Selection Panel Review Summary

Proposal No.: 027
Proposal Title: DNA Barcoding and Quantitative PCR for Zooplankton Assessment
Principle Investigator: Swee Teh
Amount Requested: $868,417.00
Recommended Amount: $0

Summary: This proposed project lists four goals that fall into two main categories:

1) Develop new molecular tools, DNA barcoding and qPCR assays, for measuring zooplankton composition and abundance (or biomass), respectively. The tools would be developed for up to 50 species in each of three sampled Bay-Delta regions (Suisun Marsh, Cache Slough, lower San Joaquin River). These potentially rapid and accurate new methods could compliment the traditional microscopic identification and enumeration techniques that are currently employed by Bay-Delta zooplankton monitoring programs. They could potentially also identify “cryptic” or microscopically unidentifiable species and be used to analyze gut contents of zooplanktivores.

2) Use the newly developed tools to analyze zooplankton composition and abundance in the gut contents of larval delta smelt and threadfin shad and compare the gut contents to ambient zooplankton at the fish sampling sites.

Assessment: While the Selection Panel felt the proposal contained good ideas and had the potential to contribute to the knowledge base, the many technical difficulties outweighed any strengths. Deficiencies included the conceptual model did not link to hypotheses, the Project Team lacked a zooplankton expert, the proposal scope was unrealistic for a team that had not demonstrated a strong knowledge of the community they are addressing, and the proposal did not meet the priorities of the PSP.

CALFED Ecosystem Restoration Program

External Scientific Review Form

Proposal Number: 027

Proposal Title: DNA Barcoding and Quantitative PCR for zooplankton assessment

Reviewer: #1

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:

   This proposal has two scientific objectives. First, assessment of zooplankton species composition and abundance in the San Francisco Estuary using DNA barcoding and quantitative PCR. Second, gut content analysis of larval delta smelt and threadfin shad using quantitative PCR. What the applicants are trying to know with these objectives is very important ecological process in the system. Also the problem that the project is addressing is well described in the proposal. Goals and hypotheses are clearly stated along with their objectives.

   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:

In theory, their experimental design should work and give us good knowledge base. However, there are several factors, which affect performance of DNA barcoding and interpretation of results they are expecting to get from qPCR analyses. 1) Primer deficiency. Mitochondrial COI gene, which is one of the target genes of DNA barcoding, of many zooplankton animals cannot amplify by conventional primer. 2) Mitochondrial pseudogene. Mitochondrial pseudogenes are nuclear copies of mitochondrial DNA. Because of sequence similarities between true and pseudogenes, often times both DNA sequences will be amplified by PCR (Bensasson et al. 2001). Frequent occurrence of mitochondrial pseudogenes is reported in animals those with large nuclear genome size (Bensasson et al. 2000). One of the major planktonic group, Calanoida Copepods also tend to have large genome size, which advocate needs of careful mitochondrial analysis in those animals. 3) PCR inhibitor. Various kinds of chemicals are known to inhibit PCR reactions (http://en.wikipedia.org/wiki/Polymerase_chain_reaction_inhibitors). Because of these effects, they might underestimate target sequence copy number in template DNA solution. Effect of PCR inhibitor is especially strong in template those extracted from environment, such as water, soil, and gut content. 3) DNA digestion in gut. Digestion of prey DNA continues until gut is preserved completely. This is potential source of underestimation. 4) Net feeding. Animals (fish larvae in this study) those collected zooplankton net tend to intake any kinds of surrounding particles. This is source of overestimation or false positive.

Applicants keep saying that they will develop qPCR assays to quantify the "abundance" of key zooplankton species. However, quantification of abundance (counting of individual numbers) is impossible from homogenized and extracted DNA solution because of size difference (target gene sequence copy number difference) of individuals from egg to adult individuals. Maybe they can use the term biomass instead of abundance.

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:

I have two concerns about feasibility of this project. First, no one in the project has ever studied zooplankton. The applicants are planning to do DNA barcoding of zooplankton. Accurate species identification, which requires quite extensive training, is critical for the performance of DNA barcoding. For the accurate DNA barcoding, it is also important to keep voucher specimen for later morphological analyses. However, most of zooplanktons are small, which makes it difficult to keep voucher specimens. It was not clear for me that how the applicants will over come this problem. They mentioned that they would take photo images of all animals. But is that mean they are going to take photo of all possible morphological characters, which can be the candidate of species identification?

Second, they do not have any preliminary results. As I listed in previous section (2. Approach), there are always taxon and sample specific difficulties in genetic analysis. If applicants have already some experience, such as development of qPCR assays in task 2-2, it would be much easier to imagine that they have good feasibility to complete the project.

Rating: Fair: Lacking in one or more critical aspects

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 provides a good conceptual model that describes the interconnections among the key ecosystem components. The applicants are trying to assess zooplankton composition in San Francisco Estuary, and compare those data to gut content of two endangered planktivorous fish larvae, delta smelt (*Hypomesus transpacificus*) and threadfin shed (*Dorosoma petenense*) using molecular data. By performing these comparisons, they might identify the possible factors, which affect survival of those fish larvae.

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:

They are expecting quantitative results and other products (Page 19). If they can perform all that they have listed, then of course it will be great out come. But it is also depending on how they can over come the difficulties I have listed above (2. Approach).

Rating: 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:

There will be products of values from the project and the individual components of the project, only if they can overcome the difficulties, which I listed in the previous sections (2 Approach and 3 Feasibility).

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:

Many projects are trying to establish zooplankton species identification system using molecular biology technique. However, the proposal is unique because it is targeting zooplankton community of San Francisco Bay Delta Estuary.

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 have very good sampling capacity and also good genetic analysis facility (5. Feasibility). One of the applicants, Mr. Randall Baxter from the CA DFG has over 22 years of experience sampling fishes and invertebrates in the San Francisco Estuary. This is very promising.

   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:

   I think it is adequate.

   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: Adequate

CALFED Ecosystem Restoration Program
External Scientific Review Form

Proposal Number: 027

Proposal Title: DNA Barcoding and Quantitative PCR for zooplankton assessment

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:

This proposal seeks to employ genetic techniques to enhance the capabilities of the plankton monitoring in the San Francisco Estuary (SFE). This would purportedly increase the taxonomic resolution of the monitoring programs and provide a means for high throughput measurement of gut contents for zooplankton predators. Overall this is a strong idea and there have been many moves in this direction from academic researchers because of the high labor costs associated with traditional taxonomic analysis. However implementation of genetic tools for taxonomic analysis has lagged, making the idea somewhat novel and potentially useful.

My concerns are that the scope of the effort seems quite unrealistic given the potential diversity and proposed organismal levels the work will address, and the authors do not seem to have demonstrated a strong knowledge of the community they are addressing. Throughout the project description “microzooplankton” are noted and yet there does not seem to be a sampling program designed to sample them. This in and of itself is not a problem, and a more focused study on mesozooplankton, and perhaps even on copepods would have strengthened the hypotheses put forth here. If by microzooplankton the authors actually mean naupliar and small

stages of copepods or other crustacean zooplankton, then there is more support for their argument, but without defining the taxonomic groups under scrutiny in more detail it is impossible to determine the potential for success. The diversity of plankton communities is enormous, particularly when considering the potential for cryptic diversity as these authors describe, which seems to suggest that pilot programs and test beds for monitoring should begin with specific defined groups to enhance the potential for success.

Further, the inclusion of fish gut content work, while intellectually useful to provide context, is not well developed. There are many problems with these methods, particularly with contamination by fish and gut fauna, as well as the degradation rates of DNA in guts. Also, the quantification of DNA in metazoans has not been well addressed in the literature for these types of methods, and their plan to relate biomass to DNA is an interesting idea, though not well developed in this proposal.

These concerns aside, the hypothesis is fairly well developed and the proposed work is generally appropriate to address it. The only major benefit to the ERP goals appears to be increased taxonomic resolution for the monitoring, as well as a potentially higher throughput method. These are not trivial benefits, but their scope may be tempered by the ability to compare the data generated by these methods with the historical data that does not have the same resolution and uses other methods for monitoring.

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:

The described approach seems appropriate to address the hypotheses, though as noted above the target organisms should be more specifically defined. The inclusion of a PI who is intimately familiar with and participating in the monitoring is a major strength of the proposal, and though more specifics on the types of sampling and preservation (95% ethanol I assume?) would be welcomed. DNA, particularly for quantification, can be degraded rapidly with poor handling of field collected samples, and more information to evaluate those procedures would have been welcomed.

If successful, this work will greatly enhance monitoring efforts and allow a means to compare the zooplankton community with other estuaries. In addition the record of the DNA barcodes can provide the means for similar projects in other locations, or enhanced temporal or spatial resolution following this effort. Also, the ability to quantitatively address fish gut contents using DNA would be a large benefit from this project, but the methodological ideas are not well developed here. There are a number of authors who have been working on these methods with limited or varied success, mainly due to issues of contamination and degradation of prey DNA. These concerns were not well addressed in the proposal.

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:

Overall the project is technically feasible in that they will be drawing samples from an existing monitoring program with technicians who have experience doing this. Further, the PIs have experience with molecular methods in general, so the tools appear to be available for the work.

My main concern about feasibility is the previously described lack of specificity about the zooplankton community, and the fact that these methods are not entirely straightforward and I imagine a great deal of method development will have to be performed before actual useable data are generated. This is particularly true for the fish gut contents, as some means of blocking the predator DNA in the PCR process will have to be developed and perfected for the particular species under scrutiny. However, the level of staffing and budget seem appropriate.

Rating: **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 provided conceptual model generally supports the hypotheses and is certainly adequate and appropriate. It could have been improved by including two possible scenarios – one as described, and one with differences between the gut contents and the prey community to show how the gut content work will enhance the current knowledge. For example, if the DNA barcoding were to uncover cryptic species complex within one of the target species, perhaps the fish prey selectivity would be reflected in only one of the cryptic species is actually preyed upon. This is more a suggestion than a criticism.

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:

Performance monitoring for this project will be difficult but not impossible. The close ties between the monitoring agency, who will collect the samples, and the PIs who will oversee the DNA barcoding suggests that performance evaluations are likely to be ongoing and useful to the project. Measures of success will be determined as the methods are developed and tested. If it is successful, information from this project has the potential to be incorporated into various monitoring and process oriented studies in the region, and could expand scope of the monitoring programs already in place.

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:

Major products from a successful program such as that described here are likely to be in the form of DNA barcodes of key species in the SFE, defined methods to use those barcodes for taxonomic analysis, DNA: biomass information for key zooplankton species, and gut content data linked to molecular analysis. All of these products will be accessible to researchers through various databases, including online DNA databases and the CA DFG monitoring programs. Even if not all of the project is successful, and the component least likely to be successful is the gut content quantification, there should be DNA barcode data for key plankton species available, as well as some quantification of the DNA:biomass ratios.

Because the gut content work is a key component of the proposal, this is somewhat of a concern. DNA barcodes are a useful product, but the proposal emphasizes gut contents, and my feeling is that there is a low probability of success with that component.

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 is certainly not a duplication of previous work; rather it represents an enhancement to ongoing monitoring programs in the region. Some of the methods have been tried or used in the past, but not in the same context or the same region.

My concern regarding related work is that the proposal presented few references on the efficacy of the major work proposed. If other researchers had not been successful using DNA to look at fish gut contents, how will the current work improve on it? If it has been successful before, how has it been used and in what systems? The same questions could be said of other parts of the proposal in particular determining the relationship between DNA and biomass.

Rating: **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:

Overall the qualifications of the team seem appropriate for this project. The skill set of the team spans all aspects of the proposed work from collection and taxonomic analysis to molecular techniques. Some of the concerns addressed in previous sections of this review relate to the specific techniques that are proposed, and they are likely to be surmounted by this team because

of their experience, however the molecular team does not seem to have direct experience with the techniques described or for the mesozooplankton targeted. Overall the assembled team seems quite capable.

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 seems reasonable for this project. The proposed work will require a great deal of labor for collection and method development, and inclusion of a graduate student on this work is a good idea. In addition, the oversight by senior PIs and direct involvement by a post-doc are positive aspects of the proposal.

If it is successful, the cost/benefit ratio is likely to be quite high, though it seems there is some risk to the project. However, if the methods can be developed to make taxonomic analysis easier, faster, and of higher resolution the risk/reward potential for funding this proposal could be quite high.

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: **Adequate**

Please provide a brief explanation of your summary rating:

This proposal addresses an important aspect of plankton community composition analysis – how to integrate molecular methods into monitoring programs. The overall makeup of the team is strong and the linkages with the monitoring agency directly (through inclusion on the PI team) are positive aspects of this proposal. My major concerns are that there seems to be less documented understanding of the actual biology and ecology of the target organisms. Some of this is necessary for a proposal like this that is methodologically driven, but it raises some concerns about the potential for success. It seems a more appropriate way to tackle the same problem would be on a smaller scale, with a specifically defined set of organisms that are relevant to the community and as prey for the target fish species.

CALFED Ecosystem Restoration Program
External Scientific Review Form

Proposal Number: 027

Proposal Title: DNA barcoding and quantitative PCR for zooplankton assessment

Reviewer: #3

Conflict of Interest Statements:

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

✓ Correct
- 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 provided excellent background and rationale for its proposed project. It clearly stated its goals, objectives, and hypotheses, and specified the ecosystem goals it is designated to address.

However, I feel odd reading the proposal. The goals, objectives and hypotheses are not formulated and organized in a conventional way. The listed four goals of the study (Page 4) are four activities proposed to complete, rather than what to achieve. The hypotheses (Page 6) are not logically set up for attaining the goals. It makes more sense to let testing those hypothesized statements be the goals, and four proposed activities as the approach to complete the hypothesis-testing.

Given the activity goals, the project does not fall into the category of hypothesis-testing research. Five hypotheses were listed as very general statements with no specific information elsewhere on how to test them. I also think the 2nd hypothesis is a questionable statement.

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 proposal clearly described its study design, methods, and time schedules. In general the approach is appropriate for attaining the goals of this project. If successfully implemented, the proposal will contribute to our knowledge base.

However, there are some intrinsic issues in DNA barcoding and its application, which I think should be addressed in the proposal and dealt with in designed approach.

1) Task 2-1: The power of DNA barcoding hinges on the goodness of taxonomy for known species and its concordance with molecular phylogeny. In reality, the poor performance of taxonomic classification and strong population genetic structuring often causes discordant patterns between the taxonomy and the molecular phylogeny. This has a profound effect on DNA barcoding and its application. Although the proposal has brought up the cryptic species issue, it did not fully consider the effect and provide a strategy to deal with it.

2) Task 2-1: The proposal has inconsistent statements about its study organisms. In its 1st goals (Page 4), it specifies the "micro- and meso-zooplankton"; in Expected Results (Page 19), it states "genetic database…… of macro and micro zooplankton species in SFE". In Table 1, it includes all the zooplankton species (micro, meso, and macro). A useful zooplankton ID system should cover as many taxa as possible, if not compete.

2) Task 2-3: I do not see how the approach to the validation of molecular tools works. Comparing species composition and abundance determined by molecular tools and morphological methods will not falsify the molecular tools. The validation will only occur when the two dataset are correlated to each other, but many reasons may cause no correlations between them.

3) Task 2-2 & 2-4: Zooplankton wet weight is a very coarse measure of biomass. It might be a good idea to obtain the ratio of qPCR results and body wet weights for different species. The ratios (or equations) is very likely species-specific. I doubt there exists a universal one for

diverse taxa. It is not appropriate to me to use a ratio from *E. affinis* for other zooplankton in Task 2-4, unless preliminary work shows a good support.

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 proposal fully documented a technically feasible approach for the project. I believe the project can be completed within any foreseeable constraints. The proposal thoroughly addressed all the requirements for completing this project. The scale of the project is consistent with 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 proposal provided a conceptual model showing the relationships between zooplankton and the environmental factors. Since the main goals of the project are develop molecular tools, rather than test scientific hypotheses, this conceptual model is not critically needed. I do not see how this model would help explaining any hypotheses to test in this proposal. The model has an identical pie chart for both zooplankton and fish gut content, suggesting that the fish food composition may reflect the ambient zooplankton composition. However, there is no such a hypothesis addressed in the text.

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:

I did not see a section addressing a performance evaluation plan. The proposal has a list of expected products and outcomes that can be used for performance evaluation but few specific quantitative measures were included in this proposal. The applicants claimed that quantitative measures are not applicable for this research and monitoring project (Line 8, Page 19). I do not fully agree. Predictable measures could be made for each goal. For example, the success of

attaining the 1st goal could be measured with an established ID system covering > 90% of zooplankton species in SFE, while a failure with a system covering only 10%.

Rating: 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:

The proposal listed 6 expected outcomes and 3 extra products (Page 19). Among them, the most valuable product is a DNA identification system for San Francisco Estuary, including all known zooplankton species, their morphological characterization, and DNA barcodes. Standard protocols for monitoring zooplankton species composition and abundance using DNA-barcoding and qPCR will be established. All the results will be readily accessible and valuable for future ecological research and management.

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 proposed DNA barcoding and qPCR assessment for zooplankton is new for San Francisco Estuary, and also among the very few ongoing attempts worldwide. Based on the scope of the research project, it is not a duplication of any work.

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:

All the applicants listed in the proposal have good tracking record for scientific research. All the required facilities, equipments, and other necessary support are available for them to accomplish the project. From their working experience, I believe the project team has sufficient working knowledge of California streams and rivers.

The project team is very strong in molecular biology and field sampling, but less strong in zooplankton ecology. As the project is focused on zooplankton, it would be more qualified to recruit a zooplankton specialist with expertise on zooplankton taxonomy and ecology.

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 adequate for the proposed project.

   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: Above Average

Please provide a brief explanation of your summary rating:

This proposal has wonderful ideas, reasonable approaches, and strong work forces for project implementation. The project, if successfully implemented, will not only contribute to our knowledge base, but also bring a revolutionary tool to our monitoring and management of marine (pelagic) system.

Meanwhile, the research topic (DNA barcoding and qPCR assay) also has intrinsic uncertainties, especially for the zooplankton, which are not addressed in sufficient details. Due to lack of this information and quantifiable measures for the goals, I am not fully confident in the quality of expected products/results.

In general, I think it is a good proposal worthy of funding.