Attachment 11. Project Narrative

Project Title: Impacts of climate change on pesticide bioavailability and sublethal effects on juvenile Chinook salmon in the Delta: Potential benefits of floodplain rearing

Applicant name: The Regents of the University of California, Riverside campus (Project Manager: Dr. Daniel Schlenk) Board of Trustees of Southern Illinois University (Co-Principal Investigators): Drs. Michael Lydy and Greg Whitledge)

Project acreage restored, enhanced, or protected: N/A

Executive Summary/Abstract

We propose a comprehensive three-year study to improve our understanding of fishery restoration options for salmonids in California. We will specifically address the impacts of climate change on pesticide bioavailability, exposure patterns and pathways, and potential sublethal effects of pesticide exposure for juvenile Chinook salmon rearing in the San Francisco Bay Delta. Although recent studies have demonstrated that several pesticides are entering Yolo Bypass floodplain and the Sacramento River, whether these pesticides are bioavailable to salmon and their prey, the principal routes by which salmon may be exposed to and accumulate pesticides, and the extent to which pesticide exposure may affect behavior and physiology (and, ultimately, growth and survival) of juvenile Chinook salmon rearing in the Delta are unknown. We hypothesize that the types of pesticides present, pesticide loadings, bioavailability, and effects on salmon may differ spatially within the Delta. We further hypothesize that pesticide bioavailability and sublethal effects on salmon will be strongly influenced by trophic pathway and water temperature and may be exacerbated by increasing temperatures resulting from climate change. Thus, pesticide bioavailability and effects may differ for salmon rearing in Yolo Bypass versus the Sacramento River due to differences in pesticide types and loadings, water temperatures, and trophic pathways in floodplain and river habitats. The proposed project will include field studies to estimate loadings and bioavailability of pesticides, concentrations of pesticide residues in salmonid prey, and the trophic basis of juvenile Chinook salmon growth (benthic vs. pelagic food web pathways) and how each of these differ between floodplain and river channel habitats in the Delta. Data from field studies will inform development of laboratory studies that will assess the potential effects of exposure to environmentally-relevant pesticide types and concentrations in prey on swimming performance, olfaction and neuroendocrinology of juvenile Chinook salmon. Laboratory studies will also evaluate how water temperature (including increased water temperatures predicted from climate change) influences these sublethal effects of pesticides on juvenile salmon. Swimming performance, olfaction and neuroendocrinology will be assessed in juvenile Chinook salmon exposed to diets representative of each habitat will provide new insight regarding the extent to which pesticide exposure may be increasing predation risk or adversely affecting growth or migration through the Delta and whether such effects might be reduced by management actions aimed at enhancing floodplain access for juvenile salmonids. The proposed project addresses a priority

need identified under Topic 2 of the proposal solicitation and goal 3 of the California Water Action Plan.

Introduction and Purpose

Rearing in floodplain habitats in the north and northeast Delta region (including Yolo Bypass) has been demonstrated to promote more rapid growth rates of juvenile Chinook salmon compared to rearing in river channel habitats[1,2]. However, an unexplored potential benefit of juvenile salmonid rearing in at least some restored floodplain habitats may be a reduction in direct and indirect effects of exposure to bioavailable pesticides. Reduced exposure to bioavailable pesticides for floodplain-rearing juvenile Chinook salmon may limit fish stress responses and partially explain increased growth. In addition, reduced pesticide exposure and body burdens may potentially promote juvenile salmon survival by limiting effects of these contaminants on predator avoidance capability, thus decreasing predation risk[3,4].

Several recent papers have detailed the presence of current-use (e.g. pyrethroids, fipronil and its metabolites), legacy (e.g. DDT) and restricted-use (e.g. chlorpyrifos and diazinon) pesticides in water and sediments in the small streams leading into wetlands and the main stem rivers within the Delta[5,6,7,8] ;however, little to no data are available concerning pesticide loadings in rearing habitats for juvenile Chinook salmon. We will assess pesticide bioavailability in Delta floodplain and Sacramento River habitats during the period of time that juvenile salmon typically reside in these areas using solid phase microextraction (SPME) samplers deployed in the water column and sediment. Solid phase microextraction samplers measure the chemical activity of hydrophobic pesticides (e.g. pesticides with Log K_{ow} >4.0), provide estimates of the freely dissolved water concentrations[9] and these estimates are directly related to bioaccumulation potential and ultimately toxicity[10]. Passive samplers will be deployed in February and collected by April (depending on the duration of natural or artificial floodplain inundation) at multiple locations in Yolo Bypass (depending on which sections are flooded), Toe Drain, Cache Slough, and in the Sacramento River channel and pesticide concentrations will be quantified on the SPMEs at equilibrium following methods developed in Dr. Lydy's lab[10,11,12].

Although pesticides are known to be present in water and sediment in streams and rivers entering the Delta, the pathways of pesticide exposure, pesticide body residue levels, and sublethal effects of pesticides on juvenile Chinook salmon rearing in the Delta are unknown; particularly under enhanced temperature regimes predicted by climate change. Additionally, whether pesticides may be differentially affecting salmon that rear in different locations within the Delta (e.g., river channel or floodplain; floodplain habitats that differ in water sources or land use) is unknown. Evaluating exposure patterns and links between trophic pathways and pesticide residues for juvenile Chinook salmon in floodplain and river channel habitats as well as temperature regimes will be accomplished using a combination of field and laboratory experiments. Pesticide concentrations will be measured in sediments, phytoplankton, zooplankton, and macroinvertebrates and in the passive samplers collected from floodplain and river channel habitats. We expect that trophic pathways may influence pesticide accumulation in juvenile Chinook salmon and will conduct stable isotope and fatty acid analyses of juvenile salmon, primary producers, and invertebrates to determine whether anticipated differences in

diet and basal energy sources for salmon in floodplain and river habitats are associated with differences in pesticide residues in their prey and estimated pesticide residues in salmon tissues. The field study will enable assessment of whether uptake of pesticides from the water column (via planktonic prey or direct water uptake, although the latter is expected to be minimal for hydrophobic compounds) or from benthic sources (especially via benthic macroinvertebrates) is the principal route of pesticide uptake for juvenile salmon and will define the most appropriate prey items and pesticide types and concentrations to use for the laboratory studies.

Climate Change Considerations

Climate change is expected to increase the occurrence and duration of droughts in the Delta region, thus reducing the frequency of late winter and spring river flooding and floodplain inundation and likely increasing water temperatures in habitats used by juvenile Chinook salmon. The proposed study will improve scientific understanding of climate change effects on juvenile Chinook salmon rearing in the Delta by assessing the combined influences of temperature and pesticide exposure on juvenile Chinook salmon swimming performance and neuroendocrinology. Understanding effects of multiple stressors is key to development of strategies to mitigate the effects of climate change on species and ecosystems. With decreasing occurrence of floodplain/floodway inundation in the Delta likely given climate change projections, provision of critical floodplain habitat for species of concern (including juvenile rearing habitat for Chinook salmon) may need to increasingly involve managed floodplain/floodway inundation or restoration efforts to increase the frequency of river-floodplain connectivity. Thus, the proposed project will also inform management responses to climate change effects by evaluating how selection of floodplain/floodway areas for inundation and enhancement of river-floodplain connectivity will influence pesticide bioavailability and sublethal effects on juvenile Chinook salmon.

Sublethal Impacts of Environmental and Chemical Stressors

To assess the potential sub-lethal effects of pesticides on juvenile Chinook salmon, we will conduct laboratory studies to determine relationships between pesticide residue levels in prey as well as fish, and several sublethal responses in the fish. Laboratory studies will include bioaccumulation experiments on juvenile Chinook salmon with designated prey species conducted at three different temperatures (12, 16, and 18 °C) determined from climate models[32]. Fish will be fed dosed-prey species determined from the habitats where salmon currently reside, and an assessment of sublethal neuroendocrine responses, fish olfaction, growth/fitness and swimming performance will be performed. Previous studies in salmonids have shown alterations in neuronal signaling gene expression, olfactory functional and sensory behavior by environmentally-relevant concentrations of pesticides that occur in the waterways of the Delta[13,14,15]. This component of the study will allow us to disentangle effects of pesticide residues from other growth- and behavioral-influencing factors and determine whether projected pesticide levels would influence growth and predation risk of juvenile salmon rearing in the Delta. Conducting laboratory studies at three different temperatures will enable assessment of

the potential impact of temperature increases (e.g., due to climate change) on sub-lethal effects of pesticide exposure for juvenile salmonids rearing in the Delta.

The proposed project will provide relevant information to managers and stakeholders on pesticide loadings, bioavailability and the impacts of temperature on uptake pathways, and exposure patterns for juvenile Chinook salmon and how these vary among locations (river vs. floodplain, floodplain habitats that differ in water source and land use) within the Delta, along with an assessment of potential effects of pesticides on Chinook salmon that rear in different habitats within the Delta. An improved understanding of spatial and temporal patterns of pesticide bioavailability and effects on Delta-rearing salmonids will complement prior and ongoing studies on the positive and negative influences of floodplain and river channel habitats as fish rearing areas. The project will address a priority need identified under Topic 2 in the Proposal Solicitation: "**Improved understanding of the effects of toxicants, including their interactions with physical parameters, on food webs and fish condition, sensory perception, and bioenergetics.**" The project will also promote goal 3 of California Water Action Plan ("Achieving the Co-Equal Goals for the Delta") by providing new knowledge of pesticide bioavailability and effects on Chinook salmon that will inform restoration and enhancement of the Delta ecosystem. Priority actions in the Delta Plan that will be informed by results of the proposed project include: 1) Developing and Implementing Comprehensive Plans to Help Recover Populations of Threatened and Endangered Species in the Delta; 2) Accelerating and Implementing Habitat Restoration; and, 3) Implementing Near-Term Delta Ecosystem Improvement Projects. Recovering Chinook salmon populations that use the Delta for juvenile rearing and restoration of floodplain habitats (such as Yolo Bypass) that promote faster growth (and likely increased survival) of juvenile salmon[1,2] will be facilitated by new information on how pesticide loadings, bioavailability, and effects differ among floodplain and river habitats in the Delta and potential approaches for limiting or mitigating effects of pesticides on fish growth and survival by manipulating. The project will enable assessment of whether effects of pesticides on fish growth and behavioral and physiological attributes that influence predation risk might be reduced by management actions aimed at enhancing floodplain access for juvenile salmonids in the Delta.

Project History / Need for CDFW Funds

This is a new project, so none of the proposed project tasks have been conducted to date. However, preliminary data with several frequently detected pesticides has indicated sublethal toxicity in salmonids[13,14,15]. The project is not related to any previous or proposed CDFW projects and is not being considered for funding under any other funding opportunities. Thus, it is unlikely that the proposed project will be conducted if the project is not funded by CDFW Prop 1 funds unless alternative funding source(s) can be identified.

Goals and Objectives

The overall goal of the proposed project is to determine whether pesticide bioavailability, exposure pathways, and potential sublethal effects differ among juvenile Chinook salmon rearing in floodplain habitats and the Sacramento River within the Delta and whether climate

change could impact expected body pesticide body residues, and subsequent sublethal effects, in exposed salmon. Specific objectives include: 1) comparing the bioavailability of pesticides to juvenile salmon in floodplain and adjacent Sacramento River channels using passive sampling devices (SPMEs); 2) determining the principal pesticide exposure routes for juvenile salmon in floodplain habitats and the Sacramento River using stable isotope and fatty acid biomarkers to infer trophic pathways (benthic vs. planktonic) supporting juvenile salmon and linking food web pathways to types and concentrations of pesticides present in salmonid prey (benthic and planktonic invertebrates); and, 3) assessing relationships between temperature and pesticide residues with growth, neuroendocrinology, and swimming performance of juvenile Chinook salmon fed dosed-prey items in a laboratory study.

Expected outcomes and benefits

The proposed project will provide new information on pesticide loadings in the Delta based on the pesticide signature in the passive samplers and concentrations in the various prey species[16,17]. The project will also determine the trophic basis of support for salmon and potential contaminant pathways in floodplain and river habitats. Knowledge of spatial variability in pesticide loadings and bioavailability in the Delta will be useful for informing potential efforts to mitigate effects of pesticides on juvenile salmonids and other species of concern. In addition, the project will determine how pesticide residues in salmon rearing in floodplain and river habitats within the Delta affect fish neuroendocrinology, growth and swimming performance, which may influence predation risk and population. Lastly, the impact of predicted temperature increases will also be evaluated allowing stakeholders to predict effect thresholds of risk, which will be altered due to climate change. Ultimately, the proposed project will contribute to recovering Chinook salmon populations that use the Delta for juvenile rearing and floodplain habitats (such as Yolo Bypass).

Site Description

Field work for the project will occur in multiple locations within the Yolo Bypass (site number and locations will depend on the duration and extent of flooding), Toe Drain (Yolo Bypass outlet) in Cache Slough/Liberty Island, and in all water inputs to Yolo Bypass and in the Sacramento River (and connected sloughs) from just upstream of the mouth of the Feather River (upstream from Sacramento) and the downstream junction of the Sacramento River and the Sacramento Deep Water Ship Channel (near Rio Vista) (see attached map). The Yolo Bypass is covered by floodway easements held by the state of California, with all other land uses subservient to flood control. Agriculture (primarily rice) is a major land use in the Yolo Bypass; other areas within the floodway are managed as irrigated pasture, kept fallow, or are managed for waterfowl habitat and hunting.

The SPME samplers will be deployed at all sites. Sediments, zooplankton, macroinvertebrates, and fin clips from juvenile, fall-run Chinook salmon will be collected from sites included in CDWR's Yolo Bypass Fish Monitoring Program and the U.S. Fish and Wildlife Service's Delta Juvenile Fish Monitoring Program.

Background and Conceptual Models

The proposed project will focus on four representative classes of pesticides including pyrethroids, organophosphates (chlorpyrifos and diazinon), fipronil and its metabolites, and the organochlorine group. The pyrethroids and the fipronil group were chosen due to their heavy usage and frequent detection in our previous studies in California[18,19,20]. Although declining in usage, Chlorpyrifos and diazinon have been an issue in California for years[21], while the organochlorines represent legacy contaminants. A list of the target analytes is presented in Table 1.

Table 1: List of target pesticides

Class	Compound
Pyrethroids	bifenthrin, lambda-cyhalothrin, cyfluthrin, cypermethrin, permethrin, esfenvalerate, deltamethrin and fenpropathrin
OCs	alpha-BHC, beta-BHC, gamma-BHC, delta-BHC, p,p'-DDE, p,p'-DDD, p,p'-DDT, aldrin, gamma-chlordane, alpha-chlordane, diedrin, endrin, endrin ketone, endosulfan I, endosulfan II, endosulfan sulfate, heptachlor, heptachlor epoxide and methoxychlor
OPs	chlorpyrifos and diazinon
Phenylpyrazoles	fipronil, fipronil-sulfide, and fipronil-sulfone

OC = organochlorine insecticides; OP = organophosphate insecticides

We expect that pesticides will be present at our study sites and that timing and magnitude of pesticide loadings may differ among locations based on water sources (agricultural drainage vs. river water), river discharge, and floodplain inundation and land use (flooded rice fields vs. fallow or non-farmed fields). Furthermore, we expect that pesticide bioavailability and pathways of pesticide uptake by juvenile Chinook salmon will likely differ between floodplain and river sampling locations due not only to differences in timing and amount of pesticide loadings, but also as a result of differences in routing of hydrophobic pesticides (accumulation in sediments vs. in phytoplankton) and in food webs supporting salmon growth in floodplain and river habitats (benthic vs. planktonic prey; detrital vs. algal trophic base). We also expect temperature to significantly alter bioaccumulation potential of the pesticides due to hormonal impacts on enzymes important for metabolism and clearance (i.e. cytochrome P450s). Combining deployment of passive samplers in the water column and sediment with information on the relative importance of benthic and planktonic trophic pathways for juvenile salmon inferred from stable isotope and fatty acid biomarkers and assays of pesticide residues in benthic and planktonic salmon prey items will enable assessment of whether direct water uptake, planktonic prey, or benthic macroinvertebrates represent the principal route of pesticide uptake for juvenile salmon rearing in the Delta. Moreover, we will assess whether pesticide uptake pathways differ among fish rearing in different floodplain and river habitats within the Delta (Fig. 1). Spatial differences in pesticide bioavailability, trophic pathways leading to juvenile salmon, and

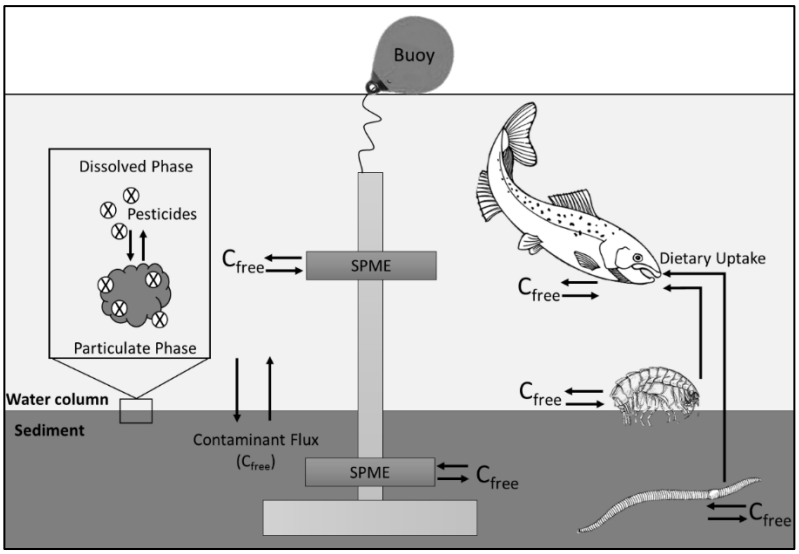
pesticide residues in various prey types are anticipated to have the potential to differentially affect growth rate, neuroendocrine function, and swimming performance of juvenile Chinook salmon rearing in different locations in the Delta.

Solid phase microextraction samplers measure the chemical activity of pesticides in sediment and in overlying water and provide estimates of the freely dissolved water concentrations[9]. In this technique, a fiber, coated with chemical sorbent is placed in the sediment or overlying water, and after a sufficiently long equilibration period (weeks to two months for pesticides), the pesticide concentration on the fiber (the solid phase) comes to equilibrium with the sediment/pore water or overlying water system. Only a small fraction of the contaminant in the system will adsorb to the fiber resulting in a "non-depletive" extraction technique. At this point, the fiber typically is removed and directly injected into an analytical instrument or the chemical is extracted from the fiber using solvents to measure the amount of contaminant on the fiber. This amount, coupled with laboratory and literature-derived equilibrium partition coefficients between the pesticide and water (K_{fw}), allow calculation of the pesticide concentration in the water[9,12]. Our previous studies[22,23,24] showed SPME measurements correlated well with body residues of organic contaminants across laboratory-spiked and field-contaminated sediments and chemical classes (Fig. 2). Therefore, SPME fiber concentration can serve as a surrogate for absorbed dose and will allow an assessment of pesticide exposure in Chinook salmon.

Stable isotopes and fatty acid biomarkers have been employed in several studies to assess the trophic basis of production and food web structure of marsh and floodplain ecosystems, including evaluation of habitat rehabilitation[25,26,27,28]. In particular, these techniques are applicable for determining the relative importance of phytoplankton, zooplankton and marsh vegetation to invertebrate and fish production. We hypothesize that the contribution of detritus derived from marsh vegetation to juvenile Chinook salmon and their invertebrate prey will be greater in floodplains compared to Sacramento River channel habitats. Juvenile Chinook salmon feed extensively on macroinvertebrates in the Yolo Bypass floodplain, whereas zooplankton dominate the juvenile salmon diet in the Sacramento River[1], suggesting a different trophic base and series of pathways leading to juvenile salmon in the two systems. In terms of dietary routes of exposure to salmon, these two pathways are distinct. First, the zooplankton community that the salmon rely on in the river habitat can be impaired due to pesticide exposure. For example, pyrethroids and chlorpyrifos have been shown to have direct acute effects as well as indirect effects on zooplankton communities[29]. The pyrethroid cypermethrin caused acute lethality to zooplankton in a freshwater lake[30], while it reduced zooplankton density and biodiversity in a marine plankton community[31]. Conversely, we expect that the increased contribution of detrital food web pathways to juvenile Chinook salmon in floodplains may result in lower body residues of pesticides in fish rearing in a floodplain habitat due to less of the pesticides being in the freely dissolved bioavailable phase. While we are aware of a recent study using stable isotopes and fatty acids to assess the trophic basis of secondary production in the Liberty Island/Cache Slough area and a study employing stable isotope techniques to elucidate relationships between trophic pathways and mercury bioaccumulation in the Delta, we are unaware of any studies using stable isotope and fatty acid biomarkers to link trophic pathways with pesticide residues and bioavailability to salmon in the Delta. Therefore, one of the project objectives will be to assess differences in the trophic basis of juvenile salmon

growth in floodplain habitats and the Sacramento River using stable isotopes and fatty acid biomarkers with the aim of determining whether the anticipated differences in basal energy sources supporting juvenile salmon in floodplain habitats and river are associated with differences in pesticide residues in juvenile Chinook salmon.

Benthic Route of Exposure



Pelagic Route of Exposure

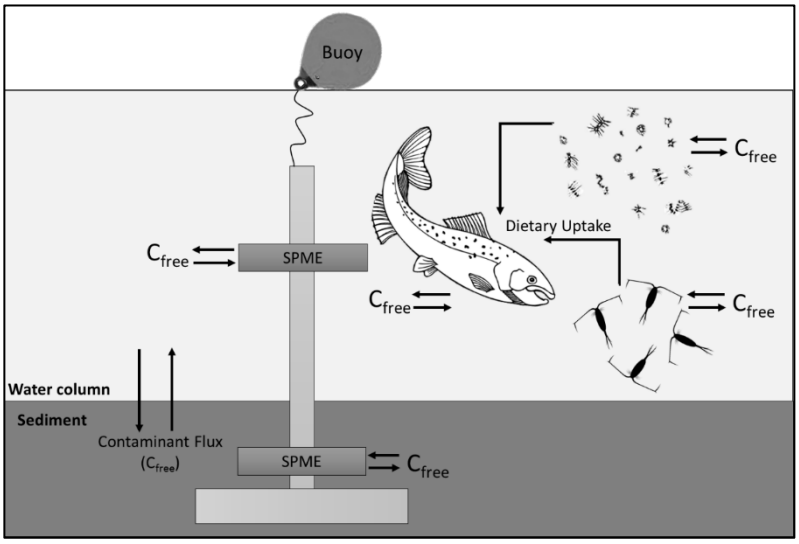


Figure 1. Conceptual models for benthic and pelagic foodwebs and transfer of hydrophobic pesticides to juvenile Chinook salmon. The freely dissolved pesticide concentration (C_{free}) will be measured using solid phase microextraction (SPME) fibers that will be attached to a pvc pipe anchored with a cement block and the SPME's will be attached to the pipe in the water column and in the sediment.

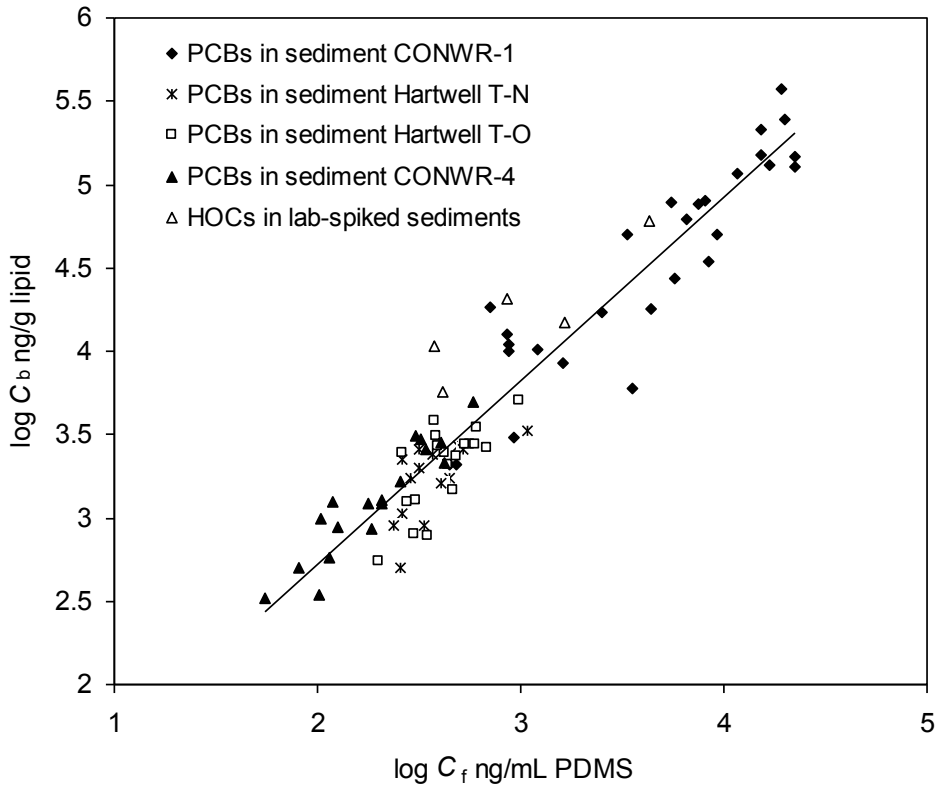


Figure 2. Relationship between matrix-solid phase microextraction fiber concentrations (C_f) and body residues in *Lumbriculus variegates* (C_b) across laboratory-spiked and field-collected sediments[22,23,24].

Increasing Temperature Along the West Coast

Global climate change generated by humans is causing physical changes in aquatic and marine systems, such as sea level rise, ocean acidification, and increasing sea surface temperatures. Climate change causes increased surface water temperatures, which includes rising water temperatures along the coast of California that is home to many marine and aquatic species. There is a projected increase in the annual temperatures of the San Francisco Delta, a heavily populated coastal region, and the rivers feeding into the Delta[32]. These increasing water temperatures have the highest increase in the summer months (Fig. 3) according to models using past monitoring data[32]. There are many uncertainties associated with the increase in surface water due to global climate change, such as the rate of water temperature increases and the effect this will have on biological communities[33]. It is evident that coastal fauna will experience a thermal shift as climate change persists. Increasing water temperatures along these coasts are inevitable, and the impacts of the new temperature regimes on many ecosystem communities remain unknown.

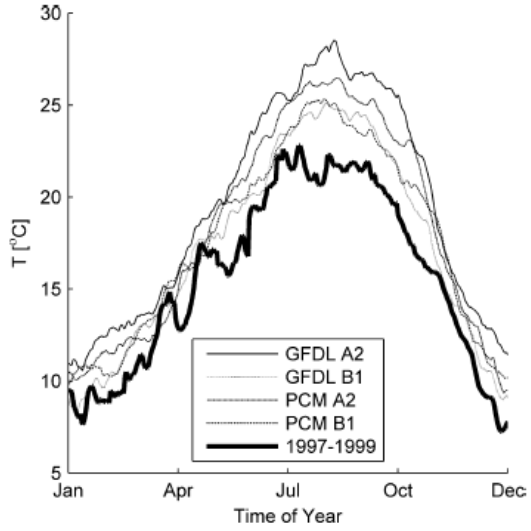


Figure 3. Comparison of projected mean temperature of the Sacramento River in 2097-2099 model years to recorded temperature averages from the years 1997-1999 (solid lines). Each shaded line represents the predicted temperatures from different global climate models, with an approximate maximum predicted temperature at 27^0C from the Geophysical Fluid Dynamics Laboratory's (GFDL) model[32].

To assess the potential sub-lethal effects of pesticides on juvenile Chinook salmon, we will conduct a laboratory study at Southern Illinois University's (SIU's) aquatic research facility to determine relationships between body pesticide residue levels and fish growth and swimming performance, as well as assess the impacts of temperature on sublethal toxicity of the pesticides. This component of the study will allow us to disentangle effects of pesticide residues from other growth- and behavioral-influencing factors inherently present in field studies. We are conducting the studies at SIU because of limited salmon availability at California hatcheries. Given the demonstrated neurotoxicity of pesticides, impairment of neuromuscular as well as neuroendocrine function represents a potential consequence of sub-lethal pesticide exposure. The Schlenk group has been interested in the toxicity of pesticides detected in the San Francisco Delta for years. Current use and organophosphate pesticides have been shown to affect the neuroendocrine system of juvenile salmonids[13,14,15,]. The pathway of interest is the hypothalamus-pituitary-gonadal axis (HPG Axis). The feedback loop of hormones in this axis helps regulate biosynthesis and signaling of downstream hormones in the HPG axis, such as estradiol and testosterone involved in sexual maturation. Dopamine is a neurotransmitter that plays an important role in regulating the HPG axis. Increases in dopamine have been shown to decrease the levels of gonadotropin releasing hormone and thyroid stimulating hormone, which are responsible for regulating levels of gonadotropins and thyroid hormones, respectively. Disruption of thyroid hormone pathways has been shown to be linked to growth, which is a critically important fitness parameter in larval and juvenile salmonids.

Previous research in the Schlenk group suggests the potential for endocrine-disrupting effects of bifenthrin due to the alterations of dopamine signaling as well as changes in the other feedback loops of the HPG axis[15]. This research also suggests that early exposure of bifenthrin can have persisting adverse outcomes on juvenile trout. Similar studies with chlorpyrifos have shown sublethal impacts on olfaction[13] which would also diminish growth. However, all of these studies were performed with aqueous exposures which we have previously shown to have limited effects to salmonids during run-off events[7]. **The truly novel aspects of our study are to examine the questions of how dietary exposure to pesticides at juvenile stages under**

varied temperature regimes affects the upstream mechanisms that control hormone biosynthesis, and how this would affect olfaction, swimming performance, energetics, growth, and population. Dietary exposure represents the most likely and realistic route of exposure to pesticides by fish as they migrate through the proposed areas.

Ecological Impacts of Combined Stressors

Temperature increases due to climate change significantly impacts the endocrine function of fish. To assess how temperature and exposure to pesticides will affect the populations of salmonids in the San Francisco Delta, olfactory function and growth will be evaluated. Survival and growth rate of juvenile salmonids will be evaluated after exposure to elevated temperature with and without pesticides, and using predator-avoidance bioassays to assess necessary survival behaviors, such as the olfactory detection of predators. Increasing thermal environments has been shown to have negative impacts on the growth, body size, and feeding behavior in salmonids[35]. Previous studies in the Schlenk group have observed inhibition of the olfactory system in salmon due to organophosphate exposure[13]. Thus, understanding the impacts of pesticide and /or temperature on olfaction and growth will provide significant threshold information for sublethal effects.

Swimming performance is a commonly-applied, ecologically-relevant measure of neuromuscular function in fishes and has direct implications for behaviors essential for survival, including maintaining position in water currents, foraging, predator avoidance, and migration. Sub-lethal exposure to pesticides has been demonstrated to reduce swimming performance in juvenile Rainbow trout and adult Coho salmon[13,36,37]. Exposure to diazinon, an organophosphate insecticide, has been demonstrated to inhibit olfactory-mediated alarm pheromone responses and homing behavior in Chinook salmon[38]. While temperature clearly affects swimming performance in salmonids[36], effects of temperature in combination with pesticides on swimming performance of juvenile Chinook salmon has not been assessed. Swimming performance differs among fish species and life stages. In addition, pesticide-induced impairment of fish swimming performance may be influenced by pesticide concentrations and timing of fish exposure, as well as the mixtures of pesticides to which fish are exposed.

Thus, understanding effects of temperature and/or pesticides on olfaction and swimming performance of juvenile Chinook salmon in the Delta will require controlled experiments to assess effects of environmentally-relevant pesticide concentrations on this species and life stage under environmental conditions representative of rearing habitats in the Delta. Knowledge of how pesticide exposure and body pesticide residue levels affect swimming performance of juvenile Chinook salmon is important for evaluating potential effects of exposure to pesticides in rearing habitats in the Delta on fish foraging ability and predation risk.

Approach and Statement of Work

Task 1 – Project Management and Administration

Quarterly progress reports and invoices will be submitted as specified in the grant agreement.

Task 2 – SPME Deployment

Passive samplers (SPMEs) will be deployed at up to 24 locations (see attached map) in Yolo Bypass (three sites, depending on which sections are flooded), Toe Drain (Yolo Bypass outlet), Cache Slough/Liberty Island, American River, the Sacramento River and connected sloughs, and all water inputs to Yolo Bypass study sites (some, e.g., Sacramento Weir, may only contain water during floods). The SPMEs will be deployed in the sediment and in the water column at each location. Study sites will be selected with assistance from our collaborators and the research coordinator (see attached letters of support). Passive samplers will be deployed in February and collected after 60 days of field exposure during 2019 and 2020. This deployment time should allow the SPME system to reach equilibrium levels based on research previously conducted in our laboratory and others[9,12,46] . Performance reference compounds that mimic the behavior of our target analytes will be used for the field-deployed samplers if it is necessary to correct for non-equilibrium conditions, but again we feel this is unlikely[9,39].

The SPME experiments will be conducted using the methods described by You et al.[22,23]. Disposable SPME fibers coated with 10 µm of polydimethylsiloxane (PDMS) from Fiberguide Industries, Stirling, NJ, USA will be used for the study. Envelopes made with 110 µm stainless steel screen will be used to protect the fragile fibers, and our previous study[44] showed that the 110 µm openings allow for adequate contact of the fiber with the pore water and sediment particles. Before use, the fiber and envelope will be sequentially rinsed with acetonitrile (ACN), ACN-water (1:1), and water, and dried at room temperature. The SPME fibers will be inserted into the sediment and in the water column (Fig. 1). After the 60-d exposure, fibers will be removed from the field for fiber concentration analysis. Fibers will be extracted with 3×1ml of hexane and the combined extract cleaned with primary/ secondary amino absorbent and copper powder prior to chemical analysis. The freely dissolved concentration of pesticides in the pore water (C_{pw}) or overlying water (C_{ow}) will be calculated from the fiber concentration (C_f) and the K_{fw} using the equation $C_{pw} = C_f / K_{fw}$ or $C_{ow} = C_f / K_{fw}$. The partition coefficients (K_{fw}) for the target pesticides have been determined previously in our laboratory and others[9,12,53].

Task 3 – Sediment, phytoplankton, invertebrate, and fish sampling

Sampling for this task will be conducted in coordination with the California Department of Water Resources (see attached letter of support). Surficial sediments will be collected by Ponar grab and homogenized to create a uniform material for chemistry determinations. Collected sediments will be placed on ice and shipped to SIU for testing. It is estimated that 0.2 kg will need to be collected from each site.

Field-contaminated sediments will be characterized for particle size, determining percentage of sand, silt, and clay, and organic carbon content will be quantified with a CHN analyzer[40]. Organic carbon associated with soot (i.e., black carbon) can influence partitioning[40] and bioaccumulation[41] of organic contaminants. Black carbon will be quantified by combusting samples with a CHN analyzer after removing carbonates with HCl following the methods of Gustafsson et al.[40] . Plant pigments within sediments, such as chlorophyll-a, will be extracted with ethanol and analyzed using fluorescence detection. In addition, other lipid-like compounds and non-polar extractable material within sediments will be quantified using gravimetric analysis.

Phytoplankton and invertebrate samples will be collected at 2-3 week intervals during the time that juvenile Chinook salmon are present in the Delta. Zooplankton samples will be collected using a 150 µm mesh zooplankton net and sorted by major taxa (e.g., clacocerans, copepods). Zooplankton nets and D-frame nets will be used to collect macroinvertebrate samples, which will also be sorted by major taxa (e.g., chironomids, amphipods, etc.). Samples of plant detritus (e.g., from terrestrial vegetation, rice stubble, or macrophytes) will also be collected, when present. Phytoplankton, sediment, plant detritus, zooplankton, and macroinvertebrate samples will be analyzed for pesticide concentrations; stable isotope and fatty acid analyses will be conducted on phytoplankton, plant detritus, zooplankton, and macroinvertebrates (see Task 5 below).

Non-lethal tissue (fin clip) samples will be obtained from juvenile, fall-run Chinook salmon collected in the Yolo Bypass and Sacramento River by CDWR's Yolo Bypass Fish Monitoring Program and the U.S. Fish and Wildlife Service's Delta Juvenile Fish Monitoring Program. These samples will be used to assess the trophic base (benthic vs. pelagic) of juvenile salmon using stable isotope fatty acid analyses.

Task 4 – Laboratory study: Relationships between pesticide residues and salmon growth, neuroendocrinology, olfaction and swimming performance

To assess the potential sub-lethal effects of pesticides on juvenile Chinook salmon, we will conduct a series of laboratory studies at SIU's aquatic research facility during the second and third years of the proposed project (2019 and 2020) to determine relationships between body pesticide residue levels and fish growth, olfaction and swimming performance. These apical endpoints will be linked to neuroendocrine responses as well to determine potential mechanisms and adverse outcome pathways for the mixtures. The rationale for using animals at SIU is that salmon are not currently available for laboratory study from California hatcheries. Bioaccumulation tests will be conducted with designated prey species from the floodplain habitats and the Sacramento River and will be determined from year one field sampling efforts. Those prey species that were most often fed upon by the salmon long with those that bioaccumulated the largest amount of pesticides will be chosen for the laboratory studies. Prey species bioaccumulation tests will be conducted for a sufficient length of time to allow for steady state levels to be achieved in the organisms. Previous studies have shown body burden levels of approximately 2.80 to 9,482 nmole/g lipid in macroinvertebrates exposed to these agents[12] so we would expect potential tropic transfer of the target pesticides into salmon. Using equilibrium rate constant assumptions, we will conduct accumulation studies under the appropriate durations to attain levels observed in earlier Tasks. These bioaccumulation tests will be run at a single temperature (e.g. 12°C) and will be conducted in an environmentally-controlled room.

As it is certain that other chemical stressors (i.e. metals; emerging contaminants) may contribute to the impact of pesticides. Additional aliquots of collected organisms at each location will be frozen and fed to salmon in order to compare "field-collected" samples with "laboratory-derived pesticide-loaded" samples. We anticipate needing enough samples not only for pesticide and lipid analyses, but also feeding 10-15 fish for 30 days. If samples are not available, first priority will be given to pesticide analyses.

Hatchery-origin, juvenile Chinook salmon will be obtained from the Illinois Department of Natural Resources' Jake Wolf Hatchery and maintained in tanks in a recirculating-water system supplied with filtered, dechlorinated municipal water (pH 6.8) and equipped with temperature control and biofiltration systems. Fish will be obtained from a source outside of California due to California hatchery-source fish not being available for use in this project. Fish will be fed a commercial pellet diet *ad libitum* daily under a 12 h light:12 h dark photoperiod; dissolved oxygen will be maintained at >90% saturation. Water temperature during initial acclimation to the laboratory (~2 weeks) will be maintained at 13 ± 0.5° C.

Individual fish will be assigned to treatment (pesticide exposure and temperature) or control groups (n=12-15 fish per group). Treatment groups will consist of fish fed to cessation prey item(s) dosed with an individual pesticide orsuite of pesticides during the 28 d exposure period. Controls will be fed un-dosed prey. Selection of target pesticides, dosing concentrations and the most pertinent prey species will be based on pesticide concentration and bioavailability data from field studies in the Delta performed in year 1 of the project. While invertebrates will be loaded at ambient Delta temperatures (12°C), fish in each treatment group will be exposed at three temperatures (12, 16 and 18°C) in separate, aerated aquaria; fish will be acclimated to experimental temperatures for at least one week prior to testing. The range of water temperatures was predicted from climate-change models[32] (see Fig. 3)

To assess swimming performance, fish from control and each treatment group (immediately following pesticide exposure) will be placed in a swim tunnel (impeller-driven respirometer) and allowed to the testing chamber for 30 minutes. Each fish will then be subjected to one of two swimming protocols to assess critical swimming speed (U_{crit}, a measure of sustainable, continuous (aerobic) swimming) and burst swimming (U_{max}) performance using procedures described in Goulding et al. (2013). Once fatigued, fish will be removed from the swim tunnel, euthanized with MS-222, measured for total length and weight, and frozen for analysis of body pesticide residues as described for fish in the field enclosure experiment. Swim tunnel trials will be conducted at 12, 16, and 18^{o}C to assess the combined influences of temperature and pesticide exposure on juvenile Chinook salmon swimming performance.

To assess olfactory behavior, Y-maze experiments will be performed. Individual fish will be used in behavioral tests just before sea water exposure, and just before being euthanized at the end of the experiment. Y-mazes have been used to test olfaction of predator avoidance by exposing fish to amino acid mixtures that have been shown to induce adverse/ avoidance reactions in salmon and trout[33]. The Y-maze consists of two arms, and one central channel filled with chilled water, as depicted in Fig 4. All exposures will be conducted at the three temperatures as above.

Fish will be acclimated in the center of a Y-maze and separated from the arms by plastic gates. The gates will be removed after 5 minutes of acclimation, and then individual fish will be exposed to the predator cue (L-serine) injected into one arm of the maze. Fish will be allowed to freely swim through the Y-maze for 15 minutes, choosing between the arm with the predator odorant, the arm with no odorant (control arm), or the central channel. Each fish will be tested once individually, and the Y-maze rinsed thoroughly before the next test to prevent exposure to residual predator odorants in the following trial. The final location of the fish will indicate if it

was able to detect the predatory cue. If the juvenile Chinook do not avoid the predatory cue, then this indicates that the pesticides have adverse effects on the olfactory system and can impact necessary survival behaviors.

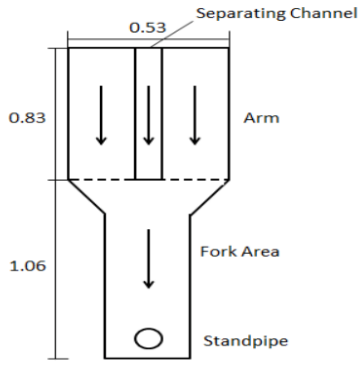


Fig 4. The design of the Y-maze behavioral test tank. Fish are acclimated in the central fork area. Predator odorant is injected into one of the arms and fish are allowed to swim freely for 15 minutes. Rehnberg et al.[42].

Following exposures, blood, brains and olfactory bulbs will be dissected from treated and control fish for measurements of neuroendocrine function.

Task 5 – Sample analyses

Pesticide concentrations in the sediments, organisms, and SPMEs fibers will be determined using GC/MS after sample extraction and cleanup. An accelerated solvent extraction method (USEPA method 3545) will be used for sediment and biota extractions. After homogenization and freeze-drying, 10 g of sediment will be mixed with pelletized diatomaceous earth and extracted on a Dionex ASE 200 using an acetone and methylene chloride mixture at 100ºC and 1500 psi. Surrogate standards (4,4'-dibromooctafluoro-biphenyl and decachlorobiphenyl) will be added into the samples prior to extraction to qualify the extraction efficiency. The filtered extracts will be concentrated and solvent exchanged to hexane. Organic interference will be removed from the extracts using solid phase extraction (SPE) cleanup methods for the pesticide analysis[43]. Copper powder will be used to remove residual sulfur from all of the sediment samples (USEPA method 3660). Tissue samples will be extracted with a methanol and ACN mixture and cleaned with tandem SPE[44]. For the tissue samples, one-tenth of the extract will be used for lipid analysis using the vanillin-based spectrophotometric method after sulfuric acid digestion[45], and the remaining extract will be used for chemical analysis.

Pesticide analyses will be performed on an Agilent 6850 gas chromatograph (GC) with a 5975C mass selective detector (MSD; Agilent Technologies). Fipronil and its metabolites will be quantified in electron impact (EI) mode, while all of the other target pesticides will be quantified in negative chemical ionization (NCI) mode using the methods developed in the Lydy laboratory[44,46,47,48]. Identification of analytes will be based on detection of the target and qualifier ions within a retention time window of 1%. Quantification will be performed using internal standard (IS) calibration with multi-point matrix-matched standards for each pesticide and surrogate.

Quality assurance and quality control for the analytical samples will consist of a laboratory control blank, a laboratory control sample, a matrix spike, and a matrix spike duplicate and will be included every 20 samples. A midlevel calibration check standard will be analyzed every 10 samples during the GC analysis. Two or three surrogates will be added to each sample prior to extraction to verify the extraction efficiency of the sample for GC/MS analysis. Acceptability limits for percentage recovery of the laboratory control sample, matrix spike, matrix spike

duplicate, and surrogates will be within 50-150% and relative percent difference of the results of matrix spike, matrix spike duplicate will be less than 25%.

Stable isotope and fatty acid analyses will be performed on phytoplankton, detritus, zooplankton, macroinvertebrate, and fin clip samples from Chinook salmon collected from the Delta. Phytoplankton samples for stable isotope and fatty acid analyses will be processed using density-gradient centrifugation on colloidal silica to aid separation of phytoplankton from detritus[55] and collected on pre-combusted glass fiber filters. All phytoplankton, detritus, invertebrate, and fish fin clip samples for stable isotope analysis will be freeze-dried, homogenized (using a commercial grinder or mortar and pestle) and analyzed for stable carbon and nitrogen isotope ratios using a Delta V Plus Isotope Ratio Mass Spectrometer interfaced with an elemental analyzer at SIU's Mass Spectrometry Facility. Stable isotope data will be expressed in standard delta notation: $\delta^{13}C$ or $\delta^{15}N$ (‰) = [(Rsample / Rstandard) – 1] x 1000, where R represents $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Mixing models (e.g., Isosource, MixSIAR) will be used to assess contributions of energy sources to juvenile Chinook salmon.

Fatty acid profiles of primary producer, invertebrate, and fish fin clip samples will be generated from crude lipids extracted from freeze-dried samples using methods described by[49.] Briefly, crude lipids will be extracted according to procedures described by Folch et al.[50] and processed to yield fatty acid methyl esters (FAME) according to acid-catalyzed transmethylation methods described by Christie[51]. The resultant FAME will be separated using a Shimadzu GC-17A gas chromatograph. Individual FAME will be identified by reference to external standards. Non-metric multidimensional scaling, analysis of similarities, and similarity of percentages analysis will be used to define and quantify trophic connections among Chinook salmon, invertebrate, and primary producer samples[52].

Expression of gene transcripts previously shown to be altered in olfactory function and neuroendocrine signaling will be evaluated in brains of fish[13,15]. In the blood, hormones responsible for growth, reproduction and behavior will be measured by ELISA assays. Endpoints will include plasma/pituitary gonadotropin releasing hormone (GnRH), plasma/pituitary gonadotropin (GtH), plasma thyroid hormones (T3/T4), cortisol, growth hormone (GH), estradiol (E2), and testosterone[14,15]. ELISA assays are a standard method of quantifying the concentrations of specific proteins and hormones, from a tissue sample. qPCR will be used to assess the transcripts of Na+/K+ ATPase (NKA), gonadotropin releasing hormone (GnRH2), and Dopamine Receptor 2A (DR2A) Calcium calmodulin kinase in the brain. Each of these genes has been shown previously in the Schlenk group to be altered by pesticide exposure[15].

Task 6 – Dissemination of research results

Research results will be presented at professional conferences, including the Bay-Delta Science Conference, American Fisheries Society, and the Society of Environmental Toxicology and Chemistry. We anticipate that at least three peer-reviewed manuscripts will be produced from the project.

Tasks 7 and 8 – Draft and final project reports and project close-out

Reports will be submitted as specified in the grant agreement.

Feasibility

Dr. Schlenk's laboratory has conducted research on the effects of pesticides on fish for more than 30 years. He is a Professor of Aquatic Ecotoxicology and has the experience in project management needed to oversee the project (Tasks 1, 6, 7, 8) has over 2000 sq ft of laboratory space. His laboratory has instrumentation (thermocylers; plate readers; qPCR) that will allow him to carry out the proposed studies (Task 4, in part). Dr. Lydy's area of expertise is environmental chemistry and aquatic toxicology. His laboratory within the Center for Fisheries, Aquaculture and Aquatic Sciences (CFAAS) contains 2,000 sq. ft. of lab space and is equipped with fume hoods, environmental chambers, analytical scales, freeze dryers, accelerated solvent extraction apparatus, four Agilent 5975C GC-MS systems and state-of -the-art aquatic rearing facilities. Similar testing and analytical methods have been performed at this facility and we anticipate little difficulty in meeting the facilities needs of this project (Tasks 2, 5, in part). Dr. Whitledge brings expertise in stable isotope and fatty acid analyses and has conducted respirometry/swim tunnel studies with a variety of fish species. An isotope ratio mass spectrometer and peripheral instrumentation required for stable isotope analysis of biological samples are available at SIU's Mass Spectrometry Facility; the SIU CFAAS also has a GC-FID that will be used for fatty acid analysis of biological samples in the proposed project (Task 3, in part). The CFAAS at SIU also maintains lab space with microscopes needed to conduct fish stomach contents analysis and >9,500 ft² of wet lab space with recirculating-water tank and aquaria systems (equipped with temperature control and biofiltration) and a swim tunnel (impeller-driven respirometer) that will be used to conduct the laboratory experiment (Task 4) in the proposed project. Equipment required to perform Tasks 2-3 (SPME deployment; and sediment/phytoplankton/invertebrate/fish sampling) will be provided through partnerships with the California Department of Water Resources and U.S. Fish and Wildlife Service, with logistical support from the Metropolitan Water District of Southern California (see Community Support and Collaboration section below). Tasks 1-5 will include personnel to be supported by the project (a post-doctoral researcher, etc... and contributions from collaborating partners (see Community Support and Collaboration section and attached letters of support).

Tasks 2 and 3 require field work in the Delta and will involve sampling of biota in both floodplain and river locations. Collection of fish and invertebrates from river and floodplain sampling sites will be conducted by partners on the project (fin clips from non-captive salmon by the U.S. Fish and Wildlife Service) or in collaboration with project partners (California Department of Water Resources; salmon and invertebrate sampling). These sampling efforts will be conducted under collecting authority/permits of these agency partners.

Schedule & Deliverables

Task No.	Task Title	Deliverables and Key Project Milestones	Estimated Completion Dates
1	Project Management and Administration	1.1 Quarterly Progress Reports 1.2 Quarterly Invoices	1.1 Due within thirty (30) days following each quarterly month following Grant execution. 1.2 Due within thirty (30) days following each month (or) quarterly month (or) semi-annual month following Agreement execution.
2	SPME deployment	2.1 Data reporting to CEDEN	2.1 Due within thirty (30) days after completion of the chemical analyses
3	Sediment, phytoplankton, and invertebrate sampling	4.1 Data reporting to CEDEN	4.1 Due within thirty (30) days of completion of sampling events
4	Laboratory study	6.1 Data reporting to CEDEN	6.1 Due within thirty (30) days of completion of the lab experiment
5	Sample analyses	5.1 Data reporting to CEDEN	5.1 Due within thirty (30) days after completion of the chemical analyses
6	Dissemination of research results	7.1 Presentations at professional meetings and publications in peer-reviewed journals	7.1 Dates of conferences listed in Task 8 section of Approach and Statement of Work; publications will be submitted within 12 months following project completion.
7	Draft and Final Project Report	8.1 Draft Final Report 8.2 Final Report	8.1 5/31/2020 8.2 6/30/2020

Task No.	Task Title	Deliverables and Key Project Milestones	Estimated Completion Dates
8	Project Close-Out	9.1 Project Close-Out Report 9.2 Final Invoice	9.1 7/31/2020 9.2 8/31/2020

Community Support and Collaboration

The Metropolitan Water District of Southern California (District), a stakeholder in the State Water Project (SWP) and the Sacramento-San Joaquin Delta (Delta), has committed to partner with us on the proposed project, with an in-kind contribution of $100,000 to assist with project logistics and coordination of sampling with other agency partners in the Delta region.

Collection of fish and invertebrates from river and floodplain sampling sites will be conducted by partners on the project (fin clips from salmon collected by the U.S. Fish and Wildlife Service) or in collaboration with project partners (California Department of Water Resources; salmon and invertebrate sampling). These sampling efforts will be conducted under collecting authority/permits of these agency partners.

The California Department of Water Resources, Division of Environmental Services, Environmental Water Quality and Estuarine Studies Branch, has pledged support for the project by assisting with acquisition of fin clips from non-listed fish species and providing logistical field support through the use of one of their Boston Whaler research vessels and one boat operator (two weeks per year) during the study ($20,000 per calendar year of cost share for labor and operating expenses). The research vessel will be used for sampling of sediment, phytoplankton, and invertebrates and fish sampling as described in the Approach and Statement of Work.

Data Management and Access

Data Plan

The data created as part of the proposed research project will include residue data in various environmental media, pesticide concentrations on passive samplers, food-web structure determined from stable isotope studies and swimming performance data for salmon. Data collected as part of this project will be compatible and consistent with other data entered into the California Environmental Data Exchange Network (CEDEN).

Data sharing

All data products used and generated will be stored using the Southern Illinois University (SIU) Conventional File Service (SalukiFile). SalukiFile is a centralized data storage facility that allows file access from either on or off campus. Though the storage is automatically backed up to protect against accidental deletion or over-writing, we will also back up the datasets using external hard drives on co-PI Lydy's file server. To facilitate requests from other researchers, we

will also store our research products in OpenSIUC, an open-access institutional repository at SIUC (http://opensiuc.lib.siu.edu/) funded and managed by SIU's Morris Library. OpenSIUC provides access to its content via any internet browser, with no user registration required. Policies and provisions for re-use and re-distribution are governed by a Creative Commons Attribution-Non Commercial-noDerivs (CC BY-NC-ND) license, allowing others to download and share the files as long as the creator is credited, but not allowing the files to be changed in any way or used commercially. Plans for archiving and preservation of access files in OpenSIUC are subject to the preservation/retention guidelines of the repository (http://opensiuc.lib.siu.edu/faq.html). OpenSIUC uses stable URLs to provide persistent linking to content, which is preserved on a failover web, database and storage servers to safeguard against main server failures. Local backups of data are made every six hours, and offsite backups are made daily. Weekly backups are also sent to a third party archival service, Iron Mountain. We do not anticipate making any software or inventions from this research, but in the event that such items are prepared, we will follow the National Science Foundation (NSF) guidelines for data sharing.

Period of data retention

Data from this investigation will be made freely available for a period of three years following timely publication of results and graduation of any students that contribute to the project as a co-author. Recognizing that access to scientific journals typically require a paid subscription, we will upon request send the journal articles and associated supplementary information in an Adobe PDF format to those who request the information at no additional charge.

Data formats and dissemination

The data sharing policy will conform to NSF published guidelines[54]. We will publish our results in well recognized scientific journals in a timely fashion, as we have done in the past. The work will be coauthored by those workers (graduate students, post-docs, scientists, and PIs) who have conducted the research. Specific data formats will include text files, Access, Excel, Word and Powerpoint files.

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UPDATED Information about the Data Management Plan Required for Full Proposals.
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Impacts of climate change on pesticide bioavailability and sublethal effects on juvenile Chinook salmon in the Delta: Potential benefits of floodplain rearing [The Regents of the University of California, Riverside Campus]
Location Map. Northwestern portion of the Sacramento Delta showing proposed SPME-only deployment sites (blue ovals) and sites where fish, invertebrate, sediment, and plankton sampling and SPME deployment will occur (red ovals) in the Sacramento River, Yolo Bypass (gray shaded area), Toe Drain, Cache Slough/Liberty Island, and drainages that flow into the floodplain/floodway.

USGS quadrangles: Knights Landing, Grays Bend, Davis, Saxon, Liberty Island, Courtland, Clarksburg, Sacramento West.