Selection Panel Review Summary

Proposal No.: 023
Proposal Title: A Systems Biology Assessment of EDCs in the Delta
Principal Investigator: Richard E. Connon
Amount Requested: $486,411
Recommended Amount: $486,411

Summary: The project will assess the genomic and proteomic responses of *Menidia beryllina* as a surrogate for Delta smelt after exposure to pyrethroid pesticides (represented by bifenthrin) and pharmaceuticals (represented by ibuprofen) and effluent from three wastewater treatment plants in the Suisun Bay area. In addition, estrogenic and anti-estrogenic activity will be assessed in these five sample types. Reproductive behavior will be assessed after exposure to bifenthrin and ibuprofen. The proposal goals are to develop monitoring tools that can be applied to assess site-specific reproductive fitness of wild populations in the Bay-Delta System.

Assessment: The Selection Panel felt this proposal would be important to improve our understanding of the impacts of the contaminants addressed in the study. It also would develop useful tools. The project is comprised of a research team that has relevant experience to conduct such a study. The primary question about this proposal was how the three waste water treatment facilities were chosen for the study. The Selection Panel found that the applicants need to justify their treatment plant selection process and that they should consider looking at tertiary treatment facilities. Additionally, Panel members wondered why the concentration of contaminants in the effluent were not being measured as it would help interpret organism response, which would have increased the power of the work.

CALFED Ecosystem Restoration Program

External Scientific Review Form

Proposal Number: 023

Proposal Title: A Systems Biology Assessment of EDCs in the Delta

Reviewer: #1

Conflict of Interest Statements:

I have no financial interest in this proposal (please mark correct response).

- Correct XX
- Incorrect

General Review Questions:

Along with your written observations in response to the questions below, please rate each using the following criteria:

Excellent: Outstanding in all respects
Very Good: High quality in nearly all aspects
Good: Quality work, but with some deficiencies
Fair: Lacking in one or more critical aspects
Poor: Serious deficiencies

1. **Problem/Goals.** Is the problem that the project is designed to address adequately described? Are the goals, objectives, and hypotheses clearly stated and internally consistent? Does the proposal describe the ecosystem goals it is designed to address (link to ERP goals)?

   Comments:

The proposal seeks to develop a microarray for Menidia and use this array to investigate the effects of bifenthrin and ibuprofen on gene expression and attempt to correlate the findings with other reproductive endpoints and cell based assays. While the applicants provide some information suggesting that bifenthrin and ibuprofen have the potential to act as endocrine disruptors, there is very little information on concentrations found in the delta or at test sites specifically that provide sound justification for the proposed studies.

The goal of developing the tools required to utilize a systems biology approach for assessing endocrine disruption in the delta is within the scope of the ERP goals. The applicants state that the research will reduce uncertainty by developing a strategy that combines data from multiple levels of organization and will be focused on restoration and monitoring. It is unlikely that the information developed in this proposal will immediately reduce uncertainty associated with assessing risks from EDCs, but may develop a framework that can reduce uncertainty in the future.

The goals and objectives and clearly stated and consistent. However, the hypotheses are somewhat nebulous (e.g. … bifenthrin and ibuprofen will have either or both estrogenic and anti-estrogenic activity…) and do not seem fully developed.

Rating: **Good**

2. **Approach.** Does the proposal clearly describe its approach (including study design and methods, if appropriate)? Is the approach well designed and appropriate for meeting the objectives of the project as described in the proposal? Will the proposal contribute to our knowledge base?

Comments:

There are several facets of the proposed research including: 1) microarray development and use; 2) in vivo testing; and 3) cell based reporter assays.

1) Microarray development and use- The general approach to development of the M. beryllina microarray is to sequence an mRNA library and use sequences to design probes to populate an array. While this approach is fairly standard, the proposal lacks details regarding what tissues/life stages will be used for RNA isolation, how the libraries will be prepared and normalized, How much sequencing will be performed (number of libraries and plates), how allelic variation arising from use of multiple individuals will be handled, what parameters will be used to assess quality of annotation, whether strand orientation (sense vs. antisense) will be determined in sequence data and how this information will be used in subsequent array design and what Agilent platform (4 x 44k vs 8 x 15k) will be utilized. These are very important issues that must be appropriately dealt with in order to ensure project success.

Overall, the approach described for isolation, labeling and hybridization of material on microarrays is fairly standard. However, the isolation of RNA from whole fish homogenates is a suboptimal approach as many of the transcripts of interest are tissue and cell type specific. Use of whole fish homogenate will markedly reduce ability to interpret results. Applicants appear to be using a two color reference design on the arrays, but the composition of the reference sample is not specified (e.g. is it control fish or a pooled reference composed of material from all groups). The analysis of gene expression is described but no methodology for identifying potential biomarkers is provided which is a stated deliverable. Similarly, it is unclear how many genes will be validated by qPCR or how these will be selected.

2) In vivo testing- Applicants will conduct 14 day static renewal bioassays with ibuprofen, bifenthrin, and water from three sewage treatment effluent outfalls. These experiments are well described, though the range of concentrations of ibuprofen and bifenthrin are not specified. Ideally there would be overlap of the concentrations in the tests with those likely to be found in the sewage outfalls. Prior experiments with ibuprofen have used very high concentrations (mg/L) that are unlikely to have environmental relevance.

Sewage effluent will be collected from three sites. These sites appear to have been selected based on the plant volume, though the rationale for this is not clear. Is it likely that they will have different concentrations of the compounds under investigation?

Behavioral response testing will be conducted with group spawning experiments. The applicants address the issue of not being able to determine gender ratios prior to initiation of the experiments. It is somewhat unclear how this will be dealt with for experiments utilizing contaminants, specifically at the level of statistical analysis. These experiments will be conducted by Dr. White in North Carolina. It is unclear why no behavioral testing is being conducted on sewage effluent samples.

3) Cell based reporter assays- These are standard experiments and the approach is likely to yield results. There is some concern that the results will not be entirely reflective of in vivo testing as there are likely to be a variety of metabolites produced by the fish that will not be tested with these procedures.

Overall, the approach should yield some dose-repsonse information on the ability of ibuprofen and bifenthrin to impair reproduction. However, the ability to ascribe any observed effects of these compounds with effects produced by sewage effluent will be limited.

Rating: Fair

3. **Feasibility.** Is the proposed project's approach fully documented and technically feasible? Can the project be completed within reasonably foreseeable constraints (e.g., acquiring permits, construction, weather, etc…)? Does the proposal thoroughly address requirements such as environmental compliance and permitting? Is the scale of the project consistent with the objectives?

Comments:

The project is quite feasible, notwithstanding details described in item 2, and is likely to be completed within the timeframe proposed. There are no construction or permitting requirements as the proposal utilizes a surrogate species.

Rating: Very good

4. **Conceptual Model.** Does the proposal provide a conceptual model that describes the interconnections among the key ecosystem components relevant to the action(s) being proposed? Does the conceptual model clearly explain the hypotheses it is testing?

Comments:

The proposal puts forward a conceptual model based on the principles of systems biology. This approach does identify some of the important ecosystem components relevant to the proposed research, but appears to ignore some important aspects of population ecology, specifically consideration of factors that control the population. It would be very useful if the applicants discussed the population dynamics of Menidia relative to the expected findings. For instance, what level of reduction in fecundity would be required to produce a substantial change in populations? This may be dealt with in the related project on mathematical modeling but would be useful to reiterate in the context of the present study.

Rating: Good

5. **Performance Evaluation Plan (Monitoring Plan and Performance Measures).** Does the proposal include a plan for project performance evaluation (monitoring to assess results and evaluate assumptions and hypotheses)? Does the project include appropriate performance measures to measure success relative to the project's goals and objectives? Will future studies or restoration projects be able to incorporate the information from this project?

Comments:

The proposal provides description of deliverables and expected quantitative results, but does not specifically issues of quality assurance or how project will measure success relative to goals. It is possible that future studies will be able to incorporate tools and data developed by this project.

Rating: Fair

6. **Expected Products/Outcomes.** Are products of value likely from the project? Are products of value also likely from the individual components of the project? Will the results of this study be readily accessible?

Comments:

The major product of value that will arise from this proposal is the development of the M. beryllina microarray. The sequence information and array data will be made available. It also appears that the array would be usable by other researchers. Some mention is made of using this array on other Menidia species. Cross species hybridization has been done and can be successful, but accurate interpretation of results requires substantial validation.

There is some question regarding the impact of a M. beryllina array on overall delta restoration research. This researcher has previously developed an array for the endangered delta smelt. It is possible that having an array for a surrogate species could enhance overall understanding of EDCs on delta smelt. However, it would appear that much analysis could be performed directly on delta smelt without further development of M beryllina arrays.

The studies should provide some dose-response information for ibuprofen and bifenthrin reproductive effects that may be useful in evaluating potential impact of these compounds in delta waters. The systems biology approach has demonstrated utility in other species and it is likely that identification of biomarkers at various levels of biological organization will be of value to decision makers in the future.

Rating: Good

7. **Previous Related Work.** Does the proposed project continue past work or include any work that could be considered a duplication of work previously done or currently being done by others?

Comments:

This work extends previous studies conducted on these compounds in other species but is not duplicative on any studies to the knowledge of this reviewer. As indicated in item 6, a

microarray for delta smelt has been developed by this researcher, but this array will not work for M. beryllina.

Rating: Very good

8. **Qualifications.** What is the track record of applicants in terms of past projects? Is the project team qualified to efficiently and effectively implement the proposed project? Do they have available the infrastructure and other aspects of support necessary to accomplish the project? Do they have working knowledge of California streams and rivers?

Comments:

The applicants and their collaborators have access to all facilities and equipment required to successfully complete the proposed research. Dr. Connon has previously developed and employed microarrays on similar species and has substantial experience with aquatic toxicology, including holding contracts to evaluate exposure and effects in delta smelt. His work on these projects has led to publication or pending publication of six manuscripts in 3-4 years. Ms. Brander has substantial experience with aquatic toxicology and has worked extensively with Menidia. Dr. Colbourne has substantial experience with genomic and transcriptomic sequencing.

Rating: Very good

9. **Cost/Benefit Comments.** Is the budget reasonable and adequate for the work proposed? If the budget is considered to be excessive or inadequate for the work proposed, please highlight areas of the budget that may be of concern.

Comments:

The operating expense budget is reasonable and well justified. The personnel budget seems excessive given the amount of work proposed. It is unclear that 50% of a post-doc time and 60% of an assistant project scientists time are required in addition to aquatic laboratory staff and student time given the scope of the proposed research.

Rating: Fair

Additional comments:

Applicants should take care with the widespread use of "genomic" assessment and sequencing as no genomic material is being analyzed in the proposal. Care should also be taken to maintain consistency in the proposal, as several places the applicants indicate that the array is being developed for delta smelt.

Overall Evaluation Summary Rating

In the space below, please provide an overall rating of the proposal using one of the following categories:

- **Superior:** Outstanding in all respects with superior technical and scientific value and no significant concerns. Expected to add substantial new thinking/concepts to our knowledge/understanding of the topic proposed.
- **Above Average:** A very good proposal with at least high technical and scientific value and no significant concerns. Will add solid basic knowledge/understanding of the topic proposed.
- **Adequate:** A reasonable proposal without serious technical deficiencies and at least adequate value scientifically. Will add some useful knowledge to the topic proposed.
- **Inadequate:** A technically deficient proposal and/or one with low value, serious impediments or concerns. Will not likely change our basic knowledge/understanding of the topic proposed.

Rating: Adequate

Please provide a brief explanation of your summary rating:

The proposal is likely to produce a usable microarray for Menidia as well as dose-response data for ibuprofen and bifenthrin on Menidia reproduction. However the technical issues identified above and the lack of clear link between test compounds and bay water quality or effects limit overall enthusiasm.

CALFED Ecosystem Restoration Program

External Scientific Review Form

Proposal Number: 023

Proposal Title: A Systems Biology Assessment of EDCs in the Delta

Reviewer: #2

Conflict of Interest Statements:

I have no financial interest in this proposal (please mark correct response).

- Correct

General Review Questions:

Along with your written observations in response to the questions below, please rate each using the following criteria:

Excellent: Outstanding in all respects
Very Good: High quality in nearly all aspects
Good: Quality work, but with some deficiencies
Fair: Lacking in one or more critical aspects
Poor: Serious deficiencies

1. **Problem/Goals.** Is the problem that the project is designed to address adequately described? Are the goals, objectives, and hypotheses clearly stated and internally consistent? Does the proposal describe the ecosystem goals it is designed to address (link to ERP goals)?

   Comments:

The project will develop molecular markers of endocrine disruption in silversides, link those molecular markers to altered behavior and reproductive output, and model those effects on population size and viability. Moreover, the project will generate tools for future assessment of the effects of contaminants on silversides by the scientific community at large and provide a mechanism linking cause and effect across levels of biological organization. The goals, objectives, and hypotheses are exceptionally clear and consistent in development.

   Rating: Excellent

2. **Approach.** Does the proposal clearly describe its approach (including study design and methods, if appropriate)? Is the approach well designed and appropriate for meeting the objectives of the project as described in the proposal? Will the proposal contribute to our knowledge base?

   Comments:

The approach is logical and well-designed. Methods are appropriate for meeting the objectives of the project. In particular, linking biomarkers of exposure (changes in mRNA expression) to effects on populations is important and something not commonly done in other projects and a particularly novel aspect of this proposal. The project will result in the development of a microarray for inland silversides, which may continued to be used in future investigations by the scientific community at large to evaluate the effects of EDC and non-EDC compounds and complex effluents on inland silversides, a potential surrogate species for the delta smelt. A couple of points for the proposal investigators to consider:

   1. The description of "Effluent water collection and exposure" for the 14-d bioassays indicates that enough water for the assays will be collected in the field and stored at 4 C for use. Though there are not many good studies of degradation rates of organic EDCs in the literature, I would be concerned that, although stored at 4 C, EDCs may degrade with time and not persist in the water sample. In the field, continuous inputs of fresh effluent into the Bay-Delta would maintain concentrations of EDCs that are readily degraded, especially under aerobic conditions. The PI may wish to consider a more frequent collection of water for use in the assay or in situ caging experiments, as have been done with fathead minnows in numerous locations in the eastern half of the United States.
   2. Though not explicitly stated in the methods that chemical analysis will be done on solutions used in the bioassays, there is $2000 requested for chemical analysis of ibuprofen and bifenthrin. This is appropriate as actual rather than nominal concentrations should be used in describing the dose-response relationship. Why are chemical concentrations of pharmaceutical and personal care products not also being measured in the effluents used in the silverside assays? This seems to me to be a critical link to evaluating the proteomic and genomic responses of effluent-treated fish to those treated with pure chemical compounds—especially with regards to the differences in the microarray "fingerprints".

3. One of the reasons stated in the proposal for the use of inland silversides is their robustness, i.e., "display a lower level of sensitivity to contaminant exposure than the delta smelt." The proposal intends that inland silversides be used as a surrogate for the endangered delta smelt. How will differences in sensitivity between these two species be resolved?

Rating: Very Good

3. **Feasibility.** Is the proposed project's approach fully documented and technically feasible? Can the project be completed within reasonably foreseeable constraints (e.g., acquiring permits, construction, weather, etc…)? Does the proposal thoroughly address requirements such as environmental compliance and permitting? Is the scale of the project consistent with the objectives?

Comments:

The approach is well documented and very feasible and there are not unreasonable obstacles that preclude success. Permits are not required as water samples are collected in public waters and cultured fish are being used for the bioassays. Project scale is appropriate for the objectives.

Rating: Excellent

4. **Conceptual Model.** Does the proposal provide a conceptual model that describes the interconnections among the key ecosystem components relevant to the action(s) being proposed? Does the conceptual model clearly explain the hypotheses it is testing?

Comments:

The conceptual model does a very good job of linking the various levels of biological organization being assessed in the assays and how the information from each assay is being used and inter-related.

Rating: Very Good

5. **Performance Evaluation Plan (Monitoring Plan and Performance Measures).** Does the proposal include a plan for project performance evaluation (monitoring to assess results and evaluate assumptions and hypotheses)? Does the project include appropriate performance measures to measure success relative to the project's goals and objectives? Will future studies or restoration projects be able to incorporate the information from this project?

Comments:

There are several intermediate products and endpoints in the project (e.g., DNA sequencing, microarray development, bioassays with pure chemical compounds, etc.). The development of the DNA sequence and microarray specific for inland silversides will be in the public domain for future researchers to use and help establish inland silversides as a focal species for use in toxicity tests assessing effects of chemicals and effluents on Delta fish species.

Rating: Very Good

6. **Expected Products/Outcomes.** Are products of value likely from the project? Are products of value also likely from the individual components of the project? Will the results of this study be readily accessible?

   Comments:

   Multiple products of value are likely from the project as a whole, as well as individual components. As mentioned previously, the development of the DNA sequence and microarray specific for inland silversides will be in the public domain for future researchers to use and help establish inland silversides as a focal species for use in toxicity tests assessing effects of chemicals and effluents on Delta fish species. Data on the potential endocrine disrupting effects of bifenthrin and ibuprofen and wastewater effluents will be examined on yeast, human cells, and the inland silversides. The consequences of endocrine disruption on fish populations will be linked to proteomic and genomic changes in the fish and provide a valuable biomarker of exposure and effects. Numerous refereed publications are anticipated.

   Rating: Excellent

7. **Previous Related Work.** Does the proposed project continue past work or include any work that could be considered a duplication of work previously done or currently being done by others?

   Comments:

   The project builds upon previous studies by the investigative team, but does not duplicate work previously done or, to my knowledge, currently being undertaken.

   Rating: Excellent

8. **Qualifications.** What is the track record of applicants in terms of past projects? Is the project team qualified to efficiently and effectively implement the proposed project? Do they have available the infrastructure and other aspects of support necessary to accomplish the project? Do they have working knowledge of California streams and rivers?

   Comments:

   The applicants are highly qualified for the projects and, though not extensive, have a solid record of accomplishments and collaboration in this area. They have a state-certified laboratory available for the project and the equipment, collaborators, and expertise necessary for successful completion of the project.

   Rating: Very Good

9. **Cost/Benefit Comments.** Is the budget reasonable and adequate for the work proposed? If the budget is considered to be excessive or inadequate for the work proposed, please highlight areas of the budget that may be of concern.

   Comments:

The budget is reasonable and costs are detailed. The products are tangible and development of the silversides array is of value to the larger scientific community.

Rating: Excellent

Additional comments:

None.

Overall Evaluation Summary Rating

In the space below, please provide an overall rating of the proposal using one of the following categories:

- **Superior:** Outstanding in all respects with superior technical and scientific value and no significant concerns. Expected to add substantial new thinking/concepts to our knowledge/understanding of the topic proposed.
- **Above Average:** A very good proposal with at least high technical and scientific value and no significant concerns. Will add solid basic knowledge/understanding of the topic proposed.
- **Adequate:** A reasonable proposal without serious technical deficiencies and at least adequate value scientifically. Will add some useful knowledge to the topic proposed.
- **Inadequate:** A technically deficient proposal and/or one with low value, serious impediments or concerns. Will not likely change our basic knowledge/understanding of the topic proposed.

Rating: Superior

Please provide a brief explanation of your summary rating:

In all most all aspects, this is an excellent proposal that addresses several Ecosystem Restoration Strategic Goals and Objectives. The objectives and hypotheses are logical and the experimental approach is well conceived with a high likelihood of success. Multiple products are likely and development of DNA sequence and microarray for inland silversides will be of value to future studies and investigators evaluating contaminant effects on Delta fish species.

CALFED Ecosystem Restoration Program

External Scientific Review Form

Proposal Number: 023

Proposal Title: A Systems Biology Assessment of EDCs in the Delta

Reviewer: #3

Conflict of Interest Statements:

I have no financial interest in this proposal (please mark correct response).

- Correct

General Review Questions:

Along with your written observations in response to the questions below, please rate each using the following criteria:

Excellent: Outstanding in all respects
Very Good: High quality in nearly all aspects
Good: Quality work, but with some deficiencies
Fair: Lacking in one or more critical aspects
Poor: Serious deficiencies

1. **Problem/Goals.** Is the problem that the project is designed to address adequately described? Are the goals, objectives, and hypotheses clearly stated and internally consistent? Does the proposal describe the ecosystem goals it is designed to address (link to ERP goals)?

   Comments:

   Goals of the project are to use a systems approach to investigate the impact of a couple of chemicals found in wastewater on fish populations in the Delta. The project also aims to develop assays that could rapidly investigate the potential endocrine disruption occurring due to these chemicals in wastewater and sediments.

   Rating: Very good

2. **Approach.** Does the proposal clearly describe its approach (including study design and methods, if appropriate)? Is the approach well designed and appropriate for meeting the objectives of the project as described in the proposal? Will the proposal contribute to our knowledge base?

   Comments:

   The approach seems somewhat appropriate to the goals in that it uses investigations The issues with the project are that the links between the population modeling and these molecular and proteomic measures are not clear. The proposal refers to another project but this proposal is not descriptive enough to determine how those other models will be used in conjunction with this project. The authors mention that a Yeast assay will be more useful in one case where the Calux in another but do not give a reason. Will the microarray focus on endocrine related pathways? Will the PI's develop pathways models to link with larger measures of fecundity, survival etc. The authors mention the issues with only testing single chemicals but by in large this project only investigates 2 chemicals so is this really an improvement? They are also doing some of the studies using effluent which would provide a broader spectrum but not all assays are being conducted on effluent so each level can be linked. Are these really the two major compounds where you suspect endocrine disruption? What about hormones and phytoestrogens? It would be nice to have a list of what you will measure in the effluent to tie to your reproductive results. Are array experiments being conducted on males or females? What is the design of the array experiment? It looks like it will be a two-color array since you mention there is no need for a dye

flip from your previous experiments but it is unclear what two types of samples will be hybridized together.

Rating: Good

3. **Feasibility.** Is the proposed project's approach fully documented and technically feasible? Can the project be completed within reasonably foreseeable constraints (e.g., acquiring permits, construction, weather, etc…)? Does the proposal thoroughly address requirements such as environmental compliance and permitting? Is the scale of the project consistent with the objectives?

Comments:

The individual components of the project seem entirely feasible and can be completed as proposed. The project is consistent with the objectives and the scale is appropriate but some of the links among the parts of the "system" are not described well enough.

Rating: Very good

4. **Conceptual Model.** Does the proposal provide a conceptual model that describes the interconnections among the key ecosystem components relevant to the action(s) being proposed? Does the conceptual model clearly explain the hypotheses it is testing?

Comments:

The conceptual model is an interesting one and linking genomic information to more population levels assessments is an important goal. This project could provide information on the endocrine disruption potential of wastewater to fish populations in the delta. The conceptual model does explain the hypotheses but the conceptual model needs to be further developed in order to fully capture what the investigators are proposing. How will the different elements fit into one model? How does the population information relate to the molecular information? How will the whole effluent data relate to the information on just these two compounds of interest when there may be hundreds of chemicals in wastewater that are influencing endocrine disruption. It would be nice to see a concrete plan for implementation of this project.

Rating: Good

5. **Performance Evaluation Plan (Monitoring Plan and Performance Measures).** Does the proposal include a plan for project performance evaluation (monitoring to assess results and evaluate assumptions and hypotheses)? Does the project include appropriate performance measures to measure success relative to the project's goals and objectives? Will future studies or restoration projects be able to incorporate the information from this project?

Comments:

Performance measures seem appropriate for the goals listed. Future studies can use this information from this project by using the different metrics (molecular, protein, behavior etc.) to determine impacted watersheds and so can be used to determine success of restoration efforts. It would be nice to see a metric that talks about the links among these different metrics and how

you would handle if one measure predicts the opposite of another or how to determine which metric is best etc. How you might use a combined metric for assessments.

Rating: Very good

6. **Expected Products/Outcomes.** Are products of value likely from the project? Are products of value also likely from the individual components of the project? Will the results of this study be readily accessible?

Comments:

Products will include a new microarray for a species that is useful for eurohaline systems important for California. In addition this project will evaluate other types of assays for their effectiveness in predicting reproductive issues. The results of this study will most likely be readily available through peer reviewed publications and the investigators have a good track record in informing managers as to the relevance of their research.

Rating: Very good

7. **Previous Related Work.** Does the proposed project continue past work or include any work that could be considered a duplication of work previously done or currently being done by others?

Comments:

The investigators on this project have each done previous work similar to that described in this project but this will not duplicate existing projects or previous projects. It will be novel in linking molecular work on a model fish species to measures of reproduction, behavior and other assays dealing with endocrine disruption.

Rating: Very good

8. **Qualifications.** What is the track record of applicants in terms of past projects? Is the project team qualified to efficiently and effectively implement the proposed project? Do they have available the infrastructure and other aspects of support necessary to accomplish the project? Do they have working knowledge of California streams and rivers?

Comments:

Investigators are highly qualified. Each is well published in the area important for this collaboration and have significant funding and research records. They each have available infrastructure and laboratory resources to complete their portions of the project. The lead PI has significant knowledge of California streams and rivers as evidenced by previously funded and published projects.

Rating: Excellent

9. **Cost/Benefit Comments.** Is the budget reasonable and adequate for the work proposed? If the budget is considered to be excessive or inadequate for the work proposed, please highlight areas of the budget that may be of concern.

Comments:

The budget proposed is reasonable for the work proposed and the number of parts and collaborators for this proposal.

Rating: Very good

Additional comments:

None.

Overall Evaluation Summary Rating

In the space below, please provide an overall rating of the proposal using one of the following categories:

- **Superior:** Outstanding in all respects with superior technical and scientific value and no significant concerns. Expected to add substantial new thinking/concepts to our knowledge/understanding of the topic proposed.
- **Above Average:** A very good proposal with at least high technical and scientific value and no significant concerns. Will add solid basic knowledge/understanding of the topic proposed.
- **Adequate:** A reasonable proposal without serious technical deficiencies and at least adequate value scientifically. Will add some useful knowledge to the topic proposed.
- **Inadequate:** A technically deficient proposal and/or one with low value, serious impediments or concerns. Will not likely change our basic knowledge/understanding of the topic proposed.

Rating: Above Average

Please provide a brief explanation of your summary rating:

This project will provide novel information on the genomic response of a potential indicator species for California for EDC research and restoration work. It will provide links among genomic markers of effect and exposure with reproduction, behavior and other EDC related tests. The couple of issues with the proposal are the lack of description as to how all the tests will be interpreted or compiled together to produce a metric and there are some issues of methodology (mentioned above) that are not clear including why just two chemicals were chosen and why these two chemicals in particular.