99D-120

## 4.5 PSP Cover Sheet (Attach to the front of each proposal)

Proposal Title: Effects of Contaminants in the Catchment of the San Francisco Bay Estuary on Reproductive Success of Adult, and Health and Fitness of Juvenile Chinook Salmon *(Oncorhynchus tshawytscha)*


Applicant Name: Applied Marine Sciences, Inc.

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Amount of funding requested: $ 745,726.00 for 3 years

Indicate the Topic for which you are applying (check only one box).

- ☐ Fish Passage/Fish Screens
- ☐ Habitat Restoration
- ☐ Local Watershed Stewardship
- ☒ Water Quality
- ☐ Introduced Species
- ☐ Fish Management/Hatchery
- ☐ Environmental Education

Does the proposal address a specified Focused Action? X yes _____ no

What county or counties is the project located in? Multiple, See List in Proposal

Indicate the geographic area of your proposal (check only one box):

- ☐ Sacramento River Mainstem
- ☐ Sacramento Trib: _____
- ☐ San Joaquin River Mainstem
- ☐ San Joaquin Trib: _____
- ☐ Delta: _____
- ☐ East Side Trib: _____
- ☐ Suisun Marsh and Bay
- ☐ North Bay/South Bay: _____
- ☐ Landscape (entire Bay-Delta watershed)
- ☒ Other: Sacramento/San Joaquin River System and Nearshore Ocean to the Farallons.

Indicate the primary species which the proposal addresses (check all that apply):

- ☒ San Joaquin and East-side Delta tributaries fall-run chinook salmon
- ☒ Winter-run chinook salmon
- ☐ Late-fall run chinook salmon
- ☐ Delta smelt
- ☐ Splittail
- ☐ Green sturgeon
- ☐ Migratory birds
- ☐ Other: _____
- ☐ Spring-run chinook salmon
- ☒ Fall-run chinook salmon
- ☐ Longfin smelt
- ☐ Steelhead trout
- ☐ Striped bass
- ☒ All chinook species
- ☐ All anadromous salmonids

Specify the ERP strategic objective and target (s) that the project addresses. Include page numbers from January 1999 version of ERP Volume I and II:

Our proposal addresses more objectives and targets than can be listed in the space provided. Please see Table 1 in the proposal for this information.

Indicate the type of applicant (check only one box):

| | |
|---|---|
| ☐ State agency | ☐ Federal agency |
| ☐ Public/Non-profit joint venture | ☐ Non-profit |
| ☐ Local government/district | ☐ Private party |
| ☐ University | ☒ Other: Private/Public/University Consortium |

Indicate the type of project (check only one box):

| | |
|---|---|
| ☐ Planning | ☐ Implementation |
| ☐ Monitoring | ☐ Education |
| ☒ Research | |

By signing below, the applicant declares the following:

1.) The truthfulness of all representations in their proposal;

2.) The individual signing the form is entitled to submit the application on behalf of the applicant (if the applicant is an entity or organization); and

3.) The person submitting the application has read and understood the conflict of interest and confidentiality discussion in the PSP (Section 2.4) and waives any and all rights to privacy and confidentiality of the proposal on behalf of the applicant, to the extent as provided in the Section.

Robert B. Spies, Ph.D.

Printed name of applicant

Signature of applicant

**Effects of Contaminants in the Catchment of the San Francisco Bay Estuary on Reproductive Success of Adult, and Health and Fitness of Juvenile Chinook Salmon (*Oncorhynchus tshawytscha)***

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April 15, 1999

## Executive Summary

**Project Location**: Chinook salmon in the Sacramento and San Joaquin River systems, the San Francisco Bay/Delta, and the nearshore ocean out to the Farallon Islands. In this proposed 3-year study, fall-run chinook will be collected from the open ocean and Coleman National Fish Hatchery; spawned out-carcasses will be collected from the mainstream and selected tributaries to the Sacramento River. Winter-run chinook will be collected by Coleman National Fish Hatchery personnel and evaluated at the Coleman National Fish Hatchery winter-run propagation facility on the mainstream of the Sacramento River. Laboratory procedures will be performed at the U.S. Fish and Wildlife Service California-Nevada Fish Health Center, Anderson, California, on the campus of the University of California Davis, and at Applied Marine Sciences, Inc., Livermore, California. Juvenile chinook salmon will be collected from multiple locations in the Sacramento/San Joaquin River system, and San Francisco Bay/Delta on their migration to the ocean.

**Primary Ecological Objectives**: This project will evaluate the effects of contaminants in the San Francisco Bay estuary and its catchment, especially the Sacramento River system and its Delta, on the reproductive success, health, and fitness of two important native fish stocks, fall-run and winter-Run chinook salmon. Successful reproduction allows continued survival of any species and this task will focus on impairment by contaminants the portion of the life cycle of chinook salmon in the estuary, from successful spawning after entering the estuary and migrating into the natal streams through survival of smoltification in the juvenile fish returning to sea.

**Relationship of Project to CALFED goals and objectives**: see Table 1.

**Cost**:– Year 1: $292.3K Year 2: $272.2K Year 3: $181.2K

**Adverse and Third Party Impacts**: None.

**Applicant Qualifications**: Dr. Robert Spies is an ecologist and aquatic ecotoxicologist with 20 years experience in evaluation of reproductive impairment of wild fish populations, contaminant analysis and biomarkers of sublethal effect. He has documented links between reproductive success and contaminant exposure in starry flounder from San Francisco Bay and described endocrinological abnormalities in kelp bass in southern California from exposure to o,p'-DDT. Jordan Gold is a marine biologist with Applied Marine Sciences. He has extensive experience in measuring contaminants and effects in multiple marine species. Dr. Gary Marty is a Board-certified veterinary pathologist specializing in contaminant and disease impacts on wild populations of fish. He has 12 years of experience in fish health research. James Haas is a wildlife and fishery biologist with the U.S. Fish and Wildlife Service, and is a Ph.D. student, Graduate Group in Ecology, UC Davis. He has designed and participated in a number of field studies on effects of contaminants on natural resources. Dr. Joe Cech is a fish physiologist, who specializes in swimming performance and metabolism studies. Dr. David Hinton is an aquatic toxicologist and professor at UC Davis in the School of Veterinary medicine. He has 30 years of experience in field and laboratory studies with fishes.



I-019153

**Monitoring and Data Evaluation**: Many fish populations in the San Francisco Bay-Delta system are in serious decline, including winter-run chinook salmon *(Oncorhynchus tshawytscha)*, the striped bass *(Morone saxatilis)*, the Sacramento Splittail *(Pogonichthys macrolepidotus)*, the Longfin Smelt *(Spirinchus thaleichthys)*, the Delta Smelt *(Hypomesus transpacificus)*, Steelhead *(Oncorhynchus mykiss)*, sturgeon *(Acipenser spp)*. and surfperch (Embiotocidae) (Herbold et al., 1992). The reasons for these declines are unclear, as there are numerous anthropogenic and natural factors that could potentially affect fish populations (e.g., see Foe, 1995; Moyle et. al, 1992; Laevastu, 1993). In order for CALFED to restore fish populations in the system, a better understanding of the mechanisms of these declines is needed.

Previous and ongoing monitoring bioassays have identified that acute toxicity to aquatic organisms occurs (Ogle et al., 1996, 1998; Fox and Miller 1996), and suggest that chronic toxicity, including both mortality and sublethal effects (e.g., reproductive effects) probably exists, and could be widespread. If widespread chronic toxicity to fish exists, then such toxicity may contribute to observed fish population declines. If these fish populations are to be restored to their former abundance and health, then such chronic toxicity must be identified and remediated.

**Hypotheses**: The null hypotheses for this project are in Table 2.

**Local Support/Coordination with other Programs, and Compatibility with CALFED Objectives**: The objectives are listed in Table 3.



I-019154

## Project Description

This proposal will assess the effects of contaminants on adult, embryonic, larval, and juvenile, and smolt chinook salmon. We will measure contaminants, various body indices, reproductive hormones, vitellogenin and chorionic (egg shell) protein (i.e., choriongenin, zona radiata protein) in males, egg hatching success, larval survival and juvenile health, inhibition of acetylcholine esterase activity, P4501A content, histopathology, and juvenile fitness. Table 4 summarizes the parameters that will be evaluated on various life stages of chinook salmon and where the fish will be captured for evaluation.

**Task I**: The objective of task 1 is to perform a thorough review of known lethal and sublethal effects of contaminants on fish in the Sacramento and San Joaquin Rivers, the Delta and San Francisco Bay. We will consult the peer-reviewed literature, available unpublished reports, as well as conduct telephone interviews with key experts to assemble all of the information. In addition, we will assemble information on concentrations of contaminants in these ecosystems and compare them with values from the literature on effects of specific contaminants on fish. This information will synthesized in an article for the peer -reviewed literature.

**Task II**: This task will evaluate the adult exposure, maternal transfer to eggs, and subsequent reproductive success of winter-run and fall-run chinook salmon. The objectives of Task II are to: 1. determine baseline contaminant concentrations in liver, gonads, and kidney, and baseline biomarker responses of returning adult salmon when they enter the estuary, 2. determine contaminant concentrations or changes in biomarker responses as the fish migrate upstream, 3. determine if the contaminant concentrations in adult fish are sufficient to adversely affect gamete or egg viability, and 4. if so, determine the effect attributable to an incremental increase in contaminant concentrations after the fish enter the estuary.

**Task III**: This task will evaluate juvenile fish migrating from the freshwater habitat through the estuary. The objective of this task will be to determine if exposures to contaminants are correlated with sublethal effects. Juvenile fish will be captured as they migrate downstream in conjunction with established programs sampling fish populations. Contaminant concentrations will be measured in liver and viscera. Several biomarkers of contaminant exposure, including acetylcholine esterase (AChE) activity in muscle, contaminants and their metabolites in gall bladder, and cytochrome P4501A induction in gill and liver will also be measured. Histopathological analyses will be done to see if structural alterations are related to contaminant concentrations. In addition, swimming performance will be evaluated and compared to contaminant concentrations.

**Project management**: This multi-investigator project will be managed jointly by various faculty and graduate student at UC Davis and by Dr. Robert B. Spies at Applied Marine Sciences. Periodic meetings will be held between all project participants. These meetings will occur at least once a month, and weekly during periods of intense work on the project. These meetings will coordinate literature review, short and long-term project objectives, upcoming activities, field work, logistics, sample distribution, data exchange, adaptive management, out-year planning, report and joint publication preparation. We have an established virtual management network with creative use of e-mail and a worldwide web site.

**Sampling**: We will carry out field collections for this project, but the majority of our collections will be in coordination with existing sampling programs in the CALFED target area. For the evaluation of reproductive fitness, adult chinook salmon returning to hatcheries will be used. Mr. James Haas has obtained permission to use returning adults to the Coleman Hatchery. The IEP

Program regularly samples juvenile salmon in the spring as they migrate down the Sacramento River and we will coordinate our sampling so that we can use fish collected in this program. In addition, we will have access to archived samples from the NMFS (Tiburon Laboratories) through recent programs of smolt evaluation in the Estuary for chemical analyses. They have also promised cooperation in obtaining adult salmon in the ocean that will be collected in their ongoing programs. We will also regularly visit fish screens at points of water diversion from the system in order to obtain live juveniles and smolt in the spring for the various proposed analyses.

**Collection and transport of field specimens**: We will coordinate with biologists from the IEP Program and NMFS for field collections of adult and juvenile chinook salmon. All fish will be collected by netting, mainly with trawls. Field collected fish (N=20 per study group) will be sacrificed immediately or, in the case of the swimming studies placed in large aerated plastic containers for transport to the Aquatics Center at UC Davis where they will be housed in circular aquaria until the time of performance analysis (within 24 hrs).

**Analytical procedures**: Space limitations prevent us from a full description of the many proposed measures, instead we summarize the methods to be used in Table 5 along with the quality assurance measures and data analysis approaches to be used.

**Biomarker selection**: We consider it appropriate to provide a rationale for the selection of some of the proposed biomarkers. In general, we propose a suite of biomarkers in this study that indicate exposure to contaminants; alterations of biochemical reactions and their hormonal controls; and, ultimately, interference of contaminants with physiological processes vital to successful reproduction, development and growth of early life history stages. Selection of measures of contaminant exposure, effects and measures of health and performance is also based on our knowledge of the contaminants in the system and their potential effects

**Acetylcholine esterase activity (AChE)**: One of the most appropriate biomarkers in the Delta, because of the widespread use of organophosphate pesticides in the Central Valley, is inhibition of AChE. Knowledge of the AChE inhibition developed concurrently with widespread use of organophosphorus and carbamate compounds. Both classes of compound were developed to kill pests by inhibiting AChE in pests, and the risk of their use is the same toxic action in non-target species, such as young fish. While the carbamates and organophosphates may have a half life less persistent the environment than organochlorine chemicals, they remain in the alkaline water of the system of 100 or more days. Both classes of compounds or their derivatives disrupt neurotransmitter processes in the central nervous system (Mayer et al. 1992). When salmonids were exposed to ChE inhibitors they alter respiration (Klaverkamp et al. 1977), swimming (Matton and Lattam 1969; Post and Leisure 1974), feeding (Wildish and Lister 1973; Bull and McInerney 1974), and social interactions ( Symons 1973). We propose to use AChE and the ChE (the butyryl analog) as biomarkers of exposure.

**P4501A**: The induction of this enzyme in fish has become accepted as one of the most sensitive and widely used markers of contaminant exposure in the aquatic environment (Stegeman et al., 1991). This enzyme system is induced in a variety of tissues from exposure to petroleum, polynuclear aromatic hydrocarbons (e.g., from urban runoff and atmospheric deposition) and a variety of organochlorine compounds. Little is known about the induction of specific P450 enzymes in fish from pesticide exposures. However, cytochrome(s) P450 convert organophosphates (OPs) to their active forms, the oxon. In Toxicant Identification Evaluations, a blocker of P450 metabolism, piperonyl butoxide, is used and results in protection of test organisms from OP toxicity. It is known that P4501A potentiates the toxicity of PAH and PCBs in fish and it may very well be involved in forming toxic intermediates in pesticide metabolism.



I-019156

The presence of elevated P450 activity in spawning female starry flounder from Central San Francisco Bay has been linked to lowered survival of embryos through hatching, indicating the this marker may provide an important link to sublethal effects in fish (Spies and Rice, 1988).

**Bile Metabolites**: Organic contaminants accumulate in liver of fish, are metabolized to polar compounds and excreated in bile for shipment to gall bladder where water absorption causes them to accumulate several orders of magnitude over their water concentrations. Fluorescence absorbing compounds are estimators of PAH body burden and we will follow NOAA protocols in making these determinations. Analysis of bile fluid has been used widely to detect presence of PAH (Krahn et al., 1984) and is also of use for detecting in a variety of other organic contaminants (Melancon et al., 1992).

**Reproductive Hormones**: Altered levels of testosterone and estradiol, or their rates of production, have been associated with reproductive impairment (Spies and Thomas, 1995). One of several toxic mechanisms may be involved including direct interference of contaminants with hormone receptor, or P450-associated hormone metabolism.

**Vitellogenin and Chorionic Protein**: The presence of these egg yolk precursors or the zona radiata protein (i.e., choriogenin) does not normally appear in plasma of males.- If present, this signals exposure to estrogen mimics (Celius et al., 1999; Purdom et al., 1994). The presence of significant quantities of these compounds in male fish, problems with female fertility, or skewed sex ratios may indicate the need for further investigation of contaminants acting as hormonal disruptors in chinook salmon.

**Histopathological Analyses**: These will be restricted to the organs listed above. Morphologic alterations often accompany exposure and are a component of adverse effects. Alterations will include necrosis, repair, hypertrophy and hyperplasia. See Hinton et (1992) for a review.



I-019157

**Ecological/Biological Benefits**

For the past 20 years, diagnoses of problems in California aquatic environments have relied on gross chemical analyses of water, tissues and sediments and short-term acute toxicity testing. These are the necessary first steps in environmental protection, but they may not have been sufficient to diagnose the subtler effects of chronic toxicity played out over multiple generations of aquatic organisms. The sublethal effects of contaminants can be expressed in larvae, juvenile and adult fish in a variety of ways: behavioral modifications, atrophied tissues, histopathological alterations, reduced fecundity, slower growth, greater energy consumption, etc. However, the maintenance of populations requires high survival of individuals through healthy growth and reproduction, particularly from gametogenesis through juvenile stages. The early stages are also the most sensitive life stages (Rosenthal and Alderdice 1976), indicating that the emphasis of any chronic fish toxicity studies should ultimately be on growth and reproduction. Survival through these stages reflects the ability of the aquatic ecosystem to maintain healthy populations. The adult stages are also subject to a variety of potentially lethal stresses (low oxygen, toxic blooms, fishing, predation, water diversions), but it is the quality of the environment for reproduction and survival through early life stages that is key to maintaining or restoring healthy fish populations. A healthy environment for fish has both good habitat and good water quality. Good water quality depends on having concentrations of contaminants that do not interfere with the general health, particularly growth and reproduction, especially through larval and juvenile stages.

Therefore, the primary ecological objective of this project is to identify any existing impairment of reproductive success in chinook salmon during its spawning migration and in the development of the offspring as they grow in the estuary and migrate out to sea.

The effects of contaminants on adult chinook salmon returning to the Sacramento River system to spawn have not been evaluated. A thorough understanding of the role of environmental contaminants and their involvement in declines of salmon and other anadromous species is therefore essential if a comprehensive watershed management plan for the Sacramento River is to be effective.

Most studies in the Sacramento-San Joaquin River system have focused on juvenile fish (Hamilton *et al.* 1990; Varanasi *et al.* 1993; Saiki *et al.* 1995). However, adult productivity can determine the abundance of an age class for the rest of its existence (Shuter 1990). Studies on lake trout (*Salvelinus namaycush*) reproduction in the Great lakes indicated a high correlation between parent and egg PCB concentrations, and a negative correlation between PCB concentrations and the number of normal fry that successfully hatched (Mac *et al.* 1991). Similar results could result from other bioaccumulative toxicants (Brooks *et al.* 1997). This proposed three year study will examine the potential effects of multiple contaminants on the reproduction of fall-run and winter-run chinook salmon (*Oncorhynchus tshawytscha*) in the Sacramento River system, with emphasis on returning adult fish, maternally-exposed eggs and larvae, and juvenile survival and fitness. Since most of the methods available to assess reproductive effects of toxicants require that the organism be sacrificed, which could be counterproductive in the assessment of rare or declining species, an attempt will be made to develop non-lethal screening parameters for reproductive impairment in winter-run salmon by correlating the results of tests on mortalities in different stages of winter-run fish with the same tests in randomly selected fall-run fish. Additional tests will be performed on fall-run fish to further elucidate possible contaminant effects on reproduction.

The results of this study will be used to evaluate and monitor contaminant effects on the native chinook salmon of the Sacramento-San Joaquin River system by focusing first on the adult fish to confirm or eliminate contaminant effects on fertility as a possible cause of species decline. Specific management actions that will be facilitated by the results of this study include the following:

1) Support ongoing efforts by the United States Fish and Wildlife Service and other resource agencies in recovering threatened and endangered anadromous fish populations in the Sacramento-San Joaquin system by facilitating restoration planning and monitoring.

2) Evaluate the utility of non-lethal screening methods (used singly and in combination) in assessing xenobiotic impacts on adult chinook salmon and generate data which can be used to assess the need for more comprehensive studies on genetic injury and reproductive success in other anadromous species using the Sacramento-San Joaquin system during parts of their life-cycles.

3) Evaluate data linking specific contaminants with adverse effects to help guide management decisions with respect to determining acceptable contaminant levels in the environment, particularly in evaluating water quality objectives and clean-up levels at CERCLA and RCRA sites.

4) Develop salmonid early life-stage mortality estimates for predictive models that can be used to evaluate management actions .

5) Further evaluate and interpret data on survival and recruitment of juvenile chinook salmon currently being collected by the National Marine Fisheries Service and develop an assessment of contaminant effects on the freshwater phase of the chinook salmon life cycle.

**Hypotheses:** The null hypotheses to be tested are outlined in Table 2.

**Adaptive Management**: Assessment of the findings from this study will be made in the annual reports prepared jointly by all investigators. On the basis of Year 1 sampling and data analyses we anticipate changing the program to both eliminate parts of the program where null hypotheses have been accepted, and to refine and revise objectives when hypotheses have been rejected. Further work in years 2 & 3 will clarify nature of initial findings where contaminants have impact on fish.

**Linkages**: Data generated by this study will enable an assessment of the effects of contaminants on individual restoration projects to evaluate whether contaminants are having a delcterious effect on individual runs or populations of chinook salmon that outweighs the benefits gained from those projects. Data from this study will complement other studies by the National Marine Fisheries Service and California Department of Fish and Game on the effects of contaminants on various life-stages of chinook salmon and aid in the development of an integrated picture of contaminant effects.

**System-Wide Ecosystem Benefits**: The sensitivity of salmonids to degraded water quality is well documented, and allows the use of salmonid species as indicators of ecosystem health. In addition, salmon recycle essential nutrients, enriching the aquatic habitat for their own offspring and to other native species. Post-spawned salmon carcasses are also a rich (and once reliable) source of food for many terrestrial species. A thorough understanding of contaminant effects on salmonid reproduction system-wide is essential if managers are to effectively assess the potential risks and benefits of specific and programmatic actions undertaken by CALFED and other large-scale, comprehensive resource programs. The information need to complete such assessments can only be gained from the detailed evaluation of the contaminant impacts of s on every stage of the salmonid life-cycle. The information gained from a study of contaminant impacts to chinook

salmon will also contribute significantly to the assessment of impacts on other native fish species.

**Compatibility with Non-Ecosystem Objectives:** This study will provide benefits to other CALFED objectives including water quality and water supply reliability; specifically, the watershed management program will benefit from elucidation of contaminant effects on a recreationally, commercially, and ecologically important resource.

**Technical Feasibility and Timing**

A modification of Investigator collecting permits will need to be obtained from the California Department of Fish and Game to allow collection of fall-run chinook salmon; activities with winter-run chinook salmon will have to be coordinated with the National Marine Fisheries Service, Santa Rosa, and the U.S. Fish and Wildlife Service Northern Central Valley Fishery Resource Office, Red Bluff, and might require a modification to the USFWS's current permit for winter-run propagation.



I-019161

## Monitoring and Data Collection Methodology

**Monitoring Parameters and Data Collection Approach:**

Winter-run. A maximum of 20 fish (depending on availability) will be identified at the winter-run propagation facility, for fecundity assessment and tissue sample collection. Sampling will be coordinated with the National Marine Fisheries Service to avoid unauthorized take.

Fall-run. Twenty adult fall-run fish will be captured by hook and line, or caught cooperatively with NMFS personnel (Tiburon), or purchased from commercial sources as they enter the estuary. The fish will be sacrificed to establish baseline contaminant concentrations and biomarker responses for adult salmon entering the estuary at the beginning of the spawning run. Tissues collected will be gills, liver, gonads, and blood. An exposed group of fish will be established by randomly selecting adult fall-run salmon (10 male, 10 female) that return to Coleman Hatchery on Battle Creek to spawn after negotiating the Sacramento River system. At the time of spawning, samples of adult tissues (muscle, liver, gonad, blood) and gametes ( fall-run only) will be collected for chemical analysis and biomarker assessment. All fish will be weighed and measured at the time of collection. Tissue samples will be analyzed for both metals and organochlorines. Ten crosses of individual male and female fish will be made and incubated separately to evaluate reproductive success and to relate that to the initial analyses.

Outmigrating juveniles and smolts. Outmigrating juveniles will be captured in the spring of the year in the mainstream Sacramento River and upper San Francisco Bay estuary. We have a goal of capturing 20 fish from each station and to have at least five stations that span the mainstream of the River through Suisun Bay. These stations will be visited monthly from March through June. All fish will be weighed and measured at the time of collection. Tissues to be collected include gills, liver and kidney. Some whole fish will be set aside for contaminant analyses. Tissue samples will be analyzed for both metals and organochlorines.

In Table 4 (4a,4b,4c,4d) are presented: The species and life stages of fishes to be used and the analyses to be conducted.

Budget: Year 1: $292.3K; Year 2: $272.2K; Year 3: $181.2K. Full budget in Appendix (Tables 3a, 3b, 3c and 4a, 4b, 4C).

**Data Evaluation Approach**: See Tables 4a, 4b, 4c and 5



I-019162

## Local Involvement

Listed below are the counties that have been notified in writing of the project.

| County Planning Department | County Board of Supervisors |
|---|---|
| Alameda | Alameda |
| San Mateo | San Mateo |
| Santa Clara | Santa Clara |
| Solano | Solano |
| Sacramento | Sacramento |
| Yolo | Yolo |
| Colusa | Colusa |
| Sutter | Sutter |
| Glenn | Glenn |
| Butte | Butte |
| Tehama | Tehama |
| Shasta | Shasta |
| Marin | Marin |
| Sonoma | Sonoma |
| Napa | Napa |
| Santa Cruz | Santa Cruz |
| San Joaquin | San Joaquin |
| Stanislaus | Stanislaus |
| Merced | Merced |
| Madera | Madera |
| Fresno | Fresno |
| Kings | Kings |

Other
Delta Protection Commission
Bay Conservation and Development Commission

Letters of Notification to the counties are located in the Appendix. For coordination with the existing programs in the area see Table 3.

## Cost

**Budget:** Year 1: $292.3K; Year 2: $272.2K; Year 3: $181.2K: The full budget is in the Appendix (Table 3a, 3b, 3c and 4a, 4b, 4c).

**Schedule:**

**Year 1**: Literature Review to be completed. Pre-field planning and coordination will be performed during the summer/fall of 1999. Winter-run salmon tissues will be collected and adult biomarkers assessed February-April 2000. Early life-stage biomarkers will be assessed beginning August 2000. Fall-run salmon will be collected in October and November 1999. All tissue samples will be taken at the times the fish are collected. Adult biomarkers, percent fertilization, hatching success, and swim-up fry survival will be assessed between November 1999 and February 2000. Health of hatchery and wild juveniles will be assessed April-June 2000. Histopathological examinations will be conducted beginning in January 2000

**Year 2:** Using an adaptive management framework, the above tasks will be repeated, except for literature review, in year 2 as appropriate to confirm and extend findings. A major emphasis will be placed on swimming performance. Any indication of hormonal disruption found in the first year's studies will initiate more investigation, possibly including receptor binding assays, gonadotropin determinations, rates of hormone production.

**Year 3**: Data analysis and report preparation will begin as results become available, with an estimated report completion time of August 2002. Additional field studies will be conducted as needed to conform findings from years 1 and 2.

**Cost-Sharing**

The Department of Toxic Substances Teaching and Research program will provide $9,000 per year for two years for salary support for Mr. Haas and supplies.



I-019165

## Applicant Qualifications

**Robert B. Spies,** Ph.D., (Univ. So. CA, 1971); President, Applied Marine Sciences; Fate and effects of contaminants in the aquatic environment, especially reproductive effects; reviews for 20 organizations, 13 journals; 2 Editorial Boards; Sample publications (from 40+):
*R. B. Spies et al.,* 1987. Benzthiazoles in estuarine sediments as indicators of street runoff. *Nature* 327: 697-699.
*R. B. Spies et al.,* 1988. The effects of organic contaminants on reproduction of starry flounder, *Platichthys stellatus* (Pallas) in San Francisco Bay. Part I. Hepatic contamination and mixed-function oxidase(MFO) activity during the reproductive season. *Marine Biology* 98, 181-189.
*R.B. Spies & D.W. Rice, Jr.* 1988. The effects of organic contaminants on reproduction of starry flounder, *Platichthys stellatus* (Pallas) in San Francisco Bay. Part II. Reproductive success of fish captured in San Francisco Bay and spawned in the laboratory. *Marine Biology* 98, 191-202.
*M.J. Melanacon, et al.* 1992 Metabolic products as biomarkers, In: *Biomarkers: Biochemical, physiological and histological markers of anthropogenic stress* (Huggett et al., Eds) Lewis Publishers, Boca Raton, Florida.

**Gary D. Marty**, Ph.D. (Univ. CA, Davis, 1993); Assistant Professor, UC Davis; Diseases of fish; Sample publications (from 12 ); Sample publications:
*Marty, G.D., et al.* 1997. Histopathology and cytogenetic evaluation of Pacific herring larvae exposed to petroleum hydrocarbons in the laboratory or in Prince William Sound, Alaska, after the *Exxon Valdez* oil spill. Can. J. Fish. Aquat. Sci 54:1846-1857.
*Marty, G.D et al.*, 1998. Viral hemorrhagic septicemia virus, *Ichthyophonus hoferi*, and other causes of morbidity in Pacific herring *Clupea pallasi* spawning in Prince William Sound, Alaska, USA. Dis. Aquat. Org. 32 (1):15-40.
*Marty, G.D. et al.*, 1998. Viral hemorrhagic septicemia virus, *Ichthyophonus hoferi*, and other causes of morbidity in Pacific herring *Clupea pallasi* spawning in Prince William Sound, Alaska, USA. Dis. Aquat. Org. 32 (1):15-40

**Joseph J. Cech, Jr.,** Ph.D. (Univ. Texas, Austin, 1973); Professor, Wildlife, Fish & Conservation Biology, UC Davis), Physiological and behavioral responses of fishes to environmental factors, including toxicants, experience with chinook salmon; Sample publications (from 90+):
*Choi, M.H., J.J. Cech, Jr., and M.C. Lagunas-Solar.* 1998. Bioavailability of methylmercury to Sacramento blackfish (*Orthodon microlepidotus*): dissolved organic carbon (DOC) effects. Env. Tox. Chem. 17:695-703.
*Swanson, C., P.S. Young, and J.J. Cech, Jr.* 1998. Swimming performance of delta smelt: maximum performance, and behavioral and kinematic limitations on swimming at submaximal velocities. J. Exp. Biol. 201:333-345. 701.
*Heath, A.G., J.J. Cech, Jr., J.G. Zinkl, B. Finlayson, and R. Fujimura.* 1993. Sublethal effects of methyl parathion, carbofuran, and molinate on larval striped bass. Amer. Fish. Soc. Symp. 14:17-28

**David E. Hinton,** Ph.D. (Univ. Miss., 1969); Professor, School of Veterinary Medicine, UC Davis; Cellular and subcellular responses of aquatic organisms to environmental pollutants; Sample publications (from 155):
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**James E. Haas**, M.S. (SF State Univ., 1995); Biologist, Fish & Wildlife Serv.; Ph.D. student, UC Davis; Sample publications:

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**Jordan Gold,** M.S. (UC Davis, 1992); Biologist, Applied Marine Sciences; Fisheries investigations, environmental monitoring programs.

## Appendix

Tables 1 - 5

Budget

Nondiscrimination Compliance Statement

Small Business Preference and Contractor Identification Number

References

Letters of Support

Letters of Notification

**Table 1. Proposal relevance to CALFED goals and objectives**

| CALFED Goal (ERP reference) | Relevance of proposal to goal |
|---|---|
| **Achieve recovery of at-risk native species dependent on the Delta and Suisun Bay as the first step toward establishing large, self-sustaining populations of these species; support similar recovery of at-risk native species in San Francisco Bay and the watershed above the estuary; and minimize the need for future endangered species listings by reversing the downward population trends of native species that are not listed. (page 1, Vol. 1 Feb. 1999 ERP)** | If chinook salmon are being impacted by contaminants, then the nature of the impact and the source of the contaminants needs to be identified as the initial step towards enabling recovery. |
| **Improve and maintain water and sediment quality to eliminate, to the extent possible, toxic impacts to organisms in the system, including humans. (page 1, Vol. 1 Feb. 1999 ERP).** | See above. |
| **Sacramento chinook Salmon: Restore winter-run, Late-fall-run, fall-run and spring-run chinook Salmon to the Sacramento river and the Bay-Delta estuary. (pages 177-178, Vol. 1 Feb. 1999 ERP)** | See above. |
| **Build an understanding of physical, chemical, and biological processes in the Bay-Delta and its watershed that are relevant to CALFED program actions. (page 9, Vol. 1 Feb. 1999 ERP)** | The potential impact of contaminants on chinook salmon is needed for a complete understanding of how the environment interacts with biological processes (e.g., reproduction). |
| **Provide information useful in evaluating the effectiveness of existing monitoring protocols and the appropriateness of monitoring attributes. (page 9, Vol. 1 Feb. 1999 ERP)** | There are existing programs to monitor juveniles leaving the system, but contaminant impacts are not fully addressed in these programs. If findings from the proposed studies indicate impacts, then it may be appropriate to monitor to see if they persist while the populations begin to recover. |
| **Test causal relationships among environmental variables identified in conceptual models. (page 9, Vol. 1 Feb. 1999 ERP)** | This proposal will address causal relationships between water quality and fish health. |
| **Reduce areas of scientific uncertainty regarding management actions. (page 9, Vol. 1 Feb. 1999 ERP)** | Whether contaminants are a significant impediment to population recovery is a major uncertainty and this proposal will address this uncertainty. |
| **Incorporate relevant new information from academic research. (page 9, Vol. 1 Feb. 1999 ERP)** | A number of new tools and approaches, biomarkers and physiological performance will be employed in this proposal. |
| **Short-term objective: Systematically identify and locate the best examples of essential fish habitats and prioritize them for conservation. Develop and begin implementation of action plans for restoring significant examples of each habitat type. (page 162, Vol. 1 Feb. 1999 ERP)** | Restoring essential habitat in the Sacramento River may require reducing contaminant inputs and this proposal will aid in that process. |

| | |
|---|---|
| **Long-term Objective: Protect and manage, on a self-sustaining basis throughout the watershed, multiple areas containing all aquatic, wetland, and riparian habitat types in the Central Valley and rivers (including the Delta and Suisun and San Francisco Bays to a point where all at-risk species that depend on the habitats are no longer at risk. (page 162, Vol. 1 Feb. 1999 ERP)** | Managing salmon habitat will require knowing what levels of contaminants place salmon at risk. |
| **Long-Term Objective: Create self-sustaining populations of winter-run chinook Salmon in both the mainstream Sacramento river and in battle Creek at abundance levels equal to or greater than those identified in the National Marine Fisheries Service (NMFS) proposed Recovery Plan for Sacramento River winter-run chinook Salmon. (page 220, Vol. 1 Feb. 1999 ERP)** | See above. |
| **Long-Term Objective: Restore wild, naturally reproducing populations of spring-run chinook Salmon to numbers or spawning densities in the Sacramento River system equal to those that existed in the 1940's (average of 70,000-80,000 per year), as measured over a period of at least 25 years. (page 220-221, Vol. 1 Feb. 1999 ERP)** | See above. |
| **Short-Term Objective: Determine the distribution, status, and habitat requirements of all native resident fishes in the Bay-Delta watershed to see if species-specific strategies are needed to reverse declines or if habitat-oriented restoration strategies will be adequate. (page 347, Vol. 1 Feb. 1999 ERP)** | See above. |
| **Long-Term Objective: Reduce concentrations and loadings of contaminants to levels that do not cause adverse affects on all organisms and ecosystems in the aquatic environment. (page 506, Vol. 1 Feb. 1999 ERP)** | Determining the levels of contaminants that affect fish is the first step in establishing what reductions may be needed. |
| Short-Term Objective: Reduce concentrations and loadings of contaminants that affect the health of organisms and ecosystems in water and sediments to the extent feasible based on benefits achieved, cost and technological feasibility. (page 506, Vol. 1 Feb. 1999 ERP) | See above |

**Table 2. Null hypotheses to be tested.** **Table 2. Null hypotheses to be tested.**

| | |
|---|---|
| Overall null hypothesis ($H_o$) | There is no effect of contaminants in the San Franciso estuary and its catchment on chinook salmon reproductive success and juvenile fitness. |
| 1. specific null hypothesis ($H_{o1}$) | Contaminants accumulated by adult chinook salmon on their return to the estuary do not affect hatchability of eggs and survival of larvae. |
| 2. specific null hypothesis ($H_{o2}$) | Health and fitness of juvenile salmon migrating to sea is unrelated to tissue concentrations of contaminants or biomarkeres of contaminant exposure and effect. |

**Table 3. Ongoing projects and studies that are integrated with our proposal**

| Study | Description of Cooperative Investigations |
|---|---|
| **1. Dr. Bruce McFarlane of the National Marine Fisheries Service has an ongoing study to investigate contaminant concentrations and other parameters in multiple life stages of chinook salmon.** | We will participate in their collections of ocean-caught chinook salmon, and incorporate their contaminant concentration data in our study. |
| **2. Dr. Peter Moyle from UC Davis conducts fish surveys in Suisun Bay and the Cosumnes river** | We will participate in their collections and retain juvenile non-winter-run chinook salmon for our study. |
| **3. Mr. Jerry Morinaka of the California Department of Fish and Game leads a project to collect entrained salmon from Delta pumping facilities and truck them to locations in the Delta where they are unlikely to become re-entrained.** | We will collect entrained non-winter-run chinook salmon in conjunction with CDF&G's fish salvage operations and use them in our study. |
| **4. Mr. Mark Pierce of the US Fish and Wildlife service conducts a monthly trawl and beach seine project for IEP to determine the number and race of juvenile chinook salmon in multiple Delta locations** | We will collect non-winter-run chinook salmon juveniles in conjunction with the USFW project and use them in our study. |

**Table 4a. Assays to be conducted on winter-run chinook salmon from hatcheries**

| pre- spawn reproductive adults | post-spawn reproductive adults | gametes | embryos | larvae | juveniles |
|---|---|---|---|---|---|
| assays to be conducted on 20 males and 20 females include:<br>1. plasma reproductive hormones; estradiol and testosterone in females; and estradiol, testosterone, vitellogenin and chorionic protein in males | assays to be conducted on 20 males and 20 females:<br>1. contaminant concentrations of Cu, Zn, Cd, Hg, Se, PCB's and pesticides in eggs, and PAH's in bile<br>2. Condition factor and HSI<br>3. biomarkers including: histopathology of gills, liver, kidney, acetylcholine esterase, and P4501A | assays to be conducted on 100 gametes:<br>1. egg # and size<br>2.%fertilization | assays to be conducted on 100 embryos<br>1. % hatching<br>2. terata (morts. only) | assays to be conducted on 20 larvae<br>1. % survival to exogenous feeding<br>2. terata (morts. only)<br>3. histopathology (morts. only), six step sections through entire fish)<br>4. contaminant concentrations (morts. only) of Cu, Zn, Cd, Hg, Se, PCB's, and pesticides | assays to be conducted on 20 juveniles<br>1 % survival to release<br>2. terata (morts. only)<br>3. condition factor, HSI |

HSI= hepatosomatic index, GSI= gonadosmatic index, NA= not applicable, PCB's= polychlorinated biphenyls, PAH's= polyaromatic hydrocarbons, CNS= central nervous system, morts= life stages that have died

**Table 4b. Assays to be conducted on fall-run chinook salmon from hatcheries**

| pre- spawn reproductive adults | post-spawn reproductive adults | gametes | embryos | larvae | juveniles |
|---|---|---|---|---|---|
| assays to be conducted on 20 males and 20 females:<br>1. plasma reproductive hormones; estradiol and testosterone in females; and estradiol, testosterone, vitellogenin and chorionic protein in males | assays to be conducted on 20 males and 20 females:<br>1. Condition factor, GSI and HSI<br>2. biomarkers including: histopathology of gills, liver, kidney ; acetylcholine esterase, and P4501A | assays to be conducted on 100 gametes<br>1. egg # & size<br>2. sperm motility<br>3.%fertilization<br>4. contaminant concentrations of Cu, Zn, Cd, Hg, Se, PCB's and pesticides in gonad, and PAH's in bile | assays to be conducted on 100 embryos<br>1. % hatching<br>2. terata | assays to be conducted on 20 larvae<br>1. % survival to exogenous feeding<br>2. terata | assays to be conducted on 20 juveniles<br>1 % survival to release<br>2. terata<br>3. biomarkers including:histopathology (six step sections through entire fish), and acetylcholine esterase, bile metabolites, P450 1A, blood comet assay, hematocrit, plasma sodium, thyroxine and cortisol and gill $Na^+$-$K^+$-ATPase activity<br>4. contaminant concentrations of Cu, Zn, Cd, Hg, Se, PCB's, PAH's and pesticides<br>5. condition factor, HSI<br>6. swimming performance |

See table 4a for key to abbreviations

**Table 4c. Assays to be conducted on chinook salmon from the wild (no analyses will be conducted on winter-run fish)**

| adults caught at sea | post-spawn reproductive adults | gametes | embryos | larvae | |
|---|---|---|---|---|---|
| assays to be conducted on 20 males and 20 females include:<br>1. plasma reproductive hormones; estradiol and testosterone in females: and estradiol, testosterone, vitellogenin and chorionic protein in males<br>2. contaminant concentrations of Cu, Zn, Cd, Hg, Se PCB's, and pesticides in liver; PAH's in bile | NA | NA | NA | NA | assays to be conducted on 20 juveniles<br>1. terata<br>2. biomarkers including:histopathology (gill, liver, kidney), and acetylcholine esterase activity, bile metabolites, P450 1A, hematocrit, plasma sodium, thyroxine and cortisol and gill $Na^+$-$K^+$-ATPase activity<br>3. contaminant concentrations of Cu, Zn, Cd, Hg, Se, PCB's, and pesticides<br>4. condition factor, HSI<br>5. swimming performance |

See table 4a for key to abbreviations

**Table 5. Summary of contaminant analyses, biomarker and health/performance measurements and data analyses**

| Analyte/Biomarker/Health /Fitness measure | Method | Quality Assurance | Data Evaluation |
|---|---|---|---|
| Organophosphate pesticides | xxx | xxx | xxx |
| PAHs | xxx | | |
| PCBs | xxx | | |
| Hg | xxx | | |
| Cu | xxx | | |
| Zn | xxx | | |
| Se | xxx | | |
| Acetylcholine esterase activity | Modified spectrophotometric (Ellman et al.,1961) | O,R, S | ANOVA |
| P4501A | Stegeman et al., 1991, Smolowitz et al., 1991, Weidmer et al., 1996 | O,R, S | ANOVA |
| Bile analysis | Krahn et al., 1984 | B, O, R | ANOVA |
| Testosterone, estradiol | Singh et al., 1988 | O, R, S, Sp | ANOVA |
| Vitellogenin, chorionic protein in males | Purdom et al., 1994; Celius et al., 1999 | O, R, S | ANOVA |
| Gonadosomatic index | Spies and Thomas, 1995 | O, R | ANOVA |
| Histopathology of liver, gills, kidney | Hinton, 1993 | BL | ANOVA |
| Swimming performance | Brett, 1964 | R | ANOVA |

Quality assurance abbreviations: B=blank, O=repeat analyses for outlier values, R=repeat analyses for precision, S=Analysis of standard reference with each batch (positive and negative), Sp=Spike recoveries, BL=Blind study plus consultation with 2nd pathologist. Data evaluation: ANOVA=analysis of variance statistical model with dependent variable being the biomarker response and independent variable(s) including site of collection, concentrations of various contaminants in tissues. Data will be transferred as appropriate to meet assumptions of normal distribution or an equivalent non-parametric test will be used.

**Table 3A. Total Budget 1999-2000**

| Task | Direct Labor Hours | Direct Salary and Benefits | Service Contracts | Material and Acquisition Costs | Miscellaneous and other Direct Costs | Overhead and Indirect Costs[1] | Total Cost |
|---|---|---|---|---|---|---|---|
| Task 1 | 200 | $8,612 | $5,000 | $0 | $650 | $10,166 | $24,828 |
| Task 2 | 90 | $3,036 | $61,500 | $507 | $1,263 | $14,649 | $81,046 |
| Task 3 | 399 | $12,822 | $109,730 | $714 | $2,027 | $33,927 | $159,619 |
| Project Management Task | 194 | $9,459 | $5,000 | $0 | $1,200 | $11,184 | $27,017 |
| Total | 883 | $33,929 | $181,230 | $1,221 | $5,140 | $69,905 | $292,309 |

**Table 3B. Total Budget 2000-2001**

| Task | Direct Labor Hours | Direct Salary and Benefits | Service Contracts | Material and Acquisition Costs | Miscellaneous and other Direct Costs | Overhead and Indirect Costs[1] | Total Cost |
|---|---|---|---|---|---|---|---|
| Task 1 | 0 | $0 | $0 | $0 | $0 | $0 | $0 |
| Task 2 | 88 | $2,636 | $63,960 | $522 | $1,301 | $14,679 | $83,187 |
| Task 3 | 354 | $11,031 | $114,119 | $735 | $2,087 | $32,832 | $161,159 |
| Project Management Task | 194 | $9,743 | $5,200 | $0 | $1,236 | $11,508 | $27,880 |
| Total | 636 | $23,410 | $183,279 | $1,258 | $4,625 | $59,019 | $272,226 |

**Table 3C. Total Budget 2001-2002**

| Task | Direct Labor Hours | Direct Salary and Benefits | Service Contracts | Material and Acquisition Costs | Miscellaneous and other Direct Costs | Overhead and Indirect Costs[1] | Total Cost |
|---|---|---|---|---|---|---|---|
| Task 1 | 0 | $0 | $0 | $0 | $0 | $0 | $0 |
| Task 2 | 49 | $1,634 | $40,707 | $538 | $1,340 | $9,425 | $53,694 |
| Task 3 | 185 | $6,044 | $74,613 | $757 | $2,150 | $20,417 | $104,167 |
| Project Management Task | 149 | $7,418 | $5,408 | $0 | $1,273 | $9,083 | $23,331 |
| Total | 383 | $15,096 | $120,728 | $1,295 | $4,763 | $38,926 | $181,192 |

[1] The overhead rate is applied to a base of direct labor dollars including fringe benefits. The overhead pool consists of such indirect expenses as office supplies, indirect labor, fringe benefits associated with that labor, postage and shipping, rent, utilities, telephone expense, insurance costs, etc. Indirect expenses included in this pool are those associated with supporting AMS direct contract effort.

The General and Administrative (G&A) expense pool is allocated on a Total Cost Input base consisting of direct material and subcontract costs, direct labor and overhead. The G&A pool consists of such expenses as management salaries, fringe benefits associated with those salaries, rent, insurance, office supplies, taxes, depreciation, maintenance, etc. Indirect expenses accumulated in this pool are those associated with running the overall company.

The overhead and G&A rates are the same for both state and federal projects. Costs deemed unallowable in accordance with the Federal Acquisition Regulation (FAR) Part 31 have been eliminated from both pools.

## Table 4A. Quarterly Budget: 1999-2000

| Task | Quarterly Budget Oct Dec 99 | Quarterly Budget Jan Mar 00 | Quarterly Budget Apr-June 0 0 | Quarterly Budget Jul-Sep 0 0 | Quarterly Budget Oct Dec 00 | Total Budget |
|---|---|---|---|---|---|---|
| Task 1 | $12,314 | $12,314 | $0 | $0 | See table 4b below | $24,628 |
| Task 2 | $20,261 | $20,261 | $20,261 | $20,261 | " | $81,046 |
| Task 3 | $0 | $39,905 | $39,905 | $39,905 | $39,905 | $159,619 |
| Project Management | $6,754 | $6,754 | $6,754 | $6,754 | See table 4b below | $27,017 |
| Total | $39,329 | $79,234 | $66,920 | $66,920 | $39,905 | $292,309 |

## Table 4B. Quarterly Budget: 2000-2001

| Task | Quarterly Budget Oct Dec 00 | Quarterly Budget Jan Mar 01 | Quarterly Budget Apr-June 0 1 | Quarterly Budget Jul-Sep 0 1 | Quarterly Budget Oct Dec 01 | Total Budget |
|---|---|---|---|---|---|---|
| Task 1 | 0 | 0 | 0 | 0 | See table 4c below | 0 |
| Task 2 | 20,797 | 20,797 | 20,797 | 20,797 | " | 83,187 |
| Task 3 | See table 4a above | 40,290 | 40,290 | 40,290 | 40,290 | 161,159 |
| Project Management | 6,970 | 6,970 | 6,970 | 6,970 | See table 4c below | 27,880 |
| Total | 27,767 | 68,056 | 68,056 | 68,056 | 40,290 | 272,226 |

## Table 4C. Quarterly Budget: 2001-2002

| Task | Quarterly Budget Oct Dec 01 | Quarterly Budget Jan Mar 00 | Quarterly Budget Apr-June 0 0 | Quarterly Budget Jul-Sep 0 0 | Quarterly Budget Oct Dec 00 | Total Budget |
|---|---|---|---|---|---|---|
| Task 1 | $0 | $0 | $0 | $0 | $0 | $0 |
| Task 2 | $10,739 | $10,739 | $10,739 | $10,739 | $10,739 | $53,694 |
| Task 3 | See table 4b above | $26,042 | $26,042 | $26,042 | $26,042 | $104,167 |
| Project Management | $4,666 | $4,666 | $4,666 | $4,666 | $4,666 | $23,331 |
| Total | $15,405 | $41,447 | $41,447 | $41,447 | $41,447 | $181,192 |

STATE OF CALIFORNIA

# NONDISCRIMINATION COMPLIANCE STATEMENT

STD. 19 (REV. 3-95) FMC

COMPANY NAME
Applied Marine Sciences, Inc.

The company named above (hereinafter referred to as "prospective contractor") hereby certifies, unless specifically exempted, compliance with Government Code Section 12990 (a-f) and California Code of Regulations, Title 2, Division 4, Chapter 5 in matters relating to reporting requirements and the development, implementation and maintenance of a Nondiscrimination Program. Prospective contractor agrees not to unlawfully discriminate, harass or allow harassment against any employee or applicant for employment because of sex, race, color, ancestry, religious creed, national origin, disability (including HIV and AIDS), medical condition (cancer), age, marital status, denial of family and medical care leave and denial of pregnancy disability leave.

## CERTIFICATION

*I, the official named below, hereby swear that I am duly authorized to legally bind the prospective contractor to the above described certification. I am fully aware that this certification, executed on the date and in the county below, is made under penalty of perjury under the laws of the State of California.*

OFFICIAL'S NAME
Robert B. Spies

DATE EXECUTED
April 15, 1999

EXECUTED IN THE COUNTY OF
Alameda

PROSPECTIVE CONTRACTOR'S SIGNATURE

PROSPECTIVE CONTRACTOR'S TITLE
President

PROSPECTIVE CONTRACTOR'S LEGAL BUSINESS NAME
Applied Marine Sciences, Inc.

I -019178

State of California
The Resources Agency
DEPARTMENT OF WATER RESOURCES

Agreement No. _____

Exhibit _____

**STANDARD CLAUSES –**
**SMALL BUSINESS PREFERENCE AND CONTRACTOR IDENTIFICATION NUMBER**

**NOTICE TO ALL BIDDERS:**

Section 14835, et. seq. of the California Government Code requires that a five percent preference be given to bidders who qualify as a small business. The rules and regulations of this law, including the definition of a small business for the delivery of service, are contained in Title 2, California Code of Regulations, Section 1896, et. seq. A copy of the regulations is available upon request. Questions regarding the preference approval process should be directed to the Office of Small and Minority Business at (916) 322-5060. To claim the small business preference, you must submit a copy of your certification approval letter with your bid.

Are you claiming preference as a small business?

_____ Yes* X No

*Attach a copy of your certification approval letter.

Not considered State small business.
Considered Federal small business.



I-019179

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United States Department of the Interior
FISH AND WILDLIFE SERVICE
Sacramento/San Joaquin Estuary Fishery Resource Office
4001 North Wilson Way, Stockton, CA 95205-2486
209-946-6400 (Voice) 209-946-6355 (Fax)

Dr. Robert B. Spies
Applied Marine Sciences
4749 Bennett Drive Suite L
Livermore, CA

Dr. Spies,

This letter is to voice support for the type of proposed work that you outlined via telephone and email, entitled "Effects of Contaminants in the Catchment of the San Francisco Bay Estuary on Reproductive Success of Adult, and Health and Fitness of juvenile Chinook Salmon (*Oncorhynchus tshawytscha*)". In my experience I have found a significant gap in information relating to the effects of contaminants and toxics on fishes in the Sacramento-San Joaquin Rivers and Delta. This information may be helpful in determining the mechanisms behind the juvenile chinook survival estimates that we generate in our ongoing studies.

Provided that the necessary take permits are provided by you, we would be happy to allow your study to "piggy back" on our standard monitoring efforts throughout the delta, as a means of cost-effectively collecting juvenile chinook for your analyses. I would appreciate a copy of your proposal once it is finalized.

Mark Pierce
Supervisory Fishery Biologist

OPTIONAL FORM 99 (7-90)

FAX TRANSMITTAL # of pages ▸ 1

| | |
|---|---|
| To Dr. RB Spies | From Mark Pierce |
| Dept/Agency | Phone # 209 946 6400 209 |
| Fax # 925 373 7834 | Fax # |

NSN 7540-01-317-7368 5099-101 GENERAL SERVICES ADMINISTRATION

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I-019183

**UNITED STATES DEPARTMENT OF COMMERCE**
**National Oceanic and Atmospheric Administration**
NATIONAL MARINE FISHERIES SERVICE
Southwest Fisheries Science Center
Tiburon Laboratory
3150 Paradise Drive
Tiburon, California 94920

14 Apr 1999 F/SWC3:RBM

MEMORANDUM FOR: CALFED

FROM: R. Bruce MacFarlane, Ph.D.
Research Fishery Biologist

SUBJECT: Proposal by Spies et al. for salmon - contaminant research

The proposal, "Effects of Contaminants in the Catchment of the San Francisco Bay Estuary on Reproductive Success of Adults and Health and Fitness of Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*)" is designed to address an important, but largely unknown, aspect of the Central Valley - San Francisco Estuary ecosystem. This is among the most agricultural and urbanized systems in the country, if not the world, thus subject to significant quantities of contaminants from virtually all sectors of society. Additionally, this ecosystem provides a home for a unique community of chinook salmon, a resource of great socioeconomic status. This is the only system containing four runs of chinook salmon, all of which are experiencing declines in natural production for a variety of reasons, some known (e.g., loss of habitat behind dams) and others unknown. The effects of contaminant exposure to salmon reproduction in this system is one of the unknown factors. Final stages of maturation during the upstream spawning migration are among the most stressful stages to any anadromous fish, and in particular semelparous, or one-time, spawners. The literature clearly shows that contaminant exposure during this stage can result in lowered reproductive success.

Our research has documented contaminant concentrations in outmigrating juveniles since 1995, but has not investigated the exposure or effects to adults. This work needs to be done to better understand the contribution of contaminants to the decline of these federally ESA-listed or candidate chinook salmon runs. The collaborators on this proposal are all well-qualified to do the work and will improve our knowledge of contaminant effects.

To the extent possible, we offer our assistance in collecting adult salmon for their research. Personnel from their project are welcome to join us on our salmon collection cruises on a space available basis (often the case). We have proposed a study of the biology and ecology of juvenile and immature salmonids in the coastal waters of California for FY 2000 and beyond to National Marine Fisheries Service (NMFS). This is a natural extension of our previous and current work that focuses on juvenile development within the estuary, and is likely to be supported since research on the influences of ocean processes on salmon growth and survival is in the forefront of NMFS Office of Protected Resources research priorities.

The requested supporting letter from the California Department of Fish and Game to allow collection of entrained salmon from several pumping facilities in the delta has been delayed until they review the full proposal. They have expressed interest in the objectives of proposal, which will entail collecting small numbers of entrained juvenile fall-run chinook salmon (20 fish). We anticipate departmental support for our proposal following their review.

APPLIED
marine
SCIENCES

April 12, 1999

San Mateo County Planning Department
590 Hamilton Street
Redwood City, CA 94063

To Whom It May Concern:

A consortium of University (UC Davis), government (USFW), and private (Applied Marine Sciences, Inc.) researchers are currently applying for CALFED funding to study effects of contaminants on Chinook salmon. CALFED requires that all proposals include notification to the counties that are within the geographic scope of the proposed research, and also requires notification of the Delta Protection Commission, and the Bay Conservation and Development Commission. This letter is intended to serve as this notification.

The research that we are proposing may elucidate potential contaminant effects on various life stages of Chinook salmon. Our aim is to be able to provide information to resource managers that will allow refinement of current management practices affecting Chinook, and by extension, other fish populations that are currently in decline. The geographic scope of our study includes much of the Sacramento/San Joaquin river system, the San Francisco Bay/Delta, and the nearshore ocean out to the Farallon islands.

Any questions that you may have concerning our proposed research may be addressed to:

Dr. Gary Marty
1 Shields Avenue
School of Veterinary Medicine
Department of Anatomy, Physiology, and Cell Biology
University of California
Davis, CA 95616
Phone: (530) 754-8062
Fax: (530) 752-7690

or,

Jordan Gold
Applied Marine Sciences, Inc.
4749 Bennett Dr. Suite L
Livermore, CA 94550
Phone: (925) 373-7142
Fax: (925) 373-7834

Thank You,

Jordan Gold
Marine Biologist

JG/dts



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APPLIED
Marine
SCIENCES

April 12, 1999

Santa Clara County Planning Department
70 W. Hedding Street
San Jose, CA 95110

To Whom It May Concern:

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Thank You,

Jordan Gold
Marine Biologist

JG/dts





I-019187

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SCIENCES

April 12, 1999

Solano County Planning Department
601 Texas Street
Fairfield, CA 94533

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Thank You,

Jordan Gold
Marine Biologist

JG/dts





I-019188

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April 12, 1999

Sacramento County Planning Department
827 Seventh Street
Sacramento, CA 95814

To Whom It May Concern:

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Thank You,

Jordan Gold
Marine Biologist

JG/dts

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SCIENCES

April 12, 1999

Yolo County Planning Department
292 West Deamer
Woodland, CA 95695

To Whom It May Concern:

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Thank You,

Jordan Gold
Marine Biologist

JG/dts





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SCIENCES

April 12, 1999

Colusa County Planning Department
220 12th Street
Colusa, CA 95932

To Whom It May Concern:

A consortium of University (UC Davis), government (USFW), and private (Applied Marine Sciences, Inc.) researchers are currently applying for CALFED funding to study effects of contaminants on Chinook salmon. CALFED requires that all proposals include notification to the counties that are within the geographic scope of the proposed research, and also requires notification of the Delta Protection Commission, and the Bay Conservation and Development Commission. This letter is intended to serve as this notification.

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Thank You,

Jordan Gold
Marine Biologist

JG/dts



I-019191

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SCIENCES

April 12; 1999

Sutter County Planning Department
1160 Civic Center Blvd.
Yuba City, CA 95993-3007

To Whom It May Concern:

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Thank You,

Jordan Gold
Marine Biologist

JG/dts





I-019192

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SCIENCES

April 12, 1999

Glenn County Planning Department
125 S. Murdock Avenue
Willows, CA 95988

To Whom It May Concern:

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Fax: (925) 373-7834

Thank You,

Jordan Gold
Marine Biologist

JG/dts

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marine
SCIENCES

April 12, 1999

Butte County Planning Department
7 County Center Drive
Oroville, CA 95965-3334

To Whom It May Concern:

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Fax: (925) 373-7834

Thank You,

Jordan Gold
Marine Biologist

JG/dts



I-019194

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marine
SCIENCES

April 12, 1999

Tehama County Planning Department
444 Oak Street, Courthouse Annex, Rm I
Red Bluff, CA 96080

To Whom It May Concern:

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Thank You,

Jordan Gold
Marine Biologist

JG/dts





I-019195

APPLIED
marine
SCIENCES

April 12, 1999

Shasta County Planning Department
1855 Placer Street
Redding, CA 96001-1759

To Whom It May Concern:

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Livermore, CA 94550
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Fax: (925) 373-7834

Thank You,

Jordan Gold
Marine Biologist

JG/dts





I-019196

APPLIED
marine
SCIENCES

April 12, 1999

Marin County Planning Department
3501 Civic Center, 308
San Rafael, CA 94903-4157

To Whom It May Concern:

A consortium of University (UC Davis), government (USFW), and private (Applied Marine Sciences, Inc.) researchers are currently applying for CALFED funding to study effects of contaminants on Chinook salmon. CALFED requires that all proposals include notification to the counties that are within the geographic scope of the proposed research, and also requires notification of the Delta Protection Commission, and the Bay Conservation and Development Commission. This letter is intended to serve as this notification.

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Thank You,

Jordan Gold
Marine Biologist

JG/dts





I-019197

APPLIED
marine
SCIENCES

April 12, 1999

Sonoma County Planning Department
2550 Ventura Avenue
Santa Rosa, CA 95403

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Marine Biologist

JG/dts





I-019198

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SCIENCES

April 12, 1999

Napa County Planning Department
1195 3rd Street, 2nd Floor, Room 210
Napa, CA 94559

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Marine Biologist

JG/dts

APPLIED
marine
SCIENCES

April 12, 1999

Santa Cruz County Planning Department
701 Ocean Street
Santa Cruz, CA 95060

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Livermore, CA 94550
Phone: (925) 373-7142
Fax: (925) 373-7834

Thank You,

Jordan Gold
Marine Biologist

JG/dts

APPLIED
marine
SCIENCES

April 12, 1999

San Joaquin County Planning Department
1810 East Hazelton
Stockton, CA 95205

To Whom It May Concern:

A consortium of University (UC Davis), government (USFW), and private (Applied Marine Sciences, Inc.) researchers are currently applying for CALFED funding to study effects of contaminants on Chinook salmon. CALFED requires that all proposals include notification to the counties that are within the geographic scope of the proposed research, and also requires notification of the Delta Protection Commission, and the Bay Conservation and Development Commission. This letter is intended to serve as this notification.

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Any questions that you may have concerning our proposed research may be addressed to:

Dr. Gary Marty
1 Shields Avenue
School of Veterinary Medicine
Department of Anatomy, Physiology, and
Cell Biology
University of California
Davis, CA 95616
Phone: (530) 754-8062
Fax: (530) 752-7690

**or,**

Jordan Gold
Applied Marine Sciences, Inc.
4749 Bennett Dr. Suite L
Livermore, CA 94550
Phone: (925) 373-7142
Fax: (925) 373-7834

Thank You,

Jordan Gold
Marine Biologist

JG/dts

APPLIED
marine
SCIENCES

April 12, 1999

Stanislaus County Planning Department
1101 H Street
Modesto, CA 95354

To Whom It May Concern:

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Thank You,

Jordan Gold
Marine Biologist

JG/dts



I-019202

APPLIED
marine
SCIENCES

April 12, 1999

Merced County Planning Department
2222 M Street
Merced, CA 95340

To Whom It May Concern:

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Thank You,

Jordan Gold
Marine Biologist

JG/dts

APPLIED
marine
SCIENCES

April 12, 1999

Madera County Planning Department
135 West Yosemite Avenue
Madera, CA 93637

To Whom It May Concern:

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Thank You,

Jordan Gold
Marine Biologist

JG/dts





I-019204

APPLIED
marine
SCIENCES

April 12, 1999

Fresno County Planning Department
2220 Tulare Street, 6th Floor
Fresno, CA 93721

To Whom It May Concern:

A consortium of University (UC Davis), government (USFW), and private (Applied Marine Sciences, Inc.) researchers are currently applying for CALFED funding to study effects of contaminants on Chinook salmon. CALFED requires that all proposals include notification to the counties that are within the geographic scope of the proposed research, and also requires notification of the Delta Protection Commission, and the Bay Conservation and Development Commission. This letter is intended to serve as this notification.

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Thank You,

Jordan Gold
Marine Biologist

JG/dts



I-019205

APPLIED
marine
SCIENCES

April 12, 1999

Kings County Planning Department
1400 W. Lacey Blvd.
Hanford, CA 93230

To Whom It May Concern:

A consortium of University (UC Davis), government (USFW), and private (Applied Marine Sciences, Inc.) researchers are currently applying for CALFED funding to study effects of contaminants on Chinook salmon. CALFED requires that all proposals include notification to the counties that are within the geographic scope of the proposed research, and also requires notification of the Delta Protection Commission, and the Bay Conservation and Development Commission. This letter is intended to serve as this notification.

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Thank You,

Jordan Gold
Marine Biologist

JG/dts

APPLIED
marine
SCIENCES

April 12, 1999

Alameda County Board of Supervisors
1212 Oak Street, Room 512
Oakland, CA 94612

To Whom It May Concern:

A consortium of University (UC Davis), government (USFW), and private (Applied Marine Sciences, Inc.) researchers are currently applying for CALFED funding to study effects of contaminants on Chinook salmon. CALFED requires that all proposals include notification to the counties that are within the geographic scope of the proposed research, and also requires notification of the Delta Protection Commission, and the Bay Conservation and Development Commission. This letter is intended to serve as this notification.

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Livermore, CA 94550
Phone: (925) 373-7142
Fax: (925) 373-7834

Thank You,

Jordan Gold
Marine Biologist

JG/dts



I-019207

APPLIED
marine
SCIENCES

April 12, 1999

San Mateo County Board of Supervisors
400 County Center
Redwood City, CA 94063

To Whom It May Concern:

A consortium of University (UC Davis), government (USFW), and private (Applied Marine Sciences, Inc.) researchers are currently applying for CALFED funding to study effects of contaminants on Chinook salmon. CALFED requires that all proposals include notification to the counties that are within the geographic scope of the proposed research, and also requires notification of the Delta Protection Commission, and the Bay Conservation and Development Commission. This letter is intended to serve as this notification.

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Livermore, CA 94550
Phone: (925) 373-7142
Fax: (925) 373-7834

Thank You,

Jordan Gold
Marine Biologist

JG/dts



I-019208

APPLIED
marine
SCIENCES

April 12, 1999

Santa Clara County Board of Supervisors
70 West Hedding Steet, 10th Floor
San Jose, CA 95110

To Whom It May Concern:

A consortium of University (UC Davis), government (USFW), and private (Applied Marine Sciences, Inc.) researchers are currently applying for CALFED funding to study effects of contaminants on Chinook salmon. CALFED requires that all proposals include notification to the counties that are within the geographic scope of the proposed research, and also requires notification of the Delta Protection Commission, and the Bay Conservation and Development Commission. This letter is intended to serve as this notification.

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Livermore, CA 94550
Phone: (925) 373-7142
Fax: (925) 373-7834

Thank You,

Jordan Gold
Marine Biologist

JG/dts



I-019209

APPLIED
marine
SCIENCES

April 12, 1999

Solano County Board of Supervisors
580 Texas Street
Fairfield, CA 94533

To Whom It May Concern:

A consortium of University (UC Davis), government (USFW), and private (Applied Marine Sciences, Inc.) researchers are currently applying for CALFED funding to study effects of contaminants on Chinook salmon. CALFED requires that all proposals include notification to the counties that are within the geographic scope of the proposed research, and also requires notification of the Delta Protection Commission, and the Bay Conservation and Development Commission. This letter is intended to serve as this notification.

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Thank You,

Jordan Gold
Marine Biologist

JG/dts

APPLIED
marine
SCIENCES

April 12, 1999

Sacramento County Board of Supervisors
700 H Street, Room 2450
Sacramento, CA 95814

To Whom It May Concern:

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Fax: (925) 373-7834

Thank You,

Jordan Gold
Marine Biologist

JG/dts

APPLIED
marine
SCIENCES

April 12, 1999

Yolo County Board of Supervisors
625 Court Street, Room 204
Woodland, CA 95695

To Whom It May Concern:

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Thank You,

Jordan Gold
Marine Biologist

JG/dts

APPLIED
marine
SCIENCES

April 12, 1999

Colusa County Board of Supervisors
546 Jay Street
Colusa, CA 95932

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Thank You,

Jordan Gold
Marine Biologist

JG/dts

APPLIED
marine
SCIENCES

April 12, 1999

Sutter City County Board of Supervisors
11520 Maidu Avenue
Nevada City, CA 95959-8617

To Whom It May Concern:

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Jordan Gold
Marine Biologist

JG/dts

APPLIED
marine
SCIENCES

April 12, 1999

Glenn County Board of Supervisors
P.O. Box 391
Willows, CA 95988

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Thank You,

Jordan Gold
Marine Biologist

JG/dts

APPLIED
marine
SCIENCES

April 12, 1999

Butte County Board of Supervisors
25 County Center Drive
Oroville, CA 95965

To Whom It May Concern:

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Thank You,

Jordan Gold
Marine Biologist

JG/dts





I-019216

APPLIED
marine
SCIENCES

April 12, 1999

Tehama County Board of Supervisors
P.O. Box 250
Red Bluff, CA 96080

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Thank You,

Jordan Gold
Marine Biologist

JG/dts



I-019217

I-019217

APPLIED
marine
SCIENCES

April 12, 1999

Shasta County Board of Supervisors
1815 Yuba Street
Redding, CA 96001

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Thank You,

Jordan Gold
Marine Biologist

JG/dts



I-019218

APPLIED
marine
SCIENCES

April 12, 1999

Marin County Board of Supervisors
3501 Civic Center Drive, Suite 329
San Rafael, CA 95403

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Thank You,

Jordan Gold
Marine Biologist

JG/dts




I-019219

APPLIED
marine
SCIENCES

April 12, 1999

Sonoma County Board of Supervisors
575 Administration Drive, Room 100
Santa Rosa, CA 95403

To Whom It May Concern:

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Any questions that you may have concerning our proposed research may be addressed to:

Dr. Gary Marty
1 Shields Avenue
School of Veterinary Medicine
Department of Anatomy, Physiology, and
Cell Biology
University of California
Davis, CA 95616
Phone: (530) 754-8062
Fax: (530) 752-7690

or,

Jordan Gold
Applied Marine Sciences, Inc.
4749 Bennett Dr. Suite L
Livermore, CA 94550
Phone: (925) 373-7142
Fax: (925) 373-7834

Thank You,

Jordan Gold
Marine Biologist

JG/dts





I-019220

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marine
SCIENCES

April 12, 1999

Napa County Board of Supervisors
1195 3rd Street, 3rd Floor, Room 310
Napa, CA 94559

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Thank You,

Jordan Gold
Marine Biologist

JG/dts



I-019221

APPLIED
marine
SCIENCES

April 12, 1999

Santa Cruz County Board of Supervisors
701 Ocean Street, Room 500
Santa Cruz, CA 95060

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Thank You,

Jordan Gold
Marine Biologist

JG/dts





I-019222

APPLIED
marine
SCIENCES

April 12, 1999

San Joaquin County Board of Supervisors
222 East Weber Avenue, Room 701
Stockton, CA 95202

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marine
SCIENCES

April 12, 1999

Stanislaus County Board of Supervisors
1100 H Street, 2nd Floor
Modesto, CA 95354

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A consortium of University (UC Davis), government (USFW), and private (Applied Marine Sciences, Inc.) researchers are currently applying for CALFED funding to study effects of contaminants on Chinook salmon. CALFED requires that all proposals include notification to the counties that are within the geographic scope of the proposed research, and also requires notification of the Delta Protection Commission, and the Bay Conservation and Development Commission. This letter is intended to serve as this notification.

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Thank You,

Jordan Gold
Marine Biologist

JG/dts



I-019224

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marine
SCIENCES

April 12, 1999

Merced County Board of Supervisors
2222 M Street
Merced, CA 95340

To Whom It May Concern:

A consortium of University (UC Davis), government (USFW), and private (Applied Marine Sciences, Inc.) researchers are currently applying for CALFED funding to study effects of contaminants on Chinook salmon. CALFED requires that all proposals include notification to the counties that are within the geographic scope of the proposed research, and also requires notification of the Delta Protection Commission, and the Bay Conservation and Development Commission. This letter is intended to serve as this notification.

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Thank You,

Jordan Gold
Marine Biologist

JG/dts



I-019225

APPLIED
marine
SCIENCES

April 12, 1999

Madera County Board of Supervisors
209 West Yosemite
Madera, CA 93637

To Whom It May Concern:

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Thank You,

Jordan Gold
Marine Biologist

JG/dts



I-019226

APPLIED
marine
SCIENCES

April 12, 1999

Fresno County Board of Supervisors
2281 Tulare Street, Room 301
Fresno, CA 93721

To Whom It May Concern:

A consortium of University (UC Davis), government (USFW), and private (Applied Marine Sciences, Inc.) researchers are currently applying for CALFED funding to study effects of contaminants on Chinook salmon. CALFED requires that all proposals include notification to the counties that are within the geographic scope of the proposed research, and also requires notification of the Delta Protection Commission, and the Bay Conservation and Development Commission. This letter is intended to serve as this notification.

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Thank You,

Jordan Gold
Marine Biologist

JG/dts



I-019227

APPLIED
Marine
SCIENCES

April 12, 1999

Kings County Board of Supervisors
1400 W. Lacey Blvd.
Hanford, CA 93230

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I-019228

APPLIED
marine
SCIENCES

April 12, 1999

Delta Protection Commission
14215 River Road
P.O. Box 530
Walnut Grove, CA 94102

To Whom It May Concern:

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I-019229

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April 12, 1999

Bay Conservation and Development Commission
30 Van Ness Avenue, Room 2011
San Francisco, CA 94102

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I-019230