CALFED Progress Report
California Sea Grant College Program

ConfirmationNumber 20100610154335

Printed: 6/10/2010 3:45:07 PM

ProjectYear_2A 1st **ProjectNo_2C** R/SF-27

TypeQuestionnaire_2B Interim Questionnaire

Preparer Information

PrepName_1A Susanne Brander
PrepEmail_1B smbrander@ucdavis.edu
PrepPhone_1C 707-227-0384

Project Information

ProjectNo_2C R/SF-27 **StartDate_3a** August 2007 **EndDate_3b** August 2011
ProjectTitle_4 The Potential for Endocrine Disruption in the Sacramento-San Joaquin Delta: Response of a Resident Fish Species

CALFed Fellow contact information

FelTitle_5A Ms. **FelLast_5B** Brander **FelFirst_5C** Susanne **FelInit_5D** M
FelInstitution_5E UC Davis, Bodega Marine Lab
FelDepartment_5F Environmental Toxicology
FelStreetAddr_5G PO Box 247
FelCity_5H Bodega Bay **FelState_5I** CA **FelZip_5J** 94923
FelPhone_5K 707-227-0384 **FelFax_5L** 707-875-2009
FelEmail_5M smbrander@ucdavis.edu
FelPositionTitle_5N Ph.D. candidate

Research Mentor (for additional please see #8)

RMTitle_6A Dr. **RMLastName_6B** Gary **RMFirstName_6C** Cherr **RMInit_6D** N
RMInstitution_6E UC Davis, Bodega Marine Lab
RMDepartment_6F Environmental Toxicology
RMStreetAddr_6G PO Box 247
RMCity_6H Bodega Bay **RMState_6I** CA **RMZip_6J** 94923
RMPhone_6K 707-875-2051 **RMFax_6L** 707-875-2009
RMEmail_6M gncherr@ucdavis.edu
RMPositionTitle_6N Professor, Bodega Marine Lab Director

Community Mentor (for additional please see #9)

CMTitle_7A Dr. **CMLastName_7B** Bruce **CMFirstName_7C** McFallon **CMInit_7D**
CMInstitution_7E NOAA, Southwest Fisheries Science Center
CMDepartment_7F Salmon Ocean Ecology
CMStreetAddr_7G 333 North Torrey Pines Court
CMCity_7H La Jolla **CMState_7I** CA **CMZip_7J** 92037
CMPhone_7K 858-546-7000 **CMFax_7L** 858-546-7003
CMEmail_7M Bruce.MacFarlane@noaa.gov
CMPositionTitle_7N Salmon Ocean Ecology Team Leader

Additional Research Mentors and Community Mentors

Additional Research Mentors_8

Dr. Inge Werner, dissertation committee member
Dr. Michael Denison, dissertation committee member

Additional Community Mentors_9

Project Objectives: Please type your responses, and answer the questions in a style appropriate for laymen.

ProjectObjectives_10

The overall goal of my study is to compare estrogenic and androgenic activity in estuarine sites exposed to varied sources of endocrine disrupting compounds at the cellular, organismal, and population level. To date I have promising results at all three scales. This work is proceeding in three steps:

1. Receptor Level: exposure of cell lines transfected with the estrogen or androgen receptor to site extracts, both alone and with estrogen or testosterone to evaluate the potential for synergism or antagonism.
- Results will indicate whether EDCs are present at these sites and will also indicate the mechanism by which EDCs will act at the organism level.

2. Organism Level: comparison of gene, choriogenin (egg coat or chorion protein) expression, gonadal lesions/tumors, and/or ovo-testes in wild-caught and outplanted male inland silversides at sites exposed to varied EDC sources (urban run-off, ranch run-off, wastewater outfall), and confirmation of results by exposing naïve fish to site water in the laboratory.
- Results will inform us as to whether receptor-level effects are influencing deleterious physiological changes at the organism level.

3. Population Level: assessment of population level demographics and extrapolation from these and results from Aims 1 & 2 to create a population model to evaluate the effects of EDCs on the population dynamics of silversides as well as other endangered or threatened fish species in Suisun Marsh and other estuaries.

Summary of progress in meeting each of these goals and objectives

ProgressSummary_11

Aim 1. Receptor Level
Cell-line Assays
Site water samples were collected from four sites exposed to varied sources of EDCs within Suisun Marsh. To determine whether estrogenic and androgenic EDCs were present, site water samples were extracted via solid phase cartridges (C18) and re-suspended in DMSO (cite). Extracts were incubated with cell-lines transfected with the estrogen or androgen hormone responsive element linked to the firefly luciferase gene (Rogers & Denison 2000). Response of the extracts alone and extracts with the addition of estrogen or testosterone were measured to evaluate the potential for activation of the estrogen or androgen receptor and for competition with endogenous hormones. Results indicate that extracts from all sites bind to the estrogen receptor but that they also attenuate the binding of endogenous estrogen. Androgenic activity is significantly higher at sites exposed to urban run-off and wastewater effluent compared to ranch run-off, and extracts from all sites add to the activity of testosterone. The implications of these initial results are that estrogenic effects at the organismal and population level may be identical at urban/wastewater and ranch run-off sites, but that higher level androgenic effects might be more likely to occur at the urban/wastewater sites. Also, there is a trend towards higher occurrence of both estrogens and androgens at the wastewater outfall and up/downstream of it, in comparison to urban sites.

Aim 2. Organism Level
Choriogenin
A random sample of male and juvenile silversides was seined from the two accessible beaches in Suisun Marsh (urban & ranch run-off) on a monthly basis from March – October 2009. Each month plasma samples were taken for measurement of choriogenin (Chg) levels via Western Blot using a commercially available choriogenin antibody (polyclonal anti-salmon zona radiata protein IgG, Biosense Laboratories). Results indicate that males seined from two beaches in Suisun Marsh are expressing choriogenin, an egg coat protein. Outplanted fish are showing a similar response to wild-caught.

Aim 3. Population Level
Population Assessment
Fish were sampled via beach seine from Suisun beach (urban/ag) and Denverton beach (ranch) on a monthly basis from February – October 2009. The following data were collected: sex, standard length (SL), body weight : gonad weight (GSI), and otoliths. Results indicate that the silverside population at Suisun beach (urban/ag) has a significantly lower proportion of females than the population at Denverton (ranch), persistent throughout the 2009 breeding season. In addition, females at the urban site are significantly smaller than those at the ranch site, and urban males are significantly larger than ranch males ($p \leq 0.05$). These preliminary results indicate

that higher levels of androgenic activity at the urban site may be contributing to increased size and number of male silversides.

PROJECT MODIFICATIONS: Please explain any substantial modifications in research plans, including new directions pursued. Describe major problems encountered, especially problems with experimental protocols and how they were resolved. Describe any ancillary research topics developed.

Modifications_12

Passive samplers: Due to funding limitations we have not used POCIS or SPME samplers. Instead we opted for a lower cost version of passive sampler (polyethylene device) available through collaboration with the USGS in Sacramento.

Field sites: Due to logistical concerns, etc. instead of using sites in the Napa and Sacramento Rivers, we decided to focus on sites within Suisun Marsh.

Gene Expression: Expression of endocrine-related genes in the livers of wild-caught silversides will be conducted. Primers designed to these genes will be run via Q-PCR to quantify differences in expression between wild fish exposed at sites with varied levels and types of endocrine disrupting compounds.

Primary Antibody Development: To improve upon the sensitivity of immunoassays, silverside chorion was isolated and used to create a polyclonal silverside chorion Ab in rabbit. The availability of a more specific antibody will allow the development of an enzyme-linked immunosorbent assay in early 2010 (ELISA) to allow higher through-put analysis of plasma samples from both monthly sampling, outplants, and bioassays. Using an ELISA will also increase the statistical power of our immunoassays as more samples can be directly compared to one another.

Otolith Analysis: The potential to have otoliths dissection and aging provided by a UC Davis laboratory would greatly enhance the population level assessment by providing information on age at maturity, which has been show to be affected by EDCs

BENEFITS AND APPLICATIONS: Suggest the relevance of these new findings to management. Describe any accomplishment, that is significant effects your project has had on resource management or user group behavior. CALFED is looking for "management cue" (see http://science.calwater.ca.gov/pdf/soemgmtcues.pdf).

BenefitsApplic_13

An ancillary benefit of this work is that the inland silverside has the potential to become an EDC sentinel species in estuaries nationwide, as it is found on the East, West and Gulf coasts. Its close phylogenetic relationship to a commonly utilized laboratory species, Japanese medaka, would allow for a large body of existing knowledge on EDC effects at the genomic and proteomic scales to be applied to more environmentally relevant studies with silversides in the field. It is easily collected at sites throughout the SSJ Delta and Suisun Marsh.

I was recently invited to speak at an Interagency Ecological Program workshop held at the Central Valley Water Quality Control Board office in Sacramento. Through communicating with workshop attendees, many of whom who represent local and federal agencies, I hope to contribute to the advancement of current techniques used to evaluate the effects of pollution on the endangered and threatened species they seek to protect. In addition, I also taught a portion of a workshop on the "Biological Effects of EDCs" at the Northern California Society of Environmental Toxicology & Chemistry meeting held in May 2010. Speaking at this venue also put me in direct contact with representatives of these agencies and may potentially influence decisions on resource management in Suisun Marsh and the greater Sacramento / San Joaquin Delta.

PUBLICATIONS: List any publications, presentations, or posters that have resulted from this funded research. Give as many details as possible, including status of paper (e.g., in review; in press), journal name, conference location and date of presentation. Please note (as outlined in the conditions of the award) that each fellow is required to submit an abstract for an oral or poster presentation at each State of the Estuary conference and CALFED Science Conference during the duration of the fellowship.

Publications_14

COOPERATING ORGANIZATIONS: List those agencies and/or persons who provided financial, technical or other assistance to your project since inception. Describe the nature of their collaboration.

CoopOrganiz_15

Dr. Richard E. Connon - UC Davis Project Scientist with expertise in the analysis of gene expression in fish.

Dr. Carol Vines - UC Davis Project Scientist assisting with general laboratory techniques.

Dr. Bryan Cole - UC Davis Post-Doctoral Researcher in the Cherr Lab assisting with the development of an ELISA using a newly created antibody specific to silverside chorion.

NSF GK-12 fellowship - provided stipend and tuition reimbursement during CALFED fellowship funding suspension.

Sacramento Sanitation District - provided supply bridge funding during funding suspension.

AWARDS: List any special awards or honors that you, or mentor or members of the research team, have received during the duration of this project.

Awards_16

NorCal Society for Environmental Toxicology & Chemistry - 1st Place Student Platform Presentation (2010)
UC Davis Graduate Student Association Travel Grant (2010)
University of California, Davis Research Scholarship (2009-2010)
NorCal Society for Environmental Toxicology & Chemistry - 1st Place Student Poster Award (2008)
Society for Environmental Toxicology & Chemistry Student Travel Award (2007)
Phi Sigma, Graduate Honors Society (2007 - present)

KEYWORDS: List keywords that will be useful in indexing your project.

Keywords_17

endocrine disruption, androgen, estrogen, multiple scales, population level effects, choriogenin, gene expression, pyrethroids

PATENTS: List any patents associated with your project.

Patents_18

not applicable

Additions: Additional information can be added here. Please begin the text with the number of the question you are adding to.

Additions_19

Project Objectives_10

Receptor level: Future Work
Future experiments will include additional exposures of both cell lines to site extracts to confirm initial results and to improve statistical power. A dose-response curve will also be generated for extracts with a dilution series. This will allow us to determine the environmental relevance of these responses and to make linkages to a demographic model with increased predictive ability.

Organism Level – Future work

Additional monthly sampling will be performed from March - October 2010 to confirm 2009 results and to increase sample size. The availability of a more specific antibody will allow the development of an enzyme-linked immunosorbent assay in early 2010 (ELISA - methods per Palumbo et al. 2008) to allow higher through-put analysis of plasma samples from both monthly sampling and outplants. Using an ELISA will also increase the statistical power of our immunoassays as more samples can be directly compared to one another. IIn addition, we have identified at least 6 endocrine-related genes in the silverside, including the receptors for estrogens, androgens, and thyroid hormone. We will compare gene expression in wild fish caught at different sites within Suisun Marsh to laboratory held controls. Finally, the potential to have histological slides of testes and ovaries from sampled fish prepared by an outside lab facility would allow us to further clarify potential connections between the receptor, organismal and population scales by permitting the quantification of gonadal malformations and/or ovo-testes (intersex fish).

Population Level - Future work
Further sampling will take place from March – October 2010, in parallel with organism level assays and water collection for receptor binding assays. The potential to have otoliths dissection and aging provided by a UC Davis laboratory would greatly enhance the population level assessment by providing information on age at maturity, which has been show to be affected by EDCs in other fish populations (McMaster et al. 1991). Having otoliths data will also allow us to make stronger links between the three scales, and will contribute an essential parameter to the population model we plan to construct.