereas some fish may

recover from *R. salmoninarum* infections (Pascho et al. 1991), it has been reported fish infected in fresh water may die after they enter salt water (Fryer and Sanders 1981; Banner et al. 1983; Sanders et al. 1992). Similarly, infectious hematopoietic necrosis virus (IHNV) is the most important viral pathogen of salmonids in the western United States (Winton 1991). Explosive losses have been recorded at hatcheries in Alaska, Washington, Idaho, Oregon and California while large outbreaks among wild fish have been observed in Oregon and Alaska. The virus is easily transmitted through the water and once infected, fish may shed high levels of virus for a substantial period (Wolf 1988). Chinook salmon released from the Coleman National Fish Hatchery on the Sacramento River have an estimated IHNV carrier rate of 10-20% and infected fish have been recovered as far as 183 miles downstream (Scott Foott, Coleman Fish Health Center, personal communication). The incidence of IHNV among fish produced in the wild or by California State hatcheries is essentially nil, leaving the possibility that fish from the Coleman Hatchery may transmit IHNV to other threatened or endangered stocks during their downstream migration.

The San Francisco Bay, together with the Sacramento and San Joaquin River Deltas, form one of the largest estuaries in the Western United States. Located in highly populated region at the mouth of the Sacramento and San Joaquin Rivers, the bay is impacted by pollutants from municipal, agricultural, mining, and industrial activities (Luoma and Phillips 1988; Setzler-Hamilton et al. 1988; Cashman et al. 1992; Long and Markel 1992). Some chemical pollutants are at levels shown to be toxic to resident invertebrates, urchins, bivalves, and bottom dwelling fishes (Reviewed by Long and Markel 1992). These have also been associated with declines in the striped bass (*Morone saxatilis*) population over the past two decades (Setzler-Hamilton et al. 1988; Cashman et al. 1992). The biological effects pollutants in the water, sediments, or biota on salmonids migrating from the Sacramento River and San Joaquin River watershed regions, however, are poorly understood. Wild and hatchery salmonids leave these areas and use the estuary to complete the physiological changes necessary for adaptation to seawater, making exposure to pollutants, or a ubiquitous marine pathogen such as *Vibrio anguillarum*, potentially very harmful.

Exposure of juvenile salmon to pollutants or chemical contaminants may result in a significant shift in the host-pathogen relationship among fish infected in fresh water (e.g. *R. salmoninarum* or IHNV) or in estuaries (e.g. *V. anguillarum*). The effect of contaminants on disease resistance of salmonids is poorly understood, and often viewed as of little importance because migrating salmonids remain in the estuary a relatively short time compared with resident species. Recent studies, however, have shown that immunodeficiencies associated with estuarine pollution can occur in salmon (Arkoosh et al. 1991, 1994), and there is evidence that exposure to copper may increase a fish's susceptibility to pathogens (Hetrick et al. 1979; Knittel 1981; McFarlane et al. 1986; Anderson et al. 1989). Therefore, the ability of fish to resist disease in the estuarine or marine environment may be greatly influenced by their ability to acclimate to both natural or anthropogenic stressors.

Many stocks of Pacific salmon are declining and over 200 naturally-spawning stocks have been identified at risk of extinction (Nehlsen et al. 1991). The declines are often attributed to loss of habitat due to urban growth, agriculture, hydroelectric power and harvesting of natural resources such as lumber and minerals, overfishing, and the introduction of nonnative species. The National Marine Service listed the Sacramento River winter-run chinook salmon (*Oncorhynchus tshawytscha*) as endangered under the Endangered Species Act. Recovery of endangered stocks through habitat restoration and natural production is considered a high priority because such an approach avoids many of the genetic, behavioral, and disease concerns associated with rearing salmonids in captivity. Kincaid (1993) cautioned, however, that the time necessary for restoration through natural production may be so long as to present an even greater risk of extinction for the population. Because the numbers of chinook salmon returning to the Sacramento River basin had been significantly reduced, captive broodstock propagation was selected for restoration of the

stock. Captive propagation differs from conventional hatchery culture in that the fish are reared for their entire life cycle in captivity. Juvenile fish produced by such a program are then used to supplement the native population.

Flagg and Mahnaken (1995), in a review of broodstock technology for Pacific salmon, highlighted the importance of understanding the complex relationships between fish nutrition, reproductive physiology, and fish health when developing fish husbandry practices. An ideal fish health management program for captive salmonid propagation, such as that now used for the Sacramento River winter-run chinook salmon, will have its foundation in a rearing environment that provides the most favorable conditions for growth and survival. Such conditions are very important because they help to maintain fish at their best physiological status to resist infection by pathogenic microorganisms, many of which are currently considered untreatable because of the lack of effective chemotherapeutics or vaccines.

In order to better understand the host defenses of salmon, and whether they are modulated by changes in season, life stage, or rearing conditions, researchers at the Northwest Biological Science Center in Seattle, Washington are developing a panel of hematological, immunological, and serological assays that can be used to measure the effects of different environmental conditions on the immune system of captively-reared salmonids, including chinook salmon. These data can provide fishery biologists and managers with important information on the effects harmful environmental conditions on the general immunological health of salmonids at various points during their life cycle.

To provide information on how, and to what extent, pollutants affect the defense mechanisms of fish, a method for *in vitro* immunization of cultured fish leukocytes is being adapted to the study of anthropogenic stressors. *In vitro* immunization is often used for the study of defense mechanisms in mice (Mishell and Dutton, 1966), and a modified version has been described for use with salmonids (Kaattari et al, 1986). Interpreting results from testing live fish is often difficult because salmonids are genetically diverse leading to a high degree of variability in their protective responses. By testing multiple samples of a leucocyte preparation from a single fish, much of that variation is removed. In addition, the researcher has greater control over the cellular environment, including temperature, the types of cells cultured, and the concentrations of specific pollutants. Previous studies with the *in vitro* immunization have demonstrated the immunomodulatory effects of copper (Anderson et al. 1989), zinc and manganese (Ghanmi et al. 1989) and aflatoxin B1 (Arkoosh and Kaattari, 1987) on salmonid fishes.

The proposed study is a new project. It will investigate the interactive effect of a natural stressor (an endemic disease, such as BKD) and an anthropogenic stressor (contaminants present in waters of the Sacramento or San Joaquin Rivers), such as a polycyclic aromatic hydrocarbon (PAH), a polychlorinated biphenyl (PCB) or a pesticide on immune function, and subsequently on the ability of salmon to resist infection by *V. anguillarum* or IHNV. The bacteria were selected because they represent common fish pathogenic microorganisms that infect fish in fresh water (*R. salmoninarum*) and in seawater (*V. anguillarum*). *Renibacterium salmoninarum* is particularly important because of the high prevalence of this organism among salmonids in the Western United States, including the winter-run chinook salmon in the captive propagation program and the Bodega Marine Laboratory. Infectious hematopoietic necrosis virus contains to be a limiting factor in the propagation of chinook salmon at Coleman NFH on Battle Creek, a tributary of the Sacramento River. The overall goals of the proposed study are to: (1) provide fishery managers with methods to assess the risk of contaminant exposure on the health and survival of anadromous salmonids, and (2) to determine the relationship between contaminant exposure, immune function, and the ability of salmonid fishes to resist infection by fish pathogenic microorganisms.

e. Proposed Scope of Work. The duration of the proposed study is 3 years. Tasks are to be completed according to the schedule outlined in Section IVb, Schedule Milestones. Interim

progress reports will be provided an annual basis.

Monitoring: Tasks 1.1 and 2.1, 2.2, and 2.3. Contaminant and pathogen monitoring will be completed each year for the duration of the study.

Contaminant exposure under laboratory conditions: Tasks 1.2 and 1.3. Contaminants for the single and multiple stressor laboratory experiments will be selected on the basis of the first years monitoring. To provide test fish, eggs from BKD-negative and IHNV-negative parents will be reared at Coleman National Fish Hatchery, Anderson, CA, or at the Northwest Biological Science Center, Seattle, WA.

Development and field testing of *in vitro* immunization assay: Tasks 1.4 and 1.5.

Optimization of the *in vitro* immunization assay will begin immediately. Screening of contaminants identified in Task 1.1 will be completed during years 2 and 3. The evaluation of tissues from fish held at the field sites will be completed during year 3; this evaluation will require installation of cell culture equipment at the Dixon Duty Station.

f. Monitoring and Data Evaluation. Analysis of variance (ANOVA) and Fisher's protected least-significant-difference test will be used to determine differences among means for contaminant parameters of fish from the various sample locations. A single factor nested ANOVA (P 0.05) will be used to compare means among treatment groups for certain nonspecific immune parameters. For disease monitoring, the G-statistic (P 0.05) will be used to test the null hypothesis that the pathogen frequency at given sample station does not differ from the average pathogen frequency for all sites sampled (for the particular pathogen being analyzed). The median lethal dose (LD50) for each anthropogenic stressor tested in the laboratory trials will be determined based on analyses with the Kaplan-Meier estimator. All reports will be peer-reviewed under the guidelines of the strategic science plan for the Biological Resources Division (USGS) before submission to CALFED.

g. Implementability. No problems are anticipated with the implementability of this research project. As a Federal Agency, our laboratory is particularly sensitive to State and Federal regulations involving trespass on private land and collection of threatened or endangered species. By agency directive, we are required to obtain written permission to enter private land and for projects involving salmon in the Columbia River, and we have worked closely with the National Marine Fisheries Service to obtain the required take permits before sampling threatened or endangered species. The monitoring portion of this project is not anticipated to involve access to private land and we will use surrogate stocks for the laboratory portions of this research. The in-river sampling of salmonids is being conducted with the assistance of Dr. Scott Foott of the USFWS who is collecting fish under a takings permit issued by the National Marine Fisheries Service as part of a wild fish survey.

IV. Costs and Schedule

a. Budget Costs. Year 1.

1. Reimbursable Salaries

Lead Technician (NWBSC-Seattle, GS-9, 12 m-m)	31,535
Benefits (30%)	9,461
Technician (Cal-Nev FHC, GS-9, 5 m-m)	13,140
Benefits (30%)	3,942
Technician (NWBSC-Seattle, GS-7, 12 m-m)	25,779
Benefits (30%)	7,734
Technician (NWBSC-Dixon, GS-7, 6 m-m)	12,882
Benefits (30%)	3,865
Technician (NWBSC-Dixon, GS-5, 6 m-m)	10,400
Benefits (30%)	3,120
Total Reimbursable Salary Costs.....	121,858

2. Travel

Transportation and per diem (coordination meetings and trips to collect samples and perform experiments):

Total costs for travel	9,791
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3. Reimbursable equipment and Supplies

1). Non-expendable

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2). Expendable

Contaminant monitoring and analysis:	
Monitoring	5,000
Organic Analyses	140,000
Metal Analyses	24,000
Fish health and physiology monitoring	6,000
Immunological assays	22,000
Total Costs for Expendable Supplies.....	197,000

4. Publication and reproduction costs.....**2,000**

5. Total Direct Costs.....**328,648**

6. Agency Support Costs (38%)**124,886**

7. Project Costs.....**453,534**

b. Cost sharing. In-kind services contributed by the USGS.

1. Contributed nonreimbursable salaries

Mr. Ronald J. Pascho (GS-12, 6 m-m)	28,900
Dr. Jim Winton (GS-14, 1 m-m)	7,350
Dr. Michael Saiki (GS-13, 6 m-m)	33,000
Total Non-Reimbursable Salary Costs	69,250

2. Equipment and facilities

Laboratory facilities: Immunology, virology, bacteriology, and toxicology	13,500
Wet laboratory facilities: support for fish rearing and experimental challenges	10,000
Total Non-Reimbursable Equipment/facilities.....	23,500

3. Total cost-sharing.....92,750

c. Schedule Milestones. The relative start and completion dates for the research tasks in Elements 1 and 2 are outlined in the following table. Further details are given in Section III.c., Proposed Scope of Work.

		Year 1	Year 2	Year 3
Element 1. Salmonid immune function				
Task 1.1	Field study; contaminant monitoring			
Task 1.2	Single stressor laboratory study			
Task 1.3	Multiple stressor laboratory study			
Task 1.4	<i>In vitro</i> immunization assay			
Task 1.5	Field study			
Element 2. Fish health and physiology				
Task 2.1	Field study; pathogen monitoring			
Task 2.2	Assessment of physiological status			
Task 2.3	Field study			

- d. **Third Party Impacts.** The completion of this study will have broad third party impacts because it will assist state and federal fisheries managers in meeting their goals for restoring listed or endangered stocks.

V. Applicant Qualifications. The overall administrative responsibility for completion of the project will be with Dr. Shipley, the Center Director of the Northwest Biological Science Center (NBSC) in Seattle, WA. Dr. Winton and Mr. Pascho will assume primary responsibility for organization and completion of all tasks, and for preparation of the annual reports. Contaminant monitoring will be coordinated by Dr. Michael Saiki at the Dixon Duty Station of the NBSC. Processing and contaminant analyses will be completed at the Environmental and Contaminants Research Center in collaboration with Dr. Carl Orazio. Fish sampling for pathogen testing and physiological measurements will in collaboration with Dr. J. Scott Foot at the California-Nevada Fish Health Center. Personnel at the fish health center will also be responsible for laboratory analyses necessary to complete these tasks.

The tasks outlined in this project proposal will be completed at three facilities in California, Washington, and Missouri:

Northwest Biological Science Center (NBSC), Seattle, WA, and Dixon, CA. The Seattle laboratory represents a state-of-the-art facility for work on infectious diseases of fish. Facilities available include over 16,000 square feet of new dry laboratory space for virology, cell, bacteriology, immunology, histology, parasitology, and molecular biology. Within the dry lab complex is a special, restricted access Biosafety Level 3 laboratory containing isolated dry and wet laboratories for work with exotic fish pathogens. The NBSC-Seattle also houses a new 9,000 square foot wet laboratory 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 Dixon Duty Station is equipped with boats and a variety of fish-sampling gear suitable for collecting juvenile salmonids and other fishes in the Sacramento-San Joaquin River systems and the San Francisco Bay-Delta. This station also contains all of the laboratory space, equipment, and supplies needed to process, store, and ship samples of whole fish and fish tissues to analytical laboratories operated by the USGS.

Environmental and Contaminants Research Center (ECRC), Columbia, MO. The ECRC has the capacity for focused and large-scale multidisciplinary studies in aquatic ecology and toxicology, and analytical chemistry. ECRC is the only USGS research laboratory capable of providing testing and analytical chemistry research for studies of highly toxic compounds, such as dioxin. A 4,000 square foot high hazard assessment laboratory is available which permits investigations of these highly toxic chemicals that are too hazardous to test in a normal laboratory situation.

California-Nevada Fish Health Center, Coleman National Fish Hatchery, Anderson, CA. This facility, operated by Region 1 of the U.S. Fish and Wildlife Service, represents a fully-equipped laboratory for the diagnosis of infectious diseases of fish and for determination of physiological indicators of fish health. Staffed by a Fish Health Specialist and technical staff, the laboratory is routinely involved with collection of fish from Federal Hatcheries and examining them for the presence of fish pathogens using the latest techniques. In addition, the laboratory is an active participant in a newly-funded USFWS wild fish health survey that will be an important part of the sampling program in this proposal.

Following are brief biosketches of the three principle investigators for the proposed study:

Dr. James R. Winton earned a Ph.D in microbiology from Oregon State University in 1981.

Dr. Winton's position at NBSC is Chief, Fish Health and Environmental Studies. During the last 10 years, the fish health research team has grown to consist of more than 25 scientists, technicians, graduate students and visiting researchers working on the most important infectious diseases of Pacific salmon and trout including infectious hematopoietic necrosis, viral hemorrhagic septicemia, bacterial kidney disease, and whirling disease. The team makes use of the newest techniques in molecular biology (including cloning, sequencing, fingerprinting, monoclonal antibodies, DNA probes and the polymerase chain reaction) to conduct research in: 1) improving the speed and precision of detection of important pathogens of salmonid fish; 2) obtaining critical information about the epidemiology of fish pathogens; and 3) developing control strategies for reducing the losses caused by infectious diseases among populations of fish. Dr. Winton is an affiliate professor of fisheries at the University of Washington and has served the Fish Health Section of the American Fisheries Society as President and as Editor of the Newsletter. He is subject editor for fish pathology for the *Journal of Applied Ichthyology* and an editorial advisor for *Diseases of Aquatic Organisms*. He is a Certified Fish Pathologist, Canadian Fish Health Officer, U.S. Title 50 Inspector and serves on the International Committee on Taxonomy of Viruses, the American Type Culture Collection Advisory Committee, and the Fish Disease Commission of the Office of International Epizooties in Paris, France. He is an author of more than 85 scientific publications.

Mr. Ronald J. Pascho has a B.S. in microbiology and a M.S. in Fisheries from the University of Washington, and been employed by the Department of Interior for over 20 years in fish microbiology. Mr. Pascho's major areas of research have focused on the development and application of new detection methods in the study of important pathogenic microorganisms of Pacific salmon. He developed a sensitive enzyme-linked immunosorbent assay (ELISA) that detects specific proteins released by *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease in trout and salmon, during an infection. This test is currently viewed as the state-of-the-art method for measuring the prevalence and levels of *R. salmoninarum* infection. The incumbent coauthored and serves as a principal investigator for a U.S. Army Corps of Engineers-funded project that examines the effect of BKD infections on the survival of juvenile salmonid fishes that are transported around dams on the Columbia and Snake Rivers. Recently, he began a new program to investigate whether the nonspecific host defenses of sockeye salmon (*Oncorhynchus nerka*) modulate with seasonal and lifestage changes, or differing rearing conditions. This research is part of a BPA-funded study investigating captive propagation as a method to restore endangered or threatened salmonid stocks.

Michael K. Saiki earned a Ph.D. in Biology (emphasis on fishery biology) in 1976 from the University of Arizona. Dr. Saiki has nearly 20 years of working experience in aquatic contaminant issues affecting chinook salmon and other fishes in the Sacramento and San Joaquin river systems. His past work involved field and laboratory studies that identified or examined contaminant hotspots and ecotoxicological effects associated with selenium and other chemical constituents in agricultural subsurface drainwater. He has also conducted field investigations of heavy metals associated with acid-mine drainage. Since 1991, Dr. Saiki has received more than \$750,000 in competitive grants and contracts to support his research program. Results from his studies have appeared in 21 peer-reviewed publications.

VI. Compliance with standard terms and conditions. All terms and conditions stated in the CALFED RFP that are applicable to federal agencies are agreeable to the applicant.

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