ERP Proposal Application Form

For DFG use only	
Proposal No.	Region

Section 1: Summary Information

1. Project title:	**Assessing Contaminant and Pathogen Susceptibility in Steelhead Trout**
2. Applicant name:	Dr. Richard Edward Connon
3. Contact person:	May Turner
4. Address:	The Regents of the University of California, Office of Research, Sponsored Programs 1850 Research Park Drive, Suite 300 University of California
5. City, State, Zip:	Davis, California 95616
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8. Email address:	vcresearch@ucdavis.edu
9. Agency Type:	Federal Agency ☐ State Agency ☐ Local Agency ☐ Nonprofit Organization ☐ University (CSU/UC) ■ Native American Indian Tribe ☐
10. Certified nonprofit organization:	Yes ■ No ☐ *(Organizational DUNS: 047120084)*
11. New grantee:	Yes ■ No ☐
12. Amount requested:	$649,340
13. Total project cost:	$706,230 including matching funds from Dr. Israel, U.S. BOR (Note: This project integrates with U.S. Bureau of Reclamation research and assessments, though not presented as matching funds)
14. Topic Area(s):	At-Risk Species Assessment (1), Ecosystem Water and Sediment Quality (2)
15. ERP Project type:	Research (1), Monitoring (2)
16. Ecosystem Element:	Contaminants (1), Riparian and Riverine Aquatic Habitats (2)
17. Water Quality Constituent:	Pesticides (1), Toxicity of Unknown Origin and Contaminants (2),
18. At-Risk species benefited:	Steelhead trout (*Oncorhynchus mykiss*)
19. Project objectives:	To investigate contaminant and pathogen susceptibility in steelhead broodstocks and families, by identifying and quantifying pathogens in steelhead, measuring responses to infection and determining susceptibility of broodstocks and families through DNA phenotyping.
20. Time frame:	3 years (July 1st, 2011, June 30th, 2014)

Section 2: Location Information

Note: *The location details provided below are applicable to sampling sites or sites at which research work will be conducted.*

1. Township, Range, Section: and the 7.5 USGS Quad map name.	**River Hatchery**: 2001 Nimbus Road, Gold River, CA 95670 **Feather River Hatchery**: 5 Table Mountain Blvd. Oroville, CA 95965 **ATL & CABA Facilities**: One Shields Avenue, VM:APC, University of California, Davis, CA 95616 *Quad map names are not applicable*
2. Latitude, Longitude (in decimal degrees, Geographic, NAD83):	**American River Hatchery**: lat/long: 38.633706,-121.225950. **Feather River Hatchery**: lat/long: 39.518145,-121.552890 **ATL & CABA Facilities**: lat/long: 38.52527,-121.77526.
3. Location description:	Steelhead hatcheries, university research facilities. Sampling will be conducted throughout the Sacramento and Delta Regions.
4. County(ies):	**American River Hatchery**: *Sacramento County*. **Feather River Hatchery**: *Oroville County*. **ATL & CABA Facilities**: *UC Davis is in both Solano and Yolo Counties.*
5. Directions:	**American River Hatchery Directions:** Off Highway 50, 18 miles east of Sacramento. Go north on Hazel Avenue 0.7 mile then west at signal onto Gold Country Blvd. Parking lot entrance is about 200 yards on right. **Feather River Hatchery Directions:** Take the Grand Nelson exit from Highway 70, east on Nelson Ave. 2 miles. South on Table Mountain Blvd. about 0.5 miles to the hatchery entrance. **ATL & CABA Facilities Directions:** Off Hwy 113, Hutchinson Dr. exit (west). Left at Campbell Rd, Right at the “T” junction at Garrod Rd, follow the road all the way to the end, making sure to stay right at the “Y’ in the road near the sheep barns.
6. Ecological Management Region:	Sacramento Region (1), Delta Region (2)
7. Ecological Management Zone(s):	Sacramento Region 3, 8, 9 and 10. Delta Region 1
8. Ecological Management Unit(s):	Sacramento Region 3.4, 8.1, 9.1, 9.2 and 10. Delta Region 1.1, 1.2 and 1.4
9. Watershed Plan(s):	**Not applicable**
10. Project area:	**Not applicable**
11. Land use statement:	**Not applicable**
12. Project area ownership:	% Private_______ % State________ % Federal________ *Enter ownership percentages by type of ownership*. **Not applicable**
13. Project area with landowners support of proposal:	**Not applicable**

Section 3: Landowners, Access and Permits

1. Landowners Granting Access for Project: (Please attach provisional access agreement[s]) **NOT APPLICABLE**	
2. Owner Interest:	
3. Permits:	
4. Lead CEQA agency:	
5. Required mitigation:	Yes ☐ No ☐

Section 4: Project Objectives Outline

1. List task information:

Goal 1: Endangered and Other At-risk Species and Native Biotic Communities (Objective 1): The goal of the proposed study is to assess how pathogens and contaminants can affect the life-cycle of steelhead trout, and ascertain differences in susceptibility between broodstocks and families. Our approach is to measure a number of parameters, at different levels of biological organization, in order to assess the degree of infection, disease condition, and stock and family susceptibility differences from steelhead trout originating from the Feather River and Nimbus hatcheries operated by California Department of Fish and Game. The data generated from our study will inform conservation management on contaminant reductions, and habitat restoration as well as provide information on life cycle monitoring of fish physiology under different stressors.

2. Additional objectives:

Goal 6: Water and Sediment Quality (objective 1): The data generated from our study will provide further evidence to support the enforcement and strengthening of the California Water Code and the Clean Water Act, particularly for the control of pyrethroid pesticides and pathogens. It will also provide a means for assessing the detection of pathogens in water and sediment samples (through the application of a resulting tool from this proposal).

3. Source(s) of above information:

Appendix D: Ecosystem Restoration Strategic Goals and Objectives

Section 5: Conflict of Interest

To assist ERP staff in managing potential conflicts of interest as part of the review and selection process, we are requesting applicants to provide information on who will directly benefit if your proposal is funded. Please provide the names of individuals who fall in the following categories:

- Persons listed in the proposal, who wrote the proposal, will be performing the tasks listed in the proposal, or who will benefit financially if the proposal is funded; and/or
- Subcontractors listed in the proposal, who will perform tasks listed in the proposal, or will benefit financially if the proposal is funded.

Primary Contact for Proposal: Dr. Richard E. Connon (UC Davis)
Primary Investigator: Dr. Richard E. Connon (UC Davis)
Co-Primary Investigator: Dr. Joshua A. Israel (U.S. Bureau of Reclamation)
Co-Primary Investigator: Dr. Bernie May (UC Davis)
Co-Primary Investigator: Dr. Frank J. Loge (UC Davis)
Co-Primary Investigator: Dr. Dolores V. Baxa (UC Davis)
Supporting Staff: SRA IV (not named)
Supporting Staff: SRA II (not named)
Supporting Staff: Junior Specialist (not named)
Supporting Staff: Student Assistant(s) III (not named)
Subcontractor: N/A

Provide the list of names and organizations of all individuals not listed in the proposal who helped with proposal development along with any comments.

Last Name	First Name	Organization	Role

Section 6: Project Tasks and Results Outline

1. Detailed Project Description

1.1. Project Purpose: This proposal directly addresses requirements specified by the Delta Regional Ecosystem Restoration Implementation Plan (DRERIP): Priority 2, *"Research that Tests Hypotheses Identified in the DRERIP Evaluation of the Bay Delta Conservation Plan (BDCP) Conservation Measures and National Research Council Operations Criteria and Plan (OCAP) Biological Opinion Review and Address Uncertainties"*, and aims to "*Address Potential Factors Affecting Productivity"*, namely the effects of a pyrethroid insecticide; bifenthrin and interactions of this and other contaminants and toxicity of unknown origin, with pathogen infections on Californian steelhead trout (*Oncorhynchus mykiss*), an at-risk species. While effects of contaminants and pathogens on juvenile steelhead are recognized to occur predominantly in tidal and floodplain habitats, and riverine habitats following early rainstorms, the DRERIP evaluations found there to be a high degree of uncertainty concerning conservation measures that reduce contaminants. This study will develop the theoretical and applied knowledge to reduce this uncertainty and inform life cycle monitoring that integrates fish physiology under different stressors and habitat restoration.

Although we generally tend to use the word contaminant to represent xenobiotic chemicals, such as pesticides and heavy metals, the term contaminant includes a number of other pollutants such as biological agents, nutrients and oxygen depleting substances, organic carbon and disinfection byproduct precursors. Hereafter, we utilize the term *contaminant* to represent xenobiotic chemicals, and *pathogens* to indicate biological agents of disease, as the two primary stress components addressed in this proposal.

Both pathogenic infections and chemical contaminants can, amongst numerous other environmental variables, greatly affect the health status of fish in the Bay-Delta System. Bacterial kidney disease, caused by *Renibacterium salmoninarum* infections, for example, is one of the major contributors to mortality of salmonids. Pathogen infections have long been a problem at fish hatcheries and facilities. There are numerous pathogens known to affect various fish populations (addressed later in this PSP), and their interactions, level of infection and subsequent effects on the immune system, are known to affect fitness, and thus (out/in)-migration success and survival. In fact, recent life history modeling of Central Valley steelhead concluded that reduced survival of emigrating smolts are the greatest management concern for preserving anadromous life history forms (Satterthwaite et al., 2010). Approximately 70% of steelhead captured at south Delta diversion fish collection facilities originated from hatcheries (adipose fin clipped) between 2000 and 2010 and approximately 90% of adults captured by anglers appear to originate from hatcheries. Levels of infections have been monitored in prior studies, by assessing physical appearance and presence/absence of pathogen classes. However, little is known about the susceptibility of different steelhead families, and the organismal responses to the various agents of disease. Furthermore, studies have indicated that contaminant exposure can increase susceptibility to pathogenic infection (Clifford et al., 2005; Eder et al., 2008), however, retrospectively, pathogenic infection will also affect an organisms ability to contend with contaminants.

The focus of this project will therefore be placed on the physiological assessment of the effect of naturally occurring pathogens and contaminants common to the Bay-Delta on steelhead trout, which will be conducted throughout life-cycle assessments. Our integrative approach will be to detect, identify and where possible quantify pathogens, profile the genomic responses to infection, examine changes in gene expression resultant from disease, and directly measure these responses to assess susceptibility across different families. We will use a specific, yet relatively straight-forward approach to assess the combined effects of pathogens and contaminant exposure. This approach will allow us to develop and apply efficient monitoring tools, which will be immediately available to biologists, to evaluate the health of steelhead and potential restored steelhead habitats in the Bay-Delta.

Our interdisciplinary team includes expertise in ecology, pathology, ecotoxicology, molecular biology, bioinformatics and modeling, key to the success of this project. The proposed study will have laboratory, hatchery and field based components, integrated to address the following research questions:

i) Are there differences in contaminant and pathogen susceptibility in different steelhead trout hatchery broodstocks and families?

ii) How do pathogenic infections and contaminant exposures affect the health status, and thus the probability of survival of steelhead trout?

We hypothesize that differences in susceptibility to pathogen infections, amongst different populations and families of steelhead trout, will affect their capacity to contend with further stressors; such as chemical contaminants. We previse that these differences can be quantified using genomic techniques, and that genomic data will be indicative of disease thresholds, and thus predictive of survival probability.

Our specific objectives are to:

1) Assess the variation in pathogen susceptibility of different stock and families of steelhead trout, and their ability to contend with subsequent contaminant exposure.
2) Apply existing and enhanced contaminant exposure and pathogen-specific molecular biomarkers to both measure and profile genomic responses in infected steelhead trout, and contrast them with pathogenic agent types.
3) Use existing genetic tools to ascertain the presence/absence and quantitation of pathogen species in steelhead trout.
4) Use data from above objectives, to model responses to survival probability, thus inferring life-cycle success
5) Monitor steelhead trout health status using a combination of tools applied to the above objectives.

1.2. Introduction: Populations of steelhead trout (*Oncorhynchus mykiss*) in the California Bay-Delta System have declined in abundance over the past decades, with causes attributed to loss of spawning and rearing habitats that are critical to juvenile growth, smolt survival, and egg incubation (Evans et al., 2004). The constriction of spawning and rearing habitats below dams has reduced the species' spatial distribution by more than 95% and likely exposes multiple life stages to abiotic and biotic processes which the species is not adapted to. The Central Valley steelhead Distinct Population Segment is listed as threatened under the U.S. Endangered Species Act (ESA) (NMFS, 1997).

Steelhead trout have an anadromous life-cycle. They spawn between December and April; with fry emergence occurring in May-June. Juveniles spend their first 1-3 years of life in freshwater, smolts out-migrate to the ocean in the spring, with a peak abundance from February to May. After spending between one to four seasons in growing in the ocean, they return to their natal spawning sites. Most steelhead present in the Central Valley, however, they originate from hatcheries. Steelhead are propagated at four hatcheries in the Central Valley and subject to many hatchery interactions while they undergo spawning, incubation, growth, and smoltification. Hatchery practices can lead to pathogen infections with varying degrees of susceptibility hypothesized amongst different families.

The value of restored tidal, riverine, and floodplain habitats for steelhead is considered to be highly uncertain due to limited monitoring data showing presence of these fish in these habitats (BDCP, 2010). The National Research Council's review on the Operations Criteria and Plan Biological Opinion suggested newly restored floodplain habitats could further mobilize contaminants (NCR, 2010), thus, the habitat conditions leading to exposure of steelhead to the interactive effects of contaminants and pathogens may have a significant effect on steelhead and other aquatic species.

Pathogenic infections in steelhead are of particular concern. Numerous viral, bacterial, fungal and parasitic diseases are associated with salmonids. The infectious hematopoietic necrosis virus (IHNV) is one of the predominant viral pathogens associated with salmonids in the western United States (Bendorf et al., 2007; Clifford et al., 2005). Bacterial kidney disease caused by *Renibacterium salmoninarum* infections (Sanders et al., 1992), and *Myxobolus cerebralis*, a microscopic parasite causative of whirling disease (Hedrick et al., 1998) are also major contributors to mortality in salmonids. Infections and subsequent effects on the immune system can hinder the ability of fish to contend with other environmental stressors, and thus affect survival. Moreover, there is growing concern that contaminant toxicity may be directly affecting salmonid populations (Baldwin et al., 2009), as exposure to contaminants can further increase susceptibility to disease (Clifford et al., 2005), consequently affecting survival and population dynamics.

Bifenthrin is one of the most frequently and widely used synthetic pyrethroid insecticides, and commonly found in surface waters and sediments of the Sacramento-San Joaquin River. It is used in agriculture, but also is increasingly used for landscaping and structural pest control (Domagalski et al., 2010). The principal mode of action of bifenthrin is interference with voltage-gated ion channels and ATPase enzymes causing hyperexcitability, tremors, convulsions and ultimately death. However, in addition, recent studies have indicated that this pyrethroid insecticide affects endocrine activity (Liu et al., 2011; Wang et al., 2010). Bifenthrin has been detected in Bay-delta urban runoff, in nearly 80% of tested samples, at concentrations as high as 30ng/L, 10 times the median lethal concentration to invertebrate organisms (Weston and Lydy, 2010), a concentration above which negative effects on fishes' swimming performance have been reported (Beggel et al., 2010). Furthermore, bifenthrin has consistently been detected; in over 95% of sediment samples in California, at concentrations as high as 542 μg/Kg for dry sediment and 122 μg/Kg for wet sediment samples in

southern California (Bondarenko et al., 2007), thus there is concern that bifenthrin mobilization resulting from restoration efforts could jeopardize fish health.

Determining the sublethal effects of environmental stressors in field-collected fish is a major challenge, yet these effects likely have a significant population-level effect. Molecular biomarkers are becoming increasingly powerful, and informative tools that can be used to assess both disease and exposure to contaminants (Ankley et al., 2006; Miller et al., 2011), along with the effects of numerous environmental stressors upon organisms at risk (Björnsson et al., 2011; Zippay and Hofmann, 2010). Gene expression profiling has received much attention in the medical arena as a therapeutic predictive tool (Auman and McLeod, 2008; Cosler and Lyman, 2009; Holmes et al., 2006; Yeoh et al., 2002) and functional classifications have enabled identification of affected biochemical pathways and the modes of action of chemicals (Ankley et al., 2009; Wang et al., 2008b). In recent years, such approaches have been successfully applied in ecological contexts, e.g. to assess effects of exposure and identify contaminants involved through known modes of action, (Poynton et al., 2007; Wang et al., 2008a). These studies have provided promising outcomes towards health status assessments in ecosystems, with some studies linking gene expression with population level effects (Connon et al., 2008; Fedorenkova et al., 2010; Heckmann et al., 2008; Miller et al., 2011). Furthermore, Connon et al. (2009) and Beggel et al. (in preparation) have identified specific genes, associated with neurological and endocrine activity in delta smelt and fathead minnow, that responded significantly to esfenvalerate and bifenthrin exposure; and current research investigating site specific genomic profiles studies on delta smelt have revealed specific gene signatures that point to the sources of contaminants, and highlighted responses to pathogen infection (Connon et al - unpublished data).

Study-specific approaches can effectively be performed using carefully selected biomarkers to assess the health status and stress responses in individuals. For example, Vanya et al. (2005; 2008), have successfully identified gene specific responses in Atlantic salmon to *Aeromonas salmonicida*, a fish bacterial pathogen that causes furunculosis. More recently, Miller et al. (2011) have published genomic profiles specific to viral infections in adult sockeye salmon and were able to differentiate between health condition and successful adult migration. Lastly,members of our integrative team have used genomic profiling to successfully differentiate between health condition of juvenile steelhead in the Columbia River (Connon et al., submitted) that were linked to outmigration success and survival probabilities (Hostetter et al., in review). Thus, by addressing adverse outcome pathways through integrating a number of levels of biological organization; histopathological, visual physiological assessments and probabilistic survival, genomic responses can be used in life-cycle assessments and population studies.

2. Background and Conceptual Models

The focus of the proposed study is to assess how pathogens and contaminants can affect the life-cycle of steelhead trout, and ascertain differences in susceptibility between broodstocks and families. Our approach is to measure a number of parameters, at different levels of biological organization, in order to assess the degree of infection, disease condition, and stock and family susceptibility differences from steelhead trout originating from the Feather River and Nimbus hatcheries operated by California Department of Fish and Game. The broodstocks at these two hatcheries are considered distinct, with the Feather River hatchery steelhead belonging to the Central Valley steelhead Distinct Population Segment (hereforth, CV Steelhead DPS) and the Nimbus hatchery steelhead being from an out-of-basin coastal stock. Genetically, steelhead from the Feather and American rivers are distinct enough that they can be identified with microsatellite DNA markers (Garza and Pearse 2009). These stocks also appear to be distinguishable in their life history diversity, spatial distribution, and phenotypes. Feather River steelhead likely spend longer as juveniles in freshwater and have a higher rate of residency, are associated with the Northern Sierra Diversity Group of the CV Steelhead DPS, and do not grow to a very large size. Historically, the Nimbus hatchery steelhead broodstock was imported from the coastal Eel River basin, but have become very well established to the dam-altered flow regime and temperatures on the American River. Nimbus steelhead grow more rapidly than other CV steelhead stocks, have a lower rate of residency, are not included in the CV steelhead DPS, and can grow to a very large size. Juvenile steelhead and resident trout in these tributaries are typically exposed to high summer and fall temperatures, and fish in the American River may be more tolerant to high temperatures than steelhead from regions further north (Myrick and Cech, 2004). This is alarming since the occurrence of a bacterial-caused anal vent inflammation has been reported by CDFG to be associated with warm water temperatures in the American River (NMFS, 2009).

Gene transcription in the assessment of pyrethroid exposure: Genomic assessments of pyrethroid exposure have provided enhanced information on the mode of action of this pesticide class. Genome-wide assessment conducted on larval delta smelt exposed to esfenvalerate (Connon et al., 2009), highlighted effects upon the neuromuscular system and immune system, along with apoptosis, redox, osmotic stress, detoxification, and growth. Swimming impairment was correlated with neuromuscular responses; e.g. expression of aspartoacylase (ASPA), an enzyme involved in brain cell function and associated with numerous human neurological diseases. In further, studies conducted on larval fathead

minnow exposures to bifenthrin, transcription responses of genes involved in detoxification, neuromuscular activity and energy metabolism could be correlated to swimming performance impairments. Bifenthrin has recently been shown to have both estrogenic and anti-estrogenic properties (Jin et al. 2008, Brander et al., unpublished data). Furthermore, pyrethroid metabolites, generated via photolysis, hydrolysis, or metabolic processing are reported to have greater endocrine activity than parent compounds (Tyler et al., 2000, McCarthy et al. 2006). Assessments in embryo-larval stages of zebrafish have indicated that bifenthrin exposure can accelerate the hatching process in a dose-dependent manner, and that genomic responses have indicated links between development and disruption of endocrine system (Jin et al., 2009). Thus, since responses to contaminant exposure are often preceded by alterations in gene expression (Connon et al., 2008; Geist et al., 2007; Heckmann et al., 2008), genomic assessment can provide species-specific and sensitive, mechanistic information on the overall health of an organism.

Gene transcription profiling in the assessment of disease condition: The application of genomic tools are not restricted to toxicology, and have been successfully applied to investigate health status and disease (Baerwald et al., 2008; Miller et al., 2011). We present below, results from a study conducted at UC Davis with collaborators at Oregon State University, and recently submitted for publication (Connon et al., submitted), that forms the proof of principal for this proposal. We used a suite of seven biomarkers developed in *O. mykiss*, preselected towards a small-scale and preliminary assessment of the effects of pathogen infection and heavy metal exposure. In this study we reported the use of molecular biomarkers applied to kidney and gill tissue samples, in the assessment of immune system responses, pathogen defense, and proteotoxicity, profiling and correlating gene expression changes in steelhead trout from the Columbia River, OR. Results were correlated with visual classifications of radio-tagged juvenile steelhead attributed upon capture, survival and out-migration probabilities (Hostetter et al., in review). In brief, genomic responses in anterior head kidney highly differentiated between visual classification groups (Fig 1.), identifying immune system responses (upregulation of NRAMP, CXC and MX) and proteotoxic effects (upregulation of HSP70 and HSP90), along with impairment of cell structure and integrity (upregulation of beta-actin), and probable metal exposure (upregulation of metallotionein) in the “poor” and “bad” condition, comparable to “good” rank condition.

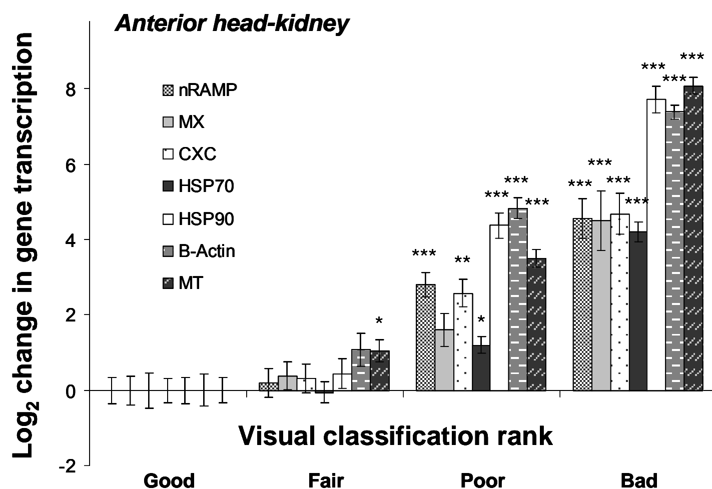

Figure 1: Mean Log_2 change in gene transcription in juvenile steelhead anterior head-kidney, per attributed physical appearance classification. NRAMP: natural resistance-associated macrophage protein, MX: myxovirus resistance, MET: metallothionein-b, CXC: alpha-chemokine, HSP70: heat-shock protein 70KDa, HSP90: heat-shock protein 90KDa, B-Actin: beta actin. Bars indicate standard errors. Levels of significance ($p<0.05$, $p<0.01$ and $p<0.001$) are represented by *, ** and *** respectively, comparable to “Good” rank classification.

Hierarchical cluster analysis using individual fish gene transcription data (Fig. 2) separated responses by tissue type (gill and kidney), and grouped overall responses by condition rank, with only a few overlapping “outliers” as determined by visual external condition classification. Profiling transcription did not separate “good” and “fair” condition ranks, however, there is a predominant cluster of individuals labeled as “bad” and “poor” expressing an overall elevated gene transcription in anterior head kidney tissue. Genomic profiling thus summarizes expression in a downward trend in health condition. Furthermore, clusters are seemingly representative of disease types, with mycotic dermatitis (Md) highlighted in the “bad” condition, and genomic profiles of fish affected by Infectious Hematopoietic Necrosis Virus (IHNV) clustered together regardless of condition classification, suggesting supporting our hypothesis that different infection types yield specific genomic response patterns (Indicated as viral or fungal in Fig 2). An overall view of the genomic response differences can be seen in figure 3, representing data per condition ranking, successfully differentiated by principal component analysis, primarily differentiating between “good”, “poor” and “bad” classification ranks, with fish labeled as “fair” overlapping with “good” and “poor” ranks. We contend that by increasing the number of genes measured, profiles will be more specific to each pathogen and/or pathogen interaction effects, and thus be more accurate in determining the health status of sampled individuals. Although this study was conducted with lethal samples, it is possible to apply this approach using specific biomarkers on non-lethal samples; such as biopsied skin and gill tissue as performed by Miller et al (2011).

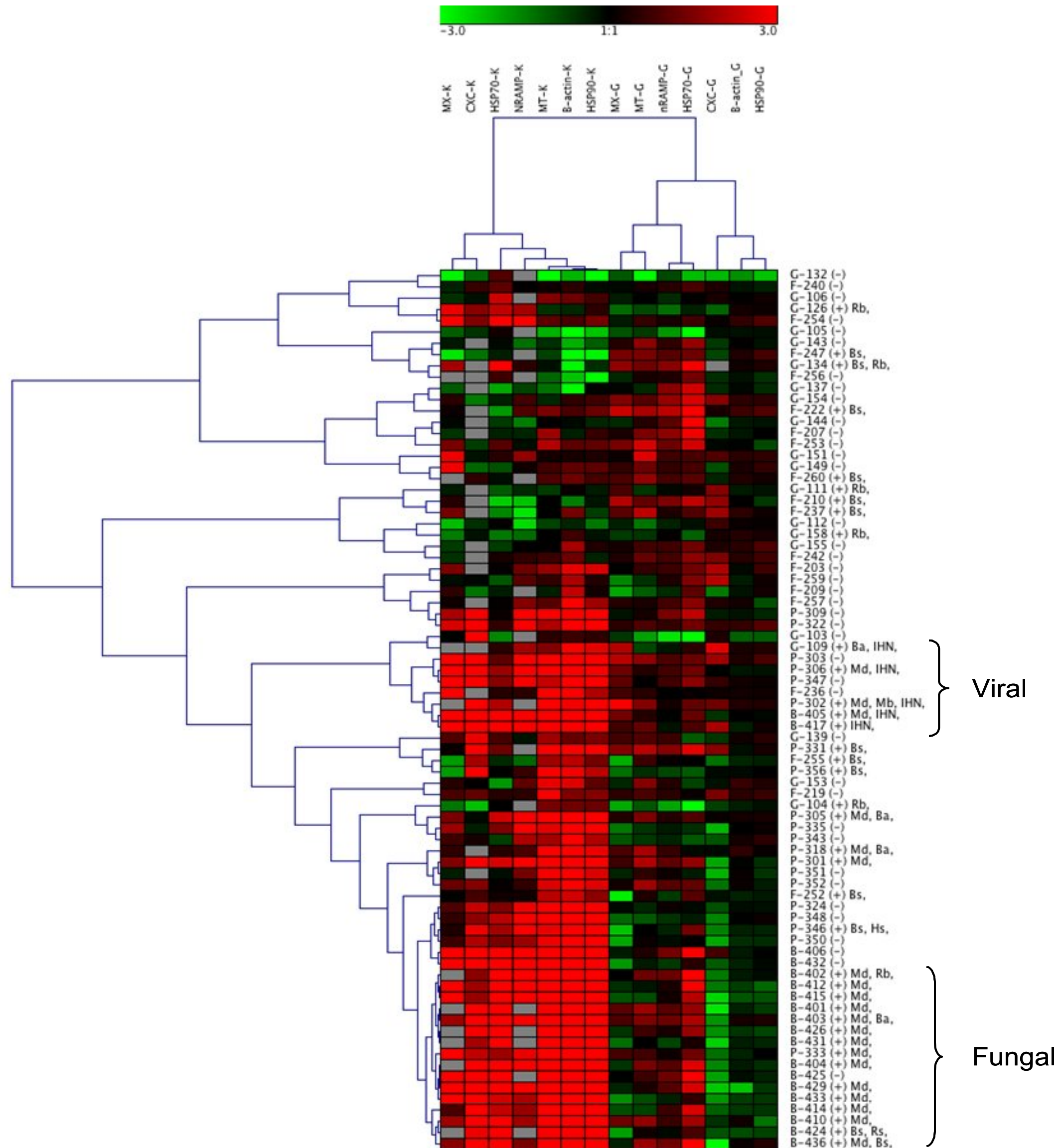

Figure 2: Genomic Profiling: Hierarchical Pearson's correlation complete clustering of gill and anterior head kidney gene expression data with individual juvenile steelhead labeled correspondingly to visually assigned health status. NRAMP: natural resistance-associated macrophage protein, MX: myxovirus resistance, MET: metallothionein-b, CXC: chemokine, HSP70: heat-shock protein 70KDa, HSP90: heat-shock protein 90KDa, B-Actin: beta actin. Grey boxes indicate missing data. Fish labels are presented as condition (G: good, F: fair, P: poor and B: bad), numbers as attributed to fish upon capture and classification, detection (+) or non-detection (-) of pathogens, and infection type/condition (Rb: Renibacterium salmoninarum, IHN: Infectious Hematopoietic Necrosis Virus, Bs: Branchial Sanguinicoliasis, Ba: Branchial Amebiasis, Md: Mycotic Dermatitis, Mb: Mycotic Branchitis, Hs: Hepatic Sanguinicoliasis, Rs: Renal Sanguinicoliasis). Specific transcription profiles attributed to fungal and viral infections are indicated.

Pathogen identification and assessment of degree of infection: RNA/DNA techniques are readily available to detect and quantify pathogens that are specific to salmonids. A number of studies have been published describing the use of molecular approaches to identify and quantify pathogen species. Warsen et al. (2004) have used 16S ribosomal DNA (rDNA) sequences to discriminate between fifteen different species of pathogens. Altinok et al. (2008) have used PCR techniques to detect 5 different bacterial strains of bacterial fish pathogens, and there are numerous other publications that depict the use of RNA/DNA samples in fish pathogen assessments (e.g. Beaz-Hidalgo et al., 2008; Dorsch et al., 1994), and others that have used quantitative PCR to ascertain the level of infection, determined from the amount of pathogen RNA/DNA in respective salmonids tissues (e.g. Chase et al., 2006). Utilizing a suite of genetic markers to detect, identify and quantify pathogens in steelhead, and many other fish species is therefore a relatively straight forward, but effective approach to perform, yet will provide invaluable support to the understanding and interpretation in the assessment of links between health condition and transcription profiles. Thus integrating pathogen identification and where possible quantification will be a key component of this proposal.

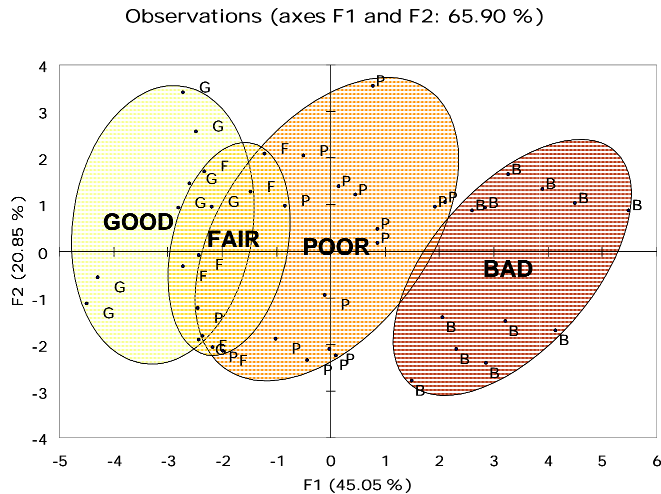

Figure 3: Principal Component Analysis (PCA) of transcription profiles of field-caught juvenile steelhead: Percentage contribution of factors F1 and F2 to variability are attributed per fish and successfully separate health conditions as designated by external characteristics of sampled fish. G: good, F; fair, P: Poor and B: Bad.

Stock and family identification using single nucleotide polymorphism (SNP): We will be able to connect our identification of pathogen infection and disease condition, for which we have developed a foundation of through laboratory investigation, to observations of individuals in the Bay-Delta through stock identification of steelhead captured during USFWS beach seining and trawls and BOR and DWR fish salvage operations at the CVP/SWP diversion facilities. As part of a larger study, funded by BOR, all steelhead hatchery broodstocks will be genetically tagged by the NOAA-Fisheries Southwest Fisheries Science Center. Parental based genetic tagging (Anderson and Garza, 2006) will be done using 96 SNP markers with genotypes of parents being provided to BOR as part of their agreement with NOAA. The publicly available program, SNPPit will be used to reconstruct parent/offspring trios for steelhead recaptured and genetically sampled in the USFWS trawls on the Sacramento River and Chipps Island and at the CVP/SWP fish collection facilities. Additionally, a profile will be taken of the fish to characterize the health condition, which will later be correlated with genomic characterization of the fishes' response to pathogens and contaminants. Using these genetic tag recaptures, a proportion of total fish recaptured from each sampling location can be characterized, and an index of stock outcomes measured, using known release numbers from each steelhead hatchery. This index will be integrated into modeling the life cycle outcomes of each hatchery steelhead stock.

Modeling pathogen-contaminant effects and survival probability: A simplistic model can be constructed that relates the probability of the level of up- or down-regulation of specific gene transcripts, to a particular exposure treatment, and subsequent probability of mortality. First, by exposing individuals to a series of exposure doses and/or infection types, obtaining non-lethal samples at more than one time-point during exposure and recording resulting mortality over the test duration, and second, by assessing genomic responses to the various treatments, and establishing a relationship between gene transcription levels and exposure dose or infection, the incidence of mortality resulting from exposure can be plotted as a function of transcription level. Conducting laboratory studies that will aid construct and calibrate this model, will result in a tool that can estimate the probability of mortality in hatchery sampled and field-caught individuals, where a dose or infection level is known, or used to estimate the probability of morality directly from quantitation of up- or down-regulated transcripts. (Please refer to Approach and Scope of Work, below, for specific experimental design and model details).

Integration of this proposal with other studies: The proposed investigation of correlations between steelhead genomic and survival responses with pathogen infection and contaminant exposure, and health condition, will provide critical tools to support the quantification of benefits, and assessments of hatchery practices, habitat restoration, and flow restoration for the CV steelhead population. These efforts are underway as part of the Comprehensive Steelhead Monitoring Plan that has been developed in coordination with the Interagency Ecological Program Steelhead Project Work Team. This monitoring plan focuses on juvenile and adult steelhead monitoring to characterize the abundance, life history diversity, and spatial distribution of these fish in Central Valley tributaries, the lower Sacramento River, and Bay-Delta restoration efforts. This study will provide biologists with insight into specific-tributary hatchery stock survival, as well as an understanding of the mechanisms behind this demographic process. Additionally, these methods will establish visual health condition measures to assess steelhead condition and survival probabilities of these fish captured in restored habitats and collected in monitoring efforts during periods when flows are being managed for fish survival. The proposal's agency co-investigator is intent of utilizing the tools developed in these studies to meet these needs in future integrated monitoring and restoration efforts.

Conceptual model: Our proposal aims to answer the following research questions: i) Are there differences in contaminant and pathogen susceptibility in different steelhead trout hatchery broodstocks and families? and ii) How do pathogenic infections and contaminant exposures affect the health status, and thus the probability of survival of steelhead trout?

Fishes' susceptibility and/or adaptive abilities to pathogenic infection are largely dependent on physiological response mechanisms; such as the adequacy of their immune system and the ability to simultaneously contend with additional environmental stressors. The major histocompatibility complex (MHC) for example is know to play an significant role in resistance to pathogenic diseases, and expression of this and other genes can influence host resistance (Arkush et al., 2002). Genomic techniques thus offer a sensitive way of measuring stressor effects upon an organism allowing for mechanistic responses. Genomics, or more explicitly transcriptomics, has the capacity to gather vast amount of information from small sample quantities, capturing the physiological responses initiated at the molecular level (messenger RNA). An effective use of genomics can be achieved in studies that use detailed functional analyses of genes in an ecologically relevant context. Transcriptional expression levels can be exploited to highlight alterations to functional networks and the biochemical pathways they are involved in, and thus the characterization of biological processes. Functional classifications offer a way of clustering together organismal responses to environmental changes (e.g. Fig. 2 – Background)

Gene transcription (or mRNA expression) provides the template for protein synthesis, thus in a simplistic manner, the level of expression indicates the quantity of protein that can be synthesized. Upregulation, for example, may result in a higher level of protein synthesis which may permit innate and adaptive immune responses offer the required protection against disease by identifying and killing respective pathogens. Induction of specific proteins can be associated with infectious pathogen classes; for example *Myxovirus resistance protein 1* (Mx) gene expression is upregulated by viral infection, responsible for resistance to influenza. Thus by measuring the transcription levels of this gene it is possible to infer viral infection levels. We hypothesize therefore, that by using a suite of genes specifically selected to assess responses to pathogen infection, and that transcription profiles will be indicative not only of level if disease, but also of pathogen classes responsible for said disease. The presence, or absence, of a pathogen can easily be determined using DNA techniques, but its presence does not mean that the organism is diseased. Thus, an integration of systems that measure both infection and effect are necessary to adequately indicate health status.

Of critical importance to our understanding of the vulnerability of organisms to environmental stress is a comprehensive understanding of the physiological "weak links" that underlie organismal susceptibility to disease. Contaminants, pathogens and other environmental stressors elicit complex physiological responses and it is often unclear which are responsible for an organism's critical point(s) of failure. With continuous concerns surrounding pelagic organism declines, along with anthropogenic impacts to the Bay-Delta, and disturbance resulting from remediation and restoration of delta habitats. In the post-genomic era, we have access to a number of sensitive genomics-enabled techniques that provide us with the capability of investigating the molecular response of many cellular pathways. Gene regulation, the process of altering mRNA transcripts for the required synthesis of proteins, is one of the most rapid and versatile ways in which an organism can respond to environmental stressors. The use of genomics tools is particularly useful and efficient in elucidating the cellular-level responses to pathogen induced disease in fish. Suites of differentially regulated genes can provide a physiological signature of organismal health condition, identify pathogen classes responsible for the disease, as well as uncover the molecular mechanisms underlying pathogen susceptibility, thus gene expression studies stand to be an excellent approach for understanding the broad and integrated responses of aquatic organisms facing environmental stressors (Hofmann and Todgham, 2010; O'Donnell et al., 2010; Todgham et al., 2007; Todgham and Hofmann, 2009). In turn, these types of data can be analyzed alongside data identifying hatchery broodstocks and family specific responses to investigate robustness and effects on steelhead life-cycles.

Infected fish may have different capacities for fending off disease. This capacity may be resultant of broodstocks, i.e. if they originate from a particular spawning site or hatchery, or it may be dependent on family susceptibility. Susceptibility differences can be assessed under laboratory conditions by infecting the test species and measuring the response, e.g my monitoring survival, which can take weeks to months to complete. Alternatively, the process can be sped up by exposing the organisms to a secondary stressor, such as a pesticide. In doing this, resulting mortality, or genomic responses, can be used to assess differences between broodstocks and families over a shorter time period. Furthermore, this represents a more environmentally relevant scenario, as organisms are generally exposed to multiple environmental stressors. Thus pathogen identification and abundance, in combination with gene expression profiles and SNP genotyping, could be used to rapidly and efficiently monitor, infection types, infection levels, effects upon the organisms and differences amongst exposed individuals (Fig. 4), parameters that can be modeled towards determining survival probability, through conducting simple but purposefully designed assessments. These monitoring systems can be applied to hatchery and field populations of steelhead trout, in order to investigate pathogen infection and level of disease in the wild (Fig. 5), where the effect of pathogenic infections can be assessed in a multiple-stressor environment, as an overall measure of health in steelhead trout. This approach would be extremely valuable in monitoring effects resulting from restoration projects in which sediment disturbance may take place, especially in areas where chemical and biological

contaminants may be present, and if so, would these disturbances could affect the life-cycle of steelhead trout? Furthermore, broodstock and family-specific differences in susceptibility could help inform managers on maintenance approaches of healthy stock populations.

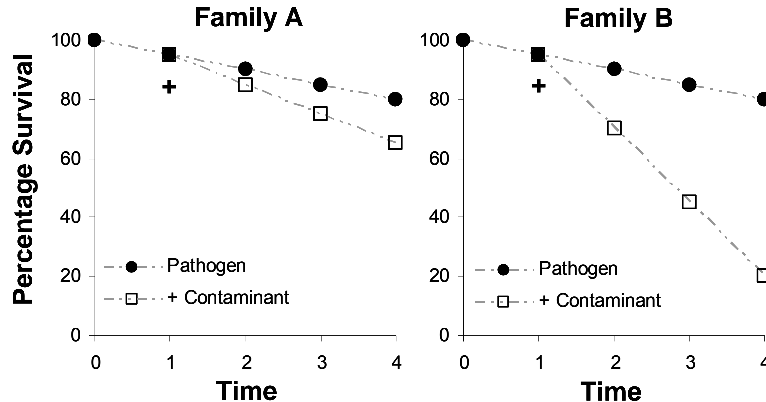

Figure 4: Conceptual model of the assessment of family specific susceptibility to pathogen infection, with added contaminant stress. The detrimental effects of pathogen infection may not me immediately noticeable, but the immune system of infected fish responds within a short time frame; from a few minutes to a few hours. In order to fully assess the effects, test durations need to be performed over weeks to months. The adding an extra stressor, such as a pesticide known to further affect the immune system is expected to enhance the detrimental effect, thus shortening the test duration time. This will allow for the rapid both a rapid assessment of susceptibility differences between fish, and also to assess the combined effect of pathogens and chemical contaminants. Family identification will be performed through SNP analysis, transcription profiling and pathogen abundance will be assessed by quantitative PCR.

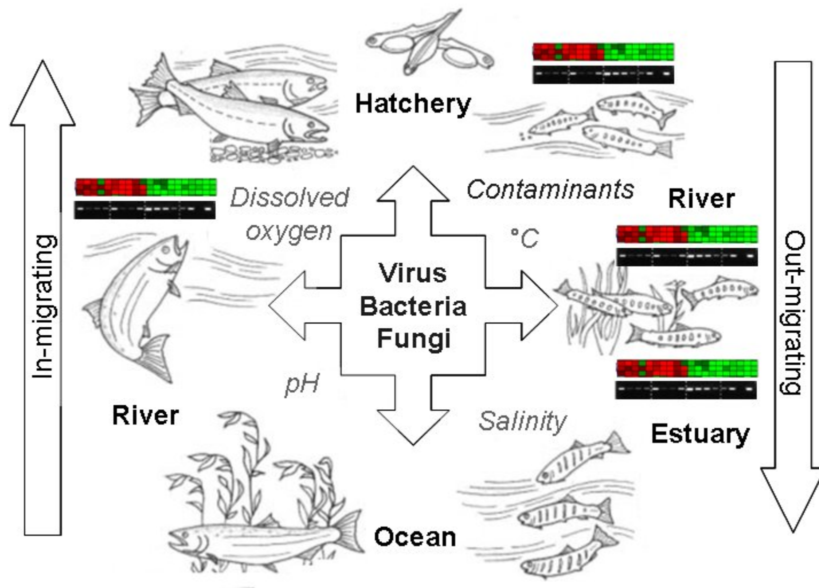

Figure 5: Schematic diagram of the hatchery and field sampling capability of this project, indicating stages at which monitoring could take place. Transcription profiling and pathogen identification can be assessed in hatchery and field-caught steelhead trout, from a multiple-stressor environment that can greatly affect steelhead life-cycle success.

3. Approach and Scope of Work:

Laboratory, hatchery and field based assessments will be conducted on larval and juvenile steelhead to investigate the effects of contaminants and pathogens. Our approach comprises the application of three primary techniques. First, we will use polymerase chain reactions (PCR) on fish samples in order to determine the presence/absence of specific pathogens, using methodology and tools described in a number of publications (Table 1). Second, we will measure transcription responses, using quantitative PCR (qPCR) techniques, of a number of carefully selected genes. Genes will

be appropriately selected from those listed in table 2 based on assessed tissue type, as transcription in some cases is tissue specific. Genes were selected as representative of known responses to pathogen exposure, and to target specific responses known to be elicited by the selected pyrethroid; bifenthrin in the proposed laboratory study. Third, we will conduct single nucleotide polymorphism (SNP) assessments to identify broodstocks and families, to ascertain any specific differences in susceptibility. We will then utilize a modeling approach to assess the predictive value of our data (details below) and conduct histopathology assessments of specific samples in order to validate transcription responses as molecular biomarkers of exposure and effect (disease).

Table 1. Published polymerase chain reaction (PCR) techniques for fish pathogen identification and quantitation

Pathogen	**Example of published PCR technique by:**
Aeromonas hydrophila	(Altinok et al., 2008; Warsen et al., 2004)
Aeromonas salmonicida	(Altinok et al., 2008; Warsen et al., 2004)
Edwardsiella ictaluri	(Warsen et al., 2004)
Escherichia coli	(Warsen et al., 2004)
Flavobacterium branchiophilum	(Warsen et al., 2004)
Flavobacterium columnare	(Altinok et al., 2008; Warsen et al., 2004)
Flavobacterium psychrophilum	(Warsen et al., 2004)
Infectious hematopoietic necrosis virus (IHNV)	(Williams et al. 1999)
Listonella anguillarum	(Hong et al., 2007)
Mycobacterium chelonae	(Warsen et al., 2004)
Mycobacterium fortuitum	(Warsen et al., 2004)
Mycobacterium marinum	(Warsen et al., 2004)
Photobacterium phosphoreum	(Warsen et al., 2004)
Renibacterium salmoninarum	(Altinok et al., 2008; Warsen et al., 2004)
Staphylococcus aureus	(Warsen et al., 2004)
Streptococcus iniae	(Warsen et al., 2004)
Tenacibaculum maritimum	(Warsen et al., 2004)
Vagococcus salmoniarum	(Warsen et al., 2004)
Viral hemorrhagic septicemia virus (VHSV)	(Williams et al. 1999)
Yersinia ruckeri	(Altinok et al., 2008; Warsen et al., 2004)

Note: many primer sets are available, for these and other pathogens, and will be added/changed within the scope of the proposal, dependent on temporal variations and pathogen concern.

Table 2. Examples of biomarkers to be used for profiling genomic responses to pathogen infections and contaminant exposure in *Oncorhynchus mykiss.*

Gene name (gene code)	Function (response to)
Tumor necrosis factor alpha (TNFa)	Regulation of immune cells and cell death (Immune system)
Transcription factor Jun-B activator protein (JunB)	Interacts with systemic inflammation processes (Immune system)
Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (NFKB1)	T-cell receptor and cell death (Immune system)
Interleukin 1, beta (IL-1B)	Mediator of the inflammatory response (Immune system)
Natural resistance-associated macrophage protein (NRAMP)	Natural resistance macrophage (Immune system)
Toll-like receptor 5 (TLR5)	Innate immune response to microbial agents (Immune system)
Interferon regulatory factor 1 (IRF1)	Regulation of cell death (Immune system)
T-cell antigen receptor, alpha polypeptide (TCR alpha)	Innate immune response to microbial agents (Immune system)
Major histocompatibility complex class I antigen (MHC1a)	Involved in the presentation of foreign antigens to the immune system (Immune system)
Proteasome (prosome, macropain) subunit, beta type, 4 (PSMB4)	Mediates the lipopolysaccharide-induced signal macrophage proteasome (Immune system)
Pituitary adenylate cyclase-activating peptide (PACAP)	Stimulates adenylate cyclase in pituitary cells (Lymphocyte)
Polyribonucleotide nucleotidyltransferase 1 (PNPT1)	Involved in mRNA degradation (Lymphocyte)
Kruppel-like factor 2 (KLF2)	Beta-globin gene promoter (Lymphocyte)
Cell division cycle 42 (CDC42)	Binds to a variety of effector proteins to regulate cellular responses (Lymphocyte)
Zeta-chain (TCR) associated protein kinase 70kDa (ZAP70)	Innate immune response to microbial agents (Lymphocyte)

Table 2 cont.

Gene name (gene code)	Function (response to)
Fushi tarazu - transcription factor 1 (FTZ-F1)	Binds to element of the enhancer II of hepatitis B virus genes, a critical cis-element of their expression and regulation (Lymphocyte)
DEP domain containing 6 (DEPDC6)	Negative regulator of the mTORC1 and mTORC2 signaling pathways. Inhibits the kinase activity of both complexes (Lymphocyte)
Interleukin 8-like chemokine (CXC)	Immune surveillance and pathogen detection (Pathogen detection)
CC motif ligand 4 (CCL4)	Immune surveillance and pathogen detection (Pathogen detection)
Elongation factor alpha-1 (EF1a)	Correlation of cell death with pathogen resistance (Immune response)
Myxovirus resistance (MX)	Antiviral activity (Viral association)
Keratin-14 (KRT14)	Associated with epidermolysis (Viral association)
Beta actin (b-actin)	Cell structure (Structural)
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Cell structure (Structural)
Collagen type X1a (Col11a)	Vertebral (Structural)
Aspartoacylase (Aspa)	Neurological (Canavan disease)
Tubulin cofactor beta (TBCB)	Neurological (Development)
Inducible nitric oxide synthase (iNOS)	Neurological (and cytokine association)
Calmodulin (CaM)	Calcium signaling (mediator of NOS and numerous other processes)
Heat shock protein (HSP 70)	General stress response (Protein stabilization)
Creatine kinase (CK)	Muscle (Activity)
Atrogin-1 (Fbxo32)	Muscle (Atrophy)
Metallothionein	Metal sequestration (Detoxification)
Cytochrome P450, family 1, subfamily A1 (Cyp1a1)	Xenobiotic metabolism (Detoxification)
Cytochrome P450, family 3, subfamily A, (Cyp3a)	Endocrine (Development and reproduction)
Choriogenin H (ChgH)	Endocrine (Development and reproduction)
Vitellogenin-1 (Vtg1)	Endocrine (Development and reproduction)
G-protein coupled glucagon receptor (GlucR)	Endocrine (Development and reproduction)

Note: genes will be added or subtracted depending on tissue types to be assessed.

To achieve our objectives, the proposal has been divided into laboratory, hatchery and field based studies upon which we will conduct genomic profiles, pathogen identification, and broodstock and family assignations (see corresponding budget justifications for each of the following sections).

3.1. Laboratory Studies: a) Assessments of the interactions between IHNV infections and bifenthrin exposure will be conducted on larval steelhead. b) Genomic profiling, IHNV infection quantitation, pesticide exposure concentration, disease and mortality, will be investigated towards generating a model to ascertain thresholds of transcription level and likely consequences. c) Transcription differences will be further validated with histopathology assessments.

a) Larval steelhead (10-15dph) from two central valley hatcheries; Feather River and American River, will be infected with IHNV and then subsequently exposed to bifenthrin. Larvae from reflecting 10-20 crosses from each hatchery (200 ea) will be infected with IHNV at 8.2×10^3 plaque-forming-units (PFU).L^{-1}, as detailed in Clifford et al (2005), by submersion for 1 h, in 5 L aquaria in groups of 10-20 individuals (dependent on available age/size). All larvae will then be transferred using a net into two 35 gallon tanks in which they will be maintained for the test duration. At 24h after IHNV infection, 80% of water will be replenished and tanks will be spiked with a sublethal dose of Bifenthrin determined from preliminary investigations, detailed below. Larvae will be exposed to bifenthrin for 2h h, after which they will be euthanized with an overdose of MS-222, snap-frozen in liquid nitrogen, and stored at -80°C for later analyses (see specific methodologies below). Controls for each test will represent untreated larvae, IHNV-bifenthrin and Bifenthrin-IHNV respectively of equal numbers. We will conduct genomic profiling by qPCR, broodstock and family assignations by SNPs and infection quantitation by qPCR utilizing IHNV-specific primers, on an estimated 100 samples, dependent on mortality rates. Specific methodologies for each assessment system are detailed below.

b) We will use juvenile steelhead (6-8 cm) towards genomic threshold modeling. All fish will be tagged with a unique identifier, 48 h prior to test start, using photonic markers. In brief, colored microscopic beads are injected into the rays of the selected fins. Multiple fin locations will be targeted (dorsal, caudal, anal) using several colors, enabling numerous combinations. Fish will be anaesthetized in 75 mg.L^{-1} of MS-222, and individually marked. Fish will be exposed to IHNV as described above, and then transferred to 16L aquaria, in triplicate, with 10 individuals per replicate. Aquaria will be maintained at 12°C, with a 16:8 hour photoperiod. At 24h after IHNV infection, 80% of water will be replenished and tanks will be spiked with three doses of bifenthrin determined from preliminary investigations (detailed below), at concentrations that represent NOEC, LC10 and LC50. Controls for each test will represent

untreated larvae, IHNV-bifenthrin and Bifenthrin-IHNV respectively in equal numbers, and replication. Following a 24 h period of pesticide exposure, approximately 50% of the surviving fish will be euthanized with an overdose of MS-222, samples of gill and head-kidney will be dissected, snap-frozen in liquid nitrogen, and stored at -80°C for later analyses of gene expression profiles and viral infection. Additionally, all remaining fish will be anaesthetized in 75 $mg.L^{-1}$ of MS-222, gill tissues will be non-lethally sampled, and appropriately stored, and fish will be returned to aquaria containing control water, where they will be maintained for a further 7 d.

Gene expression, relative to baseline values, will be plotted as a function of bifenthrin dose. Selected genes will be used to construct a model that related the probability of the quantity of up- or down-regulation of a particular gene to bifenthrin dose:

$$P_{GE} = f(D) \qquad (1)$$

where $f(D)$ is the functional relationship established from the empirical data relating gene transcript level to bifenthrin dose and P_{GE} is the probability density function of a specified transcript level (GE) given dose D. After steelhead are exposed for 24 to bifenthrin, mortality will be recorded over a 7-day period. The incidence of mortality will be plotted as a function of transcript level (GE), and the following model will be developed:

$$P(M|GE) = f(GE) \qquad (2)$$

where $f(GE)$ is the functional relationship established from the empirical data relating the incidence of mortality to gene transcript level and $P(M|GE)$ is the probability of mortality (M) given transcript level GE. The probability of mortality $P(M)$ for a fish exposed to a specified dose of bifenthrin will then be estimated as:

$$P(M) = \int P(M|GE) \cdot P_{GE} \cdot dGE \qquad (3)$$

The resulting model (Eq. 3), developed and calibrated with laboratory data, can be used to estimate the probability of mortality in both hatchery- and field-collected fish if the dose of bifenthrin were known. Alternatively, Eq. 2, developed and calibrated with laboratory data, can be used to estimate the probability of mortality in both hatchery- and field-collected fish if the gill gene transcript level (GE) is known.

At the completion of the 7-day post bifenthrin exposed, all surviving fish will be euthanized with an overdose of MS-222 and samples of gill and head-kidney will be dissected, snap-frozen in liquid nitrogen, and stored at -80°C for later analyses of gene expression profiles and viral infection. The gene expression profiles of surviving fish will be used to explore possible genes associated with survival using a similar modeling approach as outlined above.

Note: Dead fish will be removed from the aquaria as soon as identified, and stored for viral infection and quantitation analyses. Gene expression cannot be adequately measured from these samples.

c) Sub-samples of gills and kidney tissues from the above test will be will also be collected and stored for histopathological analysis, by fixing in 10% neutral buffered formalin, dehydrated in a graded ethanol series and embedded in JB-4 glycomethacrylate (Polysciences, Pennsylvania, USA), following protocols by Teh et al (2004). Histopathology assessments will be selective; and only conducted on samples in which differences in transcription responses have been measured between controls and pathogen-infected/bifenthrin-exposed samples. For these samples, serial sections of gill and kidney (4 µm thick) will be cut and stained with hematoxylin and eosin. Tissue sections will be examined under a BH-2 Olympus microscope for cytological alterations. We will conduct genomic profiling by qPCR, and infection quantitation by qPCR utilizing IHNV-specific primers, on all samples (methodologies for each system are detailed below), along with histopathology on selected samples as described herein. In order to validate and ascertain specificity of biomarker responses, direct comparisons will be made between genomic profiles, IHNV quantitation, and histopathology-assessed lesions. To do this, thresholds of transcription levels will be established for IHNV infection or disease, by contrasting and determining levels at which lesions are observable, and correlations with pathogen quantification will be performed. Although the resulting data will be specific to IHNV, information gained by this approach is expected to contribute towards the interpretation of genomic responses to other pathogen classes.

3.2. Hatchery Studies: We will assess genomic profiles, pathogen screening and family assignations, in juvenile steelhead from the Feather River and American River hatcheries. The methodologies for each analytical system are detailed below.

Gill and kidney samples, using both lethal and non-lethal approaches will be obtained from hatchery juveniles Numbers and age will be dependent on availability and hatchery-specific criteria, but we anticipate analyzing approximately 100 fish from each hatchery. Fish will be either anesthetized or euthanized in MS-222, tissue samples biopsied or dissected respectively, snap-frozen in liquid nitrogen, stored on dry ice and transported to the laboratory for storage at -80°C for subsequent analyses. (*Note: other tissue samples; such as liver, spleen, brain and muscle, will also be sampled and stored at -80°C for use in corresponding future projects and assessments*).
In addition, mucus and blood samples will be collected from all individuals, and utilized for pathogen screening and identification purposes. Specific methodologies for each assessment system are detailed below.

3.3. Field Studies: We will assess genomic profiles, pathogen screening, and broodstock and family assignations, in field-caught steelhead from the Sacramento-San Joaquin Delta. The methodologies for each analytical system are detailed below.

Gill biopsies (non-lethal sampling) will be obtained from field-caught steelhead, in association with sampling conducted on a parent project by the U.S. Bureau of Reclamation, coordinated by Dr. Israel. Sampling will be performed throughout the duration of the project, and we anticipate analyzing tissues from over 300 individuals.
Where lethal sampling will be performed by the parent project, we will obtain kidney samples on which we will assess genomic profiles. Fish will be either anesthetized or euthanized in MS-222, tissue samples biopsied or dissected respectively, placed into vials containing RNAlater™ for preservation, maintained on ice, and transported to the laboratory for storage at -80°C for subsequent analyses.
In addition, mucus and blood samples will be collected from all individuals, and utilized for pathogen screening and identification purposes. Specific methodologies for each assessment system are detailed below.

Preliminary investigations: We will conduct a series of acute and sublethal assessments on larval steelhead exposures to bifenthrin, to determine sub-lethal concentrations to be used in the pathogen/pesticide exposure described above (costs for these tests have been incorporated in the budget for the laboratory studies).

i) First, bifenthrin NOEC, LC10 and LC50 concentrations will be established using acute toxicity tests following standard procedures described for rainbow trout (USEPA, 2002). In brief, ten larval steelhead (10-20dph), will be exposed to a control, methanol control and six serial dilutions of bifenthrin, in quadruplicate, in 5L aquaria maintained at 12°C, with a 16:8 hour photoperiod, for a period of 24 h.

ii) A second exposure will then be conducted within the sublethal range to a maximum LC10, under the conditions described above. Swimming performance will be assessed using predator avoidance techniques, detailed by Beggel et al (2010). We will determine the lowest concentration at which there is a significant effect on swimming performance, and utilize this for the combined IHNV/Bifenthrin exposures described above. *Note: this concentration has proven to consistently result in peak genomic responses following contaminant exposures, thus is will be selected to represent maximum compensation to exposure (Connon et al., in review; Connon et al., 2009; Heckmann et al., 2008).*

Specific methodology:

a) *RNA and DNA extraction:* where same samples are being used for different assessments, DNA and RNA extractions will be performed simultaneously using TRIzol (Invitrogen) as per manufacturer's protocols. This system uses a phenol:chloroform extraction technique, that separates RNA, DNA and protein, by centrifugation:

- *RNA extraction*: following centrifugation, the upper aqueous phase of the Trizol extraction will be precipitated with 50% isopropanol, and purified with 75% ethanol, following centrifugation protocols that have been optimized in order to obtain maximum yield for differing fish tissues. Qualitative assessments will be carried out through visualization on 1% agarose gels to ensure product integrity. Total RNA will be quantified spectrophotometrically using a NanoDrop 2000.

- *DNA extraction*: following centrifugation, the interphase and organic phase will be mixed with 100% ethanol and homogenized, following secondary centrifugation, and sodium citrate/ethanol washing steps, the DNA will be solubilized in 8 mM NaOH and quantified spectrophotometrically using a NanoDrop 2000.

b) *cDNA synthesis:* Complementary cDNA will be synthesized from 1µg total RNA using 100 units of SuperScript III, 600 ng random random hexamer primers, 10 U RnaseOut (Rnase inhibitor), and 1 mM dNTPs (all Invitrogen, Carlsbad, CA, USA) in a final volume of 20 µL. The reverse transcription reaction will be carried out at 50°C for 50 min and

terminated by heating for 5 min to 95°C with immediate cooling on ice. Samples will be diluted with nuclease-free water to a total volume of 50µL and stored at -20 °C for subsequent qPCR assessments.

c) *Quantitative PCR:* Gene transcription will be assessed for a set of 48 TaqMan Gene Expression Assays in BioMark, 48X48 Dynamic Array chips (Fluidigm Corp). Samples and TaqMan-Primer/Probe reaction mixes will be loaded on to Dynamic arrays using a multichannel micropipettor, and qPCR reactions performed and analyzed using BioMark Real-Time PCR System and Analysis software (Fluidigm Corp), respectively. Changes in expression will be calculated the mean of the two reference genes and calibrated to control samples. Assay efficiencies criteria will be set to 100 ± 10%.

d) *Pathogen assessments:*

- *Pathogen identification (presence/absence): c*DNA and DNA from each individual will be combined in a 1:1 ratio, to provide a template from which pathogens will be identified. This combination will be performed because some pathogens are single-stranded RNA viruses (e.g. IHNV), and the combination will omit duplications, by permitting the assessments on single samples. cDNA/DNA amplification will be performed using pathogen specific primers obtained through published research (see table 2), or designed within this project, using a PCR thermocycler (Eppendorf Mastercycler), with an initial denaturation at 94◦C for 3 min followed by 30 cycles of 96◦C for 15 s, 60◦C for 1.5 min and 72◦C for 2 min. A final extension step at 72◦C for 5 min allows all amplicons to be fully extended. PCR amplified fragments will be separated by electrophoresis in a 2% agarose gel and visualized using SYBR Safe DNA stain (Invitrogen Life Technologies). Product size will be determined by comparison with a positive control and a DNA size ladder. Data will be tabulated as 1 or 0, representing presence or absence.
- *Pathogen quantitation:* will be conducted for IHNV only, and as such will use cDNA, and will be applied to laboratory studies on larval steelhead towards transcription modeling, and subsequent gene profile validation along with respective histopathology (described above). Quantitative PCR measurements will be performed using TaqMan Universal PCR Mastermix (Applied Biosystems) in a reaction containing 10mMTris–HCl (pH 8.3), 50mM KCl, 5mM MgCl2, 2.5mM deoxynucleotide triphosphates, 0.625U AmpliTaq Gold DNA polymerase per reaction, 0.25U AmpErase UNG per reaction and 5µL of DNA sample in a final volume of 12µL. Samples will be placed in 384-well plates and amplified in an automated fluorometer (ABI PRISM 7900 Sequence Detection System, Applied Biosystems). Quantitative PCR conditions are set to annealing for 2 min at 50°C, cDNA denaturing for10 min at 95°C, and product amplification will be carried out over 40 cycles of 15 s at 95°C and 60 s at 60°C. Fluorescence of samples will be measured every 7 s and signals considered positive if fluorescence intensity exceeds 10 times the standard deviation of the baseline fluorescence (threshold cycle, CT). We will use SDS 2.2.1 software (Applied Biosystems) to quantify gene amplification in real-time. Quantification will be determined by comparison to integrated pathogen specific standard curves; from empirically established concentrations.

e) *Single Nucleotide Polymorphism (SNP):* SNP detection will be done on the Fluidigm® EP1TM System that uses a Dynamic ArrayTM IFC to genotype a larger number of SNPs quickly and efficiently at a low cost. The procedure is a multi-step process that analyzes 96 samples with 96 SNP assays and ultimately yields over 9000 data points in one run. The cocktail used in Pre-Amplification consists of Qiagen QuantiTech® Multiplex NoRox and pooled assays at 0.05µM each, and the step concludes with a short 14-cycle PCR run. Separate loading reagents are used to prepare the sample and assay mixes to be loaded onto the IFC chip. Rox Reference Dye is added to the assay mix while Taqman® 2X Universal PCR Master Mix and AmpliTaq Gold® are added to the sample mix. The IFC Controller primes the chip with control line fluid and then loads each mix after the assay and sample cocktails are pipette into the appropriate wells on the chip. After the mixes are loaded onto the chip, it is transferred to a thermocycler with a vacuum attached. Pressure of the vacuum has to reach -80kPa before the thermocycler can be properly run. Results from the thermal cycling allows for end-point fluorescence detection and the analytical software provides color-coded allele maps. These allele maps will be used to perform parentage based assignment of samples using SNPPit (available at http://swfsc.noaa.gov/textblock.aspx?Division=FED&ParentMenuId=54&id=16021)

Genomic profiling, Pathogen ID, and SNP Analyses:

a) *Genomic profiling:* Cluster analysis and gene expression profiling will be performed on gene expression data. Quantitative PCR data for each individual fish from all tests will be combined into one annotated dataset and subjected to agglomerative hierarchical clustering using Genesis software version 1.7.5 (Sturn et al., 2002). A heatmap profile of gene expression will be generated using cosine correlation, with complete linkage clustering, based on data trends and mean distances, to assess response differences between species to respective chemical classes responsible for toxicity. Principal component analysis (PCA) will be conducted in conjunction to correlation heatmaps, to ascertain factors responsible for species response variability.

b) *Pathogen ID:* PCR data ran on agarose gels will result in pathogen presence/absence which will be scored as “1” or

"0", respectively. These values will then be designated to individual samples along with the corresponding pathogen ID, and will then be linked to the genomic assessments to attribute infection/disease with specific profiles.

c) *SNPS:* The Fluidigm® EP1TM Genetic Analysis hardware and the EP1TM Data Collection software collect data from the chip after the thermocycler run, and the Fluidigm SNP Genotyping Analysis software analyzes and presents the data in scatter plots.

4. Deliverables

- A detailed Study Plan will be provided within the first 30 days of starting the project.
- A biomarker suite will be developed as a tool to monitor the impacts of pathogens on steelhead trout.
- An IHNV specific model for assessing levels of diseases in steelhead trout.
- Steelhead broodstock and family-specific susceptibility data for particular hatcheries.
- A pathogen infection monitoring system, transferable to other fish species of concern.
- Progress reports will be produced as stipulated by contract with the Department of Fish and Game.
- Staff will participate in Contaminants Workshops of the Interagency Ecological Program
- Research will be presented at State and National Congresses of the Society for Environmental Toxicology and Chemistry, The American Fisheries Society, and Delta Stewardship Program meetings (obtaining necessary authorization where applicable).
- Three or more peer-reviewed journal publication(s) are anticipated to result from this research.
- Final Report

5. Feasibility

Feasibility of this project is extremely high as our pilot study (Connon et al., submitted) linked with recently submitted research (Hostetter et al., in review) was recently supported by published research on sockeye salmon (Miller et al., 2011) who were also successful in demonstrating that molecular profiles can be used to ascertain not only the level of disease, but indicate the infection types. This linked with the proposed identification and presence/absence of pathogens, will enable effects on life-cycle to be determined. Drs. Connon, Israel, May, Loge and Baxa, along with other staff in the Aquatic Toxicology lab at UC Davis, are highly qualified, and suitably experienced to carry out all aspects of this investigation (see qualifications, below). The proposed work will benefit considerably from results of previous and ongoing studies on a number of Californian fish species on which genomic studies are being conducted by Dr. Connon and staff and students in his lab, along involvement alongside Dr. Loge, in research conducted on steelhead in the Columbia River, OR, genetic variance studies performed by researchers at Dr. May's lab and pathological assessments carried out by Dr. Baxa who works in Dr. Swee Teh's lab at UC Davis. The integration and interaction with ongoing steelhead studies involving Dr. Israel, at the U.S. Bureau of Reclamation, will permit direct links to be construed between genomic biomarker responses and life-cycle perspectives in steelhead.

Facilities and equipment: Work will be conducted at Drs. Connon and May's research laboratories, using other facilities throughout UC Davis; including the Center for Aquatic Biology and Aquaculture (CABA) and the Aquatic Toxicology Laboratory (ATL).

Dr. Connon's Molecular and Biochemical lab, at the UC Davis, School of Veterinary Medicine (http://www.vetmed.ucdavis.edu/apc/wernerlab/subpage/connon.html), is fully equipped to conduct molecular and biochemical biomarker analyses. Routine RNA extractions, cDNA synthesis and quantitative polymerase chain reaction (qPCR), poly acrylamide gel electrophoresis (PAGE), and a number spectrophotometric protein assays, are performed by staff and students. Available equipment includes, but is not limited to an Eppendorf thermocycler, vacuum centrifuge DNA concentrator, refrigerated centrifuge, nano-drop for nucleotide and protein concentration, molecular and biochemical electrophoresis systems, gel densitometer, UV imaging system, various cell incubators, 96-well spectrophotometer, 384 and 86-well fluorometer, a sterilizing biosafety and an externally vented flow hood.

Dr. May's Genomic Variation lab, at UC Davis (http://genome-lab.ucdavis.edu/People/May), is equipped with two state of the art capillary sequencers (one 16 and one 96) for sequencing and genotyping, 19 thermocyclers including four for qPCR, microcentrifuges, refrigerated centrifuges, agarose gel electrophoresis, imaging systems, Fluidigm 96x96 SNP detection, and other standard molecular genetic laboratory equipment.

The Center for Aquatic Biology and Aquaculture is located within the UC Davis, College of Agriculture and

Environmental Sciences http://caba.ucdavis.edu), and the was established to provide leadership, focus, and support to University of California Davis researchers in addressing problems associated with California's cultured and wild aquatic biological resources. CABA and its aquatic research facilities provide the basic infrastructure to allow departments within the College of Agriculture and Environmental Sciences, as well as campus-wide, to conduct multidisciplinary and interdepartmental research and associated programs. These activities provide the scientific base to sustain California's natural populations of aquatic species, support the technological framework of the state's marine and freshwater aquaculture industries, and create sustainable aquaculture production. The heart of CABA's aquatic research and student training program is a five-acre facility housing laboratories and aquatic animal containment resources. There is research and student training space for a wide range of programs, including fish ecology, reproduction, nutrition, genetics, endocrinology, disease and pathology, aquaculture engineering, aquatic toxicology, and general aquatic biology. About 15 species are under study at any one time. CABA also has two buildings with approximately 21,000 square feet of animal-holding space are located along the south fork of Putah Creek.

The Aquatic Toxicology Laboratory is located within the UC Davis, School of Veterinary Medicine (www.ucdavis.edu/apc/atl), is a State-certified lab with over 20 years of experience in investigating surface water quality and aquatic ecosystem health in watersheds throughout California. ATL activities focus on conducting aquatic organism toxicity tests, as defined by the United States Environmental Protection Agency (US EPA), using standard as well as resident aquatic species. ATL follows protocols and quality assurance criteria established by the US EPA and the State of California's Surface Water Ambient Monitoring Program (SWAMP).

The ATL is a 3200 ft^2 facility consisting of four individual laboratories and is fully equipped to conduct toxicant exposure and surface water monitoring studies. Available equipment includes multiple temperature and light controlled environmental chambers (32 ft^3 each) for incubation and experiments, orbital shaker tables, vacuum pumps for sample filtration, a Coulter Counter for the enumeration of cells, a fluorometer for chlorophyll A analysis and two compound microscopes. The 1500 ft^2 wet lab is equipped with multiple temperature controlled 32 ft^3 environmental chamber for culture, maintenance and experimentation of small fish and invertebrate experiments. A 375 ft^2 section is reserved for conducting the EPA *P. promelas* and *O. mykiss* tests and contains two temperature controlled 29 ft^2 water baths and two 13.5 ft^3 environmental chambers. There are also 320 ft^3 of refrigerated storage with an additional 8 ft^3 of explosion proof refrigerated storage. Additional equipment housed in the wet lab includes pH meters, electrical conductivity meters, dissolved YSI oxygen meters, hardness and alkalinity titration stations and an autoclave. A 280 ft^2 negative pressure room is used primarily for exposure experiments with hazardous chemicals, and equipped with a 6 ft^2 fume hood and a vented, temperature controlled exposure chamber containing 14 individual tanks. A 180 ft^2 photo period and temperature controlled room is used for the EPA *C. dubia* bioassays. Bench space and light boards provide space for daily water renewals and shelves provide incubation areas for the organisms.

Computers: Computer systems with statistical, graphics and text processing software packages (SigmaPlot, SigmaStat, Systat, Statview, ARC GIS, Excel, Word, Powerpoint, Adobe Photoshop) are available for data analysis, data storage, publications and presentations. Computer systems with specialized bioinformatics software (Primer express, R statistics with LIMMA GUI for microarray analyses, Genesis, GenePixPro, SDS, Chromas, BioEdit,) along with image software analyses (EthoVision XT version VI) are available for data acquisition and analysis.

Office: Approx. 1200 ft^2 of office space is available for PIs, UCD-ATL staff, and graduate students. All offices are equipped with computers, printers, copiers and other necessary office equipment, and all computers have internet access. Office, publishing and accounting support are provided by the department.

Other: Dr. Connon has access on a fee per service basis to core facilities at the UCD Genome Center (http://genomics.ucdavis.edu/corefacilities.html) and other UCD based state-of-the-art service facilities. The purpose of the Genome Center is to serve the campus scientific community by providing state-of-the-art genomic resources. The Lucy Whittier Molecular & Diagnostic Core Facility [http://www.vetmed.ucdavis.edu/vme/taqmanservice/] at UCD offers services for qPCR analyses and support, which are regularly used by this team.

6. Relevance to the CALFED ERP

Relevance to this PSP: The integrated research study detailed in this proposal is particularly relevant to the CALFED ERP towards better understanding the Delta system and linkages between restoration management, species, and water quality. This proposal focuses on previously identified uncertainties regarding BDCP Conservation Measures and the OCAP Biological Opinion by holistically evaluating adverse outcome pathways through integrating a number of levels of biological organization; histopathological, visual physiological assessments, probabilistic survival, and genomic responses can be used in life-cycle assessments and population studies. Our proposal addresses and incorporates questions regarding potential factors affecting productivity (e.g. contaminants) and assessing the response of fauna to ecological characteristics of the Bay-Delta. Additionally, our proposal is interdisciplinary, collaborative, and integrative, which will results in synergies amongst academic and agency scientists, and focus

results towards application for restoration and performance monitoring.

Relevance to CALFED Issues Outside this PSP: One of the main goals of this study is to develop a useful suite of biomarker tools for steelhead trout, with a particular focus on pathogenic infections, and interactions with contaminants, that can be interpreted in the context of ecologically relevant adverse outcomes. The overall relevance overall Bay-Delta issues, is that these tools will be transferable to numerous other important native species. An important aspect of this study is the validation of the tools from a systems biology perspective, including measurements at different levels of biological organization. Furthermore, this research is highly positively executable and will also provide valuable monitoring tools to assess the temporal and spatial distribution of pathogens throughout the Sacramento-San Joaquin Bay-Delta system.

7. Expected quantitative results (project summary):

The proposed research is both of a quantitative and qualitative nature, and is targeted at measuring health parameters in steelhead trout that originate primarily from two hatchery facilities operated by California Department of Fish and Game; the Feather River and American River (Nimbus) hatcheries. We will assess pathogenic infections in juvenile steelhead, both under hatchery conditions and in the field, using polymerase chain reaction (PCR) techniques whereby detection and identity will be established, and quantified as a function of infection concentration determined by the DNA copies per sample. We also will perform carefully designed laboratory tests to investigate responses to infection from a genomic perspective, and identify levels of response that are indicative of disease, quantified as gene copy number per infection level, per sample. The latter will be validated through qualitative histopathology assessments. Furthermore, within this laboratory study, we will determine the effects of a pyrethroid pesticide; bifenthrin, in combination with infection and determine thresholds of disease through quantitative PCR (qPCR) techniques; specifically quantifying IHNV infection in steelhead infected under laboratory conditions, relating this to gene transcription levels, and model estimates of survival probability dependent on this data. We will qualitatively (using SNPs) identify different families and broodstocks of juvenile steelhead obtained from each hatchery, and use qPCR to ascertain any difference in susceptibility. This proposal will permit the development and validation of simple monitoring tools, which will be immediately available to biologists, to evaluate the health of steelhead and potential restored steelhead habitats in the Bay-Delta. Thus this project will provide quantitative and qualitative data and information that will aid management and monitoring efforts on the status and life history assessments of this at-risk species.

The quantitative units for the specific parameters measures will be:

Transcriptomics:

- Gene expression, as copy number per sample
- Profiles of gene copy numbers from a multitude of genes, based on correlations.

Pathogen ID:

- Presence or absence, as 1 or 0
- Level of infection as, gene copy number, representing biomass.

8. Other products and results:

- Genomic profiling tools will be developed to specifically assess health status, and level of disease in steelhead trout, however, these tools will be directly applicable to other salmonids species.

- Fish pathogen screening tools that are transferable and applicable to all fish species, water and sediment samples (beyond the proposed scope this study), will be developed and validated, towards monitoring efforts. This genetic approach will be suitable as an early warning tool of emerging pathogens, and for profiling temporal variation of pathogens.

9. Qualifications (See also Biosketches)

The researchers included in this proposal are highly qualified and suitably experienced to carry out all aspects of this investigation, and the proposed methodologies are of common use at the respective UC Davis laboratories. Our individual research expertise has been applied to contaminant, physiological, genetic, disease and ecological issues with respect to the Pelagic Organism Decline (POD) and other projects of concern. Dr. Connon's research on the Bay-Delta System, since his involvement in the POD program began 2007, has so far resulted in six publications concerning the effect of

contaminants and disease on various fish species (published and submitted) and a further three manuscripts in preparation. Dr. Israel participated in the DRERIP evaluations of BDCP conservation measures and has recently received funding from the POD program to work on conservation genetics of longfin smelt, which has resulted in a recent publication. Dr. Israel's other work on species in the Bay-Delta system has resulted in multiple publications related to aquatic species propagation, water diversions, and population demography and spatial structure in green sturgeon. Dr. May is well known for his studies on genetic variation in fish population in a wide variety of organisms, but of important to this proposal is his expertise on salmonids, and assistance with genotyping steelhead families and broodstocks. Dr. Loge is co-author, with Dr. Connon on the manuscript recently submitted to Environmental Science and Technology, entitled Molecular biomarkers in environmental diagnostics: health assessments in Columbia River steelhead (Oncorhynchus mykiss), on which this proposal is based and his expertise in modeling will form an essential part the study and Dr. Baxa is a specialist in infectious diseases and parasitology.

Dr. Richard Connon is a project scientist working at the aquatic toxicology laboratory at UC Davis, currently researching sublethal molecular and behavioral endpoints in fish at the University of California Davis. He will act as Project Director and will be responsible for performing and overseeing experiments and analyses at UC Davis, and is expected to become a Research Scientist as from July 1, 2011. Trained as an Environmental Scientist and Ecotoxicologist, he is experienced in conducting toxicological tests on microorganisms, and both invertebrates and vertebrates. His past research involves ecological, toxicological, biochemical, molecular and a large number of environmentally relevant parameters, including reproduction and population growth rate investigations in *D. magna* carried out at the University of Reading, UK. Published studies on exposure of *D. magna* to a number of stressors (agrochemicals, pharmaceuticals and urban contaminants) have demonstrated the ability to link transcriptional responses with those observed at higher levels of organization; reproductive value, development and population growth rates. Dr Connon has developed biochemical biomarkers in *D. magna* that were successfully applied field studies on jet fuel spillages at Heathrow Airport, London, UK and linked detrimental effects of exposure between the biomarker activity and reproduction. Studies investigating molecular responses linked to population growth rates on a heavy metal (cadmium) and a pharmaceutical (ibuprofen) were published in 2008. Further work carried out in characterizing the ecological niche of *D. magna* using population growth rate was published in 2007. Recent work by Dr. Connon, developing microarrays for a Californian endangered endemic fish species; delta smelt, successfully linked gene expression with swimming behavior anomalies and was published in 2009 and 2010. He has also identified key genes relating to ammonia toxicity (submitted) and urban wastewater contaminants, and has successfully identified disease-specific genomic profiles in outmigrant juvenile steelhead in the Columbia River (submitted). Dr Connon is highly experienced at molecular, microbiological and ecological aspects of the proposed study. Current and upcoming contracts awarded to Dr. Connon, as PI, include: the Refinement and Application of Novel Molecular and Biochemical Biomarkers to Determine Sublethal Contaminant "Exposure and Effects in Archived Delta Smelt Samples" (IEP contract number: R10AC20097, 09/2010-12/2011, and "Physiological Mechanisms of Environmental Tolerance in Delta Smelt (Hypomesus Transpacificus): From Molecules To Adverse Outcomes" (Bay Delta Stewardship contract in review).

Dr. Joshua Israel is a Fish Biologist working in the Applied Science Branch of the Mid-Pacific Region of the Bureau of Reclamation and is expected to become a Visiting Researcher at UC Davis in spring 2011. He will be a co-PI on this project, and will be responsible for performing and coordinating field sampling for this study, as well as co-supervise a graduate student. Trained in conservation ecology, he is experienced in undertaking risk assessment, conducting genetic analyses at multiple scales, and employing telemetric techniques to study animal behavior and survival. At UC Davis, his research involved ecological, telemetric, and genetic data on green strurgeon to evaluate the population biology of green sturgeon (*Acipenser medirostris*). Published articles by Dr. Israel on sturgeon and salmonids have shown relationships between numerous risks (i.e. fishing, diversions, hatcheries, habitat loss) to populations. In 2011, Dr. Israel has been responsible for a multi-agency investigation of steelhead survival in the San Joaquin River and south Delta, proposing genetic and telemetric hatchery evaluation studies of American River steelhead as part of the Nimbus Hatchery and Genetic Management Plan, and developing a life-cycle model for the comprehensive steelhead monitoring program. These studies are applied in nature, and are required parts of the Operations Criteria and Plan NMFS biological opinion. Dr. Israel is highly experienced at ecological and field aspects of the proposed project

Dr Bernie May has been the Director of the Genomic Variation Laboratory at UC Davis for the past 15 years and has significant experience in project management. He currently has eight doctoral students, three technicians, two post-docs, and two project scientists working in his laboratory who use a variety of molecular techniques to study genomic variation in natural and aquacultural populations. He has published over 175 scientific papers on questions related to genomic structure, linkage of markers to QTLs, population analysis, mixed stock analysis, genomic manipulation, effects of non-indigenous species/populations, effects of toxicants on gene pools, and isolate identification in a wide range of fish, fungi, birds, mammals, plants, and invertebrates. Current target organisms include: salmonids (golden trout, redband trout, Chinook salmon, rainbow trout, cutthroat trout), jellyfish, lion paw scallops, tui chub, fairy shrimp, delta smelt, Sacramento perch, Shasta crayfish, and sturgeon (lake, green, and white). The following were all CALFED contracts on which Dr. May was a co-PI or PI:

Contract #	Title	PI	Outcome
unavailable	Biological assessment of green sturgeon in the Sacramento-San Joaquin watershed	J. Cech	*completed as contracted*
P014004	San Joaquin River basin Fall-run chinook salmon genetic baseline and discrimination	B. May	*completed as contracted*
1132321G005	Biological assessment of green sturgeon in the Sacramento-San Joaquin watershed	A. Klimley	*completed as contracted*
02P34	Restoration of Sacramento perch to San Francisco Estuary	P. Moyle	*completed as contracted*
113322J006	Sex-reversal in Central Valley Chinook salmon: occurrence and population genetic consequences	B. May	*completed as contracted*
4600002763	Population genetics of splittail	B. May	*completed as contracted*
02DP57	Biological assessment of green sturgeon in the Sacramento-San Joaquin watershed	A. Klimley	*completed as contracted*
05WRGR0012	Are apparent sex-reversed Chinook salmon a symptom of genotoxicity?	B. May	*completed as contracted*
1036	Predicting the effects of invasive hydrozoa (jellyfish) on pelagic organisms under changing saline and temperature regimes	B. May	*partially completed*
E078004	Population biology, life history, distribution, and environmental optima of green sturgeon	A. Klimley	*partially completed*

Dr. Frank Loge's research focuses on three principal areas: water reuse, fate and transport of contaminants in the environment, and optimization of existing treatment technologies and design of new treatment systems. The underlying theme of his research is to better understand the relationship between the structure and function of engineered/natural systems and human/environmental health. Current research topics include an assessment of the impact of environmental stressors on the dynamics of disease transmission in outmigrant juvenile salmon in the Columbia River basin (NOAA), and correlations between spatial ecology and contaminants with the health of outmigrant salmon populations in the Columbia Estuary (Bonneville Power Administration), a novel environmentally benign manufacturing process utilizing renewable biopolymers to manufacture natural fiber reinforced thermoplastic composites (National Science Foundation), distributed physical and molecular separations for selective harvest of higher value wheat straw components: pilot-scale testing (US DOE), and a multi-community intervention with UV light disinfection for estimating the risk of gastrointestinal illness from drinking groundwater (US EPA).

Dr. Dolores Baxa is a project scientist at UC Davis and is the lead researcher at the Aquatic Toxicology Program on studies involving the key interplay between infectious diseases and toxicants and how they impact fish health. Dr. Baxa has a broad range of background and training in infectious diseases of fish for the last 20 years with over 30 peer-reviewed publications. She has maintained rigorous research projects in bacteriology, parasitology, and molecular biology that assess the transmission, interaction, and detection of disease agents in various fish and other secondary hosts in fresh and marine water environments. Her recent project involved the development of molecular-based techniques to evaluate the dynamics of toxic *Microcystis* in the San Francisco Estuary.

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Section 7: Project Budget

1. **Detailed Project Budget**

BUDGET	Total 3 years	Year 1			Year 2			Year 3		
Staff Level		Hourly Rate	Total Hours	Total Year 1	Hourly Rate	Total Hours	Total Year 2	Hourly Rate	Total Hours	Total Year 3
Asst. Researcher; R. Connon (PI)	$74,551	$33.17	720	$23,882	$34.50	720	$24,838	$35.88	720	$25,831
Adjunct Prof.; B. May (Co-PI)	$36,453	$67.50	173	$11,678	$70.20	173	$12,145	$73.01	173	$12,630
Assoc. Prof.; F. Loge (Co-PI)	$25,549	$47.31	173	$8,185	$49.20	173	$8,512	$51.17	173	$8,852
Asst. Project Scientist II; D. Baxa (Co-PI)	$33,083	$30.63	346	$10,598	$31.86	346	$11,022	$33.13	346	$11,463
SRA IV	$18,037	$33.40	173	$5,778	$34.74	173	$6,009	$36.13	173	$6,250
SRA II	$21,440	$19.85	346	$6,868	$20.64	346	$7,143	$21.47	346	$7,429
Junior Specialist	$51,503	$16.19	1019	$16,501	$16.84	1019	$17,158	$17.51	1019	$17,844
Student Assistants III	$13,798	$8.50	520	$4,420	$8.84	520	$4,597	$9.19	520	$4,781
Personnel Subtotal	$274,414			$87,910			$91,424			$95,080
Fringe benefits										
Asst. Researcher; R. Connon (PI)	$21,879		27.0%	$6,448		30.2%	$7,501		30.7%	$7,930
Adjunct Prof.; B. May (Co-PI)	$10,698		27.0%	$3,153		30.2%	$3,668		30.7%	$3,877
Assoc. Prof.; F. Loge (Co-PI)	$7,498		27.0%	$2,210		30.2%	$2,571		30.7%	$2,718
Asst. Project Scientist II; D. Baxa (Co-PI)	$9,709		27.0%	$2,861		30.2%	$3,329		30.7%	$3,519
SRA IV	$7,810		40.2%	$2,323		44.2%	$2,656		45.3%	$2,831
SRA II	$9,283		40.2%	$2,761		44.2%	$3,157		45.3%	$3,365
Junior Specialist	$0		0.0%	$0		0.0%	$0		0.0%	$0
Student Assistants III	$179		1.3%	$57		1.3%	$60		1.3%	$62
Benefits Subtotal	$67,057			$19,813			$22,942			$24,302
Personnel Total (Salary and benefits)	$341,471			$107,723			$114,366			$119,382
Operating Costs										
Lab studies										
Genomic biomarkers (whole organism)	$12,500			$12,500						
DNA - pathogen quantitation (single)	$500			$500						
Genetics - SNPs system	$14,000			$14,000						
EH&S waste disposal	$4,000			$4,000						
Chemical analyses	$1,000						$1,000			
Histopathology	$5,000						$5,000			
Field studies										
Genomic biomarkers (gill and kidney)	$37,500			$12,500			$12,500			$12,500
DNA - pathogen identification (multiple)	$4,500			$1,500			$1,500			$1,500
Genetics - SNPs system	$21,000			$7,000			$7,000			$7,000
Hatchery studies										
Genomic biomarkers (gill and kidney)	$25,000						$25,000			
DNA - pathogen identification (multiple)	$3,000						$3,000			
Genetics - SNPs system	$14,000						$14,000			
Project Administration										
Office supplies/publications	$3,500			$500			$1,500			$1,500
Travel and per diem	$7,500			$2,500			$2,500			$2,500
Equipment lease and maintenance	$25,000			$9,000			$9,000			$7,000
Other Costs Subtotal	$178,000			$64,000			$82,000			$32,000
Total overhead @ 25%	$129,869			$42,931			$49,092			$37,846
Total Costs for Project	$649,340			$214,654			$245,458			$189,228

2. Budget Justification

Total budget costs: $649,340 for three years.

a) **Personnel Services Cost: $341,471** plus overheads applied to all personnel at 25% ($85,368) as required by the University of California at Davis (Total: $426,839**)**.

Personnel: Included in the funds requested are salary and benefits Dr. Connon (PI), [720h/year, total $96,430] representing 35% effort, towards overall project management activities including, supervision of the project, performance and training of molecular work, data handling, report preparation and project oversight and outreach. Funds are requested for Dr. May (Co-PI), Director of the Genomic Variation Lab at UC Davis [173h/year, total $47,151] and Dr. Loge (Co-PI), Dept. of Civil and Environmental Engineering at UC Davis [173h/year, total $33,047] representing 8% effort, towards project oversight, involvement in experimental design and specifics pertaining to Steelhead family and stock assessments, and modeling of genomic response integration with higher level of biological organization. We also request salary and benefits for Dr. Baxa (Co-PI), Project Scientist, Aquatic Toxicology Program at UC Davis [346h/year, total $42,792] for 16% effort, towards assistance with pathogen identification techniques and support with histopathology assessments, two Staff Research Assistants at the Aquatic Toxicology Lab UC Davis [SRA IV: 173h/year, total $25,847; and SRA II: 346h/year, total $30,723] for 8% and 16% effort respectively, and Student Assistants [SA III: 520h/year, totaling $13,977] to assist with sampling, collections, exposures, dissections, sample preparation and other aspects of the laboratory and field work as required. Also included in personnel requests is funding for a Junior Specialist [1019h/year, total $51,503; with no benefits], at 49% effort, towards cost of living of a graduate student from the University of Munich, (major professor: Dr. Juergen Geist; Technische Universität München), Germany, who will conduct research under Dr. Connon's direct supervision, towards a PhD thesis, and as such will be involved in all aspects of the study. As the student has concluded all required course work at his home university, he does not need to be registered as a UC student and fees are therefore not requested. We are matching funds in the form of the salary and benefits of Dr. Israel, (Co-PI), United States Bureau of Reclamation [346h/year, total $56,890] representing 16% effort, who will assist with project oversight, provide expertise and support on hatchery and field sample collections, and specifics pertaining to Steelhead family and stock assessments, and co-supervise the graduate student. Although not presented as matching funds, this proposal forms part of a wider research program, funded by the United States, Bureau of Reclamation, in which steelhead hatchery broodstocks will be genetically tagged by the NOAA-Fisheries Southwest Fisheries Science Center, thus allowing field caught individuals to be traced back to family and broodstocks origins. Time spent on each task has been calculated on an hourly basis however these are represented as percentage total time in respective task budget justification. Benefit rates are calculated based on current benefits cost as percentage of salaries, as requested by the University of California. Dr. Connon is expected to be appointed Assistant Researcher as of July, 2011, his salary and benefits have been calculated accordingly.
Fringe Benefits: Fringe benefits rates and annual increases are defined by the University's composite fringe benefit rates for fiscal years 2011-2014.

b) Operating Expenses Cost: $178,000 plus overheads applied at 25% ($38,250) less $25,000 equipment, as required by the University of California at Davis (Total: $216,250).

Laboratory studies: (Year 1-2)

Cost: **$37,000** plus overheads at 25% ($9,250) applied to supplies as required by the University of California at Davis (Total: $46,250**)**.

Supplies: We request funds towards performing contaminant/pathogen interaction exposures in larval steelhead, and analyses of 200 samples, as whole organisms, consisting of: a) genomic profiling of 24 biomarkers ($12,500), b) DNA identification of single test pathogen ($500), c) family and broodstock identification by SNPs ($14,000) and d) histopathology assessments on sub-samples ($5,000) which will be conducted in year 2, samples will be selected dependent on results from year 1. Additional to these costs are funds towards Environmental Health & Safety waste disposal costs ($4,000). Materials required for sample processing have been factored into the assessment costs. Funds towards Bifenthrin concentration analyses are also requested ($1,000).

Field Studies: (Years 1-3)

Cost: **$63,000** plus overheads at 25% ($15,750) applied to supplies as required by the University of California at Davis (Total: $78,750).

Supplies: We request funds towards the analyses of up to 300 samples field-caught steelhead, of gill and kidney tissue where applicable, consisting of: a) genomic profiling of 24 biomarkers ($37,500), b) DNA identification of multiple pathogens ($4,500), c) family and broodstock identification by SNPs ($21,000).

Hatchery studies: (Year 2)

***Cost:* $42,000** plus overheads at 25% ($10,500) applied to supplies as required by the University of California at Davis (Total: $52,500**)**.

Supplies: We request funds towards the analyses of up to 200 samples, of gill and kidney tissue where applicable, consisting of: a) genomic profiling of 24 biomarkers ($25,000), b) DNA identification of multiple pathogens ($3,000), c) family and broodstock identification by SNPs ($14,000).

Project Administration: (Year 1-3)

***Cost:* $11,000** plus overheads at 255 ($2,750) applied to supplies and travel, as required by the University of California at Davis (Total: $13,750).

Supplies: We request office supplies and funds towards publication costs ($3,500).

Travel: Funds are requested to support domestic travel ($7,500) over the three year period, including costs of field sample collection ($1,000), in addition to team member's attendance at scientific conferences ($6,500) to present research findings. Targeted annual conferences include Delta Science Program related conferences and workshops, and the annual meeting of the Society for Environmental Toxicology and Chemistry, California State and National Congress, and attendance at the American Fisheries Society meetings. Respective authorization will be sought for any travel outside the State of California, if deemed appropriate.

Equipment lease and maintenance: (Years 1-3)

***Cost:* $25,000** plus overheads at 25% ($6,250) as required by the University of California at Davis (Total: $31,250). These funds are requested towards the lease and maintenance of Fluidigm high-throughput quantitative PCR system (BioMark™). The use of this equipment for genomic analyses more than halves the costs of reagents, as such it's reduces the overall cost of performing the proposed molecular work.

c) **Administrative Overhead Cost: $129,869.** The current indirect cost rate for VM:APC is 25% with all California State Agencies (Waiver # 03R-135).

· Class Waiver	No.: **03R-135**	Date Approved: **5/9/2003**	Sponsor Code:
Campus: **OP**	Reason: **C** [A=vital interest; C=sponsor policy]		
Sponsor Name: *CALIFORNIA STATE AGENCIES*			
Project Title: **CALIFORNIA STATE AGENCY AGREEMENTS****			
Waiver Rate: **25.00% MTDC***			
Notes: *UNLESS OTHERWISE SET FORTH IN STATUTE, REGULATION, OR PUBLISH POLICY THAT APPLIES TO ALL RECIPIENTS. C&G MEMO 03-02. SEE OTHER STATE CLASS WAIVERS FOR SPECIFIC PROGRAMS.			