Responses to Review Comments about Draft Report (dated 20 March 2009): "A Framework for Research Addressing the Role of Ammonia/Ammonium in the Sacramento-San Joaquin Delta and the San Francisco Bay Estuary Ecosystem"

Responses prepared by the report authors:
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PREFACE

As the Expert Panel for the Bay-Delta ammonia workshop, our task was to recommend a framework for research addressing the role of ammonia/ammonium in the Sacramento-San Joaquin Delta and the San Francisco Bay Estuary Ecosystem. Given the time and budgetary constraints on our effort, we have provided a general outline of the types of research that we believe will (1) help elucidate the causes of the pelagic organism decline (POD) and major food web changes in the Bay-Delta ecosystem and (2) support future management decisions. We thank the numerous reviewers who carefully read our draft report and spent considerable time preparing thoughtful comments and questions.

In response to the draft report, some reviewers requested more specific details about study designs or a prioritization of the recommended research topics. We do not believe our task is to provide detailed study designs, because many researchers and other stakeholders in the Bay-Delta region know this system and the vast body of previous and current research better than we do. Therefore, it will be preferable to have the details decided by those who know the system best.

Additionally, detailed prioritization of research projects should be an iterative process that takes into account available resources (personnel, time, and money) in the context of current knowledge and understanding of a system. That is why an integrative analysis of available information and a resulting overarching model of the Bay-Delta ecosystem are important, to help guide the prioritization of additional research. To respond to reviewer requests, we have added overall recommendations for research priorities to our report. However, we leave detailed prioritization to those more knowledgeable of the pertinent constraints (e.g., staff of the CALFED Science Program, in consultation with stakeholders).

REVIEW COMMENTS AND THE PANEL'S RESPONSES

Reviewer: Dr. Thomas R. Mongan, P.E., Consultant to the San Luis and Delta-Mendota Water Authority

Before spending money on "an overarching integrative model" (page 6), there should be focused research to answer three basic questions:

1. What fraction of the ammonia measured in Suisun Bay and the lower Sacramento River comes from the Sacramento wastewater treatment plant?

2 Are ambient ammonia levels measured at Hood (and in Suisun Bay) toxic to Eurytemora affinis, a key zooplankton food of juvenile delta smelt?

3. Are ambient ammonia levels at Hood (and in Suisun Bay) toxic to early larval stage delta smelt? In this connection, Finlayson claims to have done testing with 9 to 14 day old delta smelt.

Response: We do not agree that a decision to develop an overarching integrative model should be contingent on the outcome of the three questions posed above. Without an overarching integrative model, we do not believe the role of ammonia/um in the POD declines and in the food web changes can validly be placed in perspective, relative to all the other stressors in the Bay-Delta ecosystem. Additionally, even if ammonia/um could magically be removed from these waters, an overarching integrative model would be an important tool for analyzing, understanding, and managing the effects of other current and potential future stressors. Therefore, the overarching integrative model should be developed concurrently with research to address the highest priority questions concerning ammonia/um in the Bay-Delta region.

We basically agree with Question 1, and we believe that question is implicit in the central question that we posed in the first sentence under Research Topic 2.

We agree that Question 2 might be important. To address that question, toxicity data would be needed for *Eurytemora affinis* and other major prey items of POD species. Therefore, we have added a paragraph to Research Topic 11 (now Topic 10), mentioning that acute and chronic toxicity tests could also be conducted with major prey items of the POD species (e.g., *Eurytemora affinis*, *Neomysis mercedis*), although invertebrates tend to be less sensitive to ammonia than fish.

The toxicity tests we have already recommended in Research Topic 11 (now Topic 10) are intended to provide data to help answer Question 3. We have added a paragraph under Research Topic 11 (now Topic 10), indicating that those toxicity results should be compared to ambient ammonia concentrations reported downstream of major N dischargers and in other locations throughout the Bay-Delta region. However, we were not able to locate any reports by Finlayson about ammonia toxicity tests conducted with delta smelt; the only Finlayson report we could locate about delta smelt only deals with herbicides (Riley, F. and S. Finlayson. 2004. Acute Toxicities of Herbicides Used to Control Water Hyacinth and Brazilian Elodea on Larval Delta Smelt and Sacramento Splittail. California Department of Fish and Game, Elk Grove, CA.). Therefore, we have not mentioned those tests in the first paragraph in Research Topic 11 (now Topic 10).

Finally, the POD involves only JUVENILE striped bass (page 2).
Response: We believe that distinction is not necessary for the generalized statement we made in that sentence.

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Reviewer: Alex Parker

Overall, I would emphasize that much of what we know about phytoplankton primary production rates in the SFE are based on light-biomass models (Cole and Cloern, 1984, Jassby et al 2002), and are driven entirely by biomass and light attenuation – these models overlook the potential for physiological changes in phytoplankton (or phytoplankton community shifts) which have likely occurred since the original empirical relationships were developed. I know of two theses (Lorenzi 2006, Lidstrom 2009) that measured 14C primary production in Central SFE and in the western delta / Suisun Bay and found that the Cole and Cloern model overestimate primary production by a factor of ~2. This indicates that declining primary production rates are not only driven by decreased biomass due to grazing.
Response: This and other arguments presented by other reviewers with different perspectives demonstrate that considerable uncertainty still exits about the factors driving phytoplankton composition, productivity, and biomass in the SFE. That is why we recommended additional research along several general lines, as well as the need for an integrative analysis of the numerous competing hypotheses and available data.

My main comment relates to Research topics 9
Inhibition versus preferential uptake of NH_4.

I think that there are important distinctions between the use of the words "preference" and "inhibition" in the relationship between NH_4 and NO_3 use. Preference of NH_4, as suggested by the McCarthy's RPI, relates N substrate uptake to N substrate concentration and total N concentration, and is therefore biased by relatively low ambient NH_4 concentrations. The NH_4 "preference" argument is based on the increased energetic requirements for NO_3 transport/uptake compared to NH_4 and implies that there should be enhanced phytoplankton growth on NH4 compared to NO_3. I am not aware of experimental results to support this (Thompson et al 1989 that showed no evidence for enhanced growth on NH_4 or reduced growth on NO_3 – except maybe at very low light).

The "Inhibition" argument is based on the inhibition of NO_3 reductase activity by NH_4 which has been shown in several studies (see Falkowski review in Nitrogen in the Sea, 1983 (and references therein) and Mulholland and Lomas review in updated Nitrogen in the Sea 2008). Figure 1 of Falkowski shows NH_4 inhibition of NO_3 reductase. NH_4 is described as "repressing" NO_3 reductase activity. More recently, Song and Ward (2004) showed that NO_3 reductase activity is inhibited by NH_4. This suggests that NH_4 use over NO_3 use is not due to "preference" or energetics but it because the enzymes required for NO_3 uptake are shut down by the presence of NH_4.
Response: We do not dispute the general applicability of the NH_4 inhibition mechanism in marine phytoplankton; and it is plausible that the same mechanism occurs in the phytoplankton

community in Suisun Bay. However, based on the information available to us, conclusive evidence has not yet been provided. Therefore, we believe the proposed inhibition mechanism remains a hypothesis that needs to be tested against alternative, competing hypotheses that apply to *in situ* conditions [e.g., the preference (based on uptake energetics) mechanism, grazing, flushing].

Also in Research topic 9 – the question about whether there is a change in productivity, C:N uptake ratios and growth per unit N uptake on NH_4 and NO_3 is, to me, the right questions to be asked. The Dugdale et al 2007 paper shows pretty clearly that VN on NO_3 (after NH_4 inhibition is overcome) is higher than VN for NH_4 for the SFE phytoplankton community. This is likely due to the induction of NO_3 reductase by the presence of NO_3 (Song and Ward, 2004, Dugdale et al 1991 "Shift-up"). The question of whether the higher NO_3 uptake that is observed is coupled with higher VC (or uncoupled as reported by Lomas and Glibert, 1999) has not been published yet, but we in fact see that C uptake follows elevated VNO_3 (i.e. higher primary production rates during NO_3 supported growth) as phytoplankton balance C and N growth.

Response: Based on the information available to us, we concluded that this question has not yet been adequately resolved. The information alluded to in the reviewer's comments should be taken into account in the integrative analysis we recommended; and, if substantiated, this process should be included in the overarching model of the Bay-Delta ecosystem. However, we do not believe it is necessary to revise our report to address this point.

Research Topic 10

I agree that clam grazing rates need to be better constrained during the POD years. I am a little confused about when clams are actually present in Suisun Bay and whether grazing impacts are likely during the spring.

Response: We believe this uncertainty supports our recommendation for an integrative analysis of available information about the Bay-Delta ecosystem.

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Reviewer: Michele M. Pla, Executive Director, Bay Area Clean Water Agencies

We are pleased to have the opportunity to offer our comments on the Draft Report entitled, "A framework for research addressing the role of ammonia/ammonium in the Sacramento-San Joaquin Delta and the San Francisco Bay Estuary Ecosystem," prepared by Drs. Meyer, Mullholland, Paerl, and Ward (the Panel). The report offers a succinct summary of the issues that the San Francisco Bay Estuary ecosystem is facing, as well as offering a well thought out research program to begin to understand the role that ammonia/ ammonium nitrogen may play in determining changes in primary production, phytoplankton species composition, and food web effects. The proposed research program is comprehensive and multi-faceted. The Panel strongly encourages CALFED to build the research program on existing field monitoring programs, existing research and existing tools such as the DSM-2 hydrodynamic model and we strongly encourage this.

We also offer the following comments on specific Research Topics identified by the authors in their report.

Research Topic 1:
The development of a comprehensive modeling framework that includes site specific information in order to determine species composition and grazing effects is dependent on other proposed Research Topics, specifically Topics 4 through 10. Would the Panel comment on whether and how the modeling effort should be staged, i.e., What modeling work could proceed while others Research Topics are being conducted? Would it make sense to utilize the particle tracking component of DSM-2 to address residence time issues for the spring algal bloom in Suisun Bay and perhaps the potential for using freshwater flow management strategies to determine if *Microcystis* could be flushed from the Delta system?

Response: The integrative analysis and subsequent development of an overarching model should be initiated as soon as possible and receive high funding priority, as we recommend in the "Research Prioritization" subsection we have added to the "Major Research Needs" section. Models are not constructed, tested, and refined overnight; and often they must first walk before they can run. A hydrologic model like DSM-2 could provide the basis for an initial effort, onto which other components (e.g., nutrient loading and transformations, phytoplankton growth) could subsequently be added. Although the details of such modeling are best left to experts in the Bay-Delta region, the use of the particle-tracking component of DSM-2 recommended by the reviewer makes sense as an early modeling effort.

The Panel did not mention including aquatic macrophytes in the modeling framework, perhaps recognizing the complexity and difficulty of performing such a task. Would the Panel offer any comments about how the impact of aquatic macrophytes on nutrient dynamics be parameterized in the modeling framework?

Response: At the current level of knowledge about the effects of aquatic macrophytes on nutrient dynamics, detailed modeling does not appear to be warranted. Instead, at best, a simple black-box model with generic first-order uptake and release rates would probably suffice. However, the major impediment would be finding and/or generating adequate data to parameterize such a model. This component of an overarching model might have to remain mostly qualitative until the perceived importance of aquatic macrophytes in the POD and food web dynamics (or in other concerns that might emerge in the future) justifies the additional research that will be needed to parameterize such a submodel.

Research Topic 2:
The panel offers three approaches to fill data gaps concerning sources and fate of nitrogen and phosphorus in the Delta. Recognizing potential budget limitations of conducting more than one of these approaches would the Panel comment on the prioritization of the approaches.

Response: Because Research Topic 2 is central to determining whether N discharge from the SacWWTP is contributing a to the POD and/or food web changes, all of the research aspects we mentioned will be needed to help answer that question. Therefore, in the face of resource constraints, it would be better to limit the extent of the analyses (e.g., a less extensive survey of N sources) than to eliminate an aspect of the recommended research.

Research Topic 3:
Would the panel offer any comments on how changes in aquatic macrophytes might result in changes in habitat for POD species and whether any additional research should be conducted in conjunction with this Research Topic?

Response: This is a good question. Therefore, we have added it as a new research topic (the new Research Topic 9).

Research Topic 7:
The Panel offers an interesting research question, "Could threshold responses to changes in nutrient (N and P) loading also play a role in these recent "state changes" – instead of or in addition to the roles of invasive filter feeders and hydrologic changes?" Does the Panel have any suggestions as to how or what research could be conducted to address this question other than perhaps the use of mathematical modeling?

Response: We have deleted that research topic and added discussion of threshold responses into Research Topic 1, where it appropriately belongs as a data-analysis need. We believe that a threshold response to gradual increases in nutrient loading over the years is a distinct possibility, and new experimental work (e.g., microcosms or mesocosms in which phytoplankton from Suisun Bay are exposed to a range of NH_4^+ concentrations in appropriate light intensities) and modeling might help elucidate this process.

Research Topics 8-10:
These Research Topics are all closely related and to some degree address potential artifacts of analysis conducted by Dugdale et al. (2007) and Wilkerson et al. (2006). While the Panel suggests conducting nutrient addition bioassays using water samples from the Delta and Suisun Bay, would the Panel offer specific recommendations as to the magnitude of the nutrient additions recommended. For example, Pennock et al. (2006) and Takabayashi et al. (2006) performed such bioassays using significant additions of ammonium versus the relatively small ammonium addition of Dugdale (2007).

Response: We believe this detail would be best left to individual researchers and research coordinators to determine, because both approaches can provide informative results. The challenge is to appropriately interpret the results in the context of the Bay-Delta ecosystem.

One last thought about the framework and the potential scientific inquire that it proposes; BACWA recommends that the monitoring of pH be re-instituted within the existing monitoring programs for the Delta system to provide this basic and necessary piece of information which can help us understand overall toxicity.

Response: We agree and have added several sentences under Research Topic 11 (now Topic 10), strongly recommending that pH be measured in all water quality monitoring programs in the Bay-Delta system.

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Reviewer: JamesM.Kelly, General Manager, Central Contra Costa Sanitary District

Central Contra Costa Sanitary District (CCCSD) is an independent special district that collects, treats, disposes and reclaims wastewater for nearly half of Contra Costa County's population, approximately 450,000 residents. CCCSD's plant is a secondary treatment facility and our final effluent enters the southwest corner of Suisun Bay. As a secondary treatment facility on the very edge of the Delta-Suisun Bay Study Area of the research framework, we have a strong interest in the framework development and the potential future implications of the research. CCCSD has always been a leader in environmental issues and shares your interest in implementing meaningful changes that will positively affect the health of the Delta-Suisun Bay ecosystem.

CCCSD recognizes the draft research framework is a presentation of the panel's perception of the major concerns related to ammonia/um in the Bay-Delta ecosystem. CCCSD also recognizes that the suggested research topics proposed in this framework would require significant funding, which will likely limit actual research considerably. Therefore, the comments in this letter exceed the scope of the draft research framework purposely in an effort to reconsider the most favorable approach to better understanding the concerns facing the Bay-Delta ecosystem and discovering potential solutions to those concerns. The following are our comments on the draft research framework dated March 20, 2009.

Project Scope

During the second day of the CALFED Science Program workshop, a presentation was given breaking down the panel's understanding of two major concerns in the Delta-Suisun Bay. These were: (1) the pelagic organism decline (POD), and (2) undesirable changes in community structure and productivity of phytoplankton, which were said to be separate yet perhaps inter-related issues. Given these two major concerns, the project scope should be revised, with the research from the framework addressed to all potential drivers of these two issues rather than taking ammonialum as a starting point. The title of the research framework should be altered to read **"A Framework for Research Addressing Drivers for the Pelagic Organism Decline and Undesirable Changes in Community Structure and Productivity of Phytoplankton."** All of the potential drivers to unwanted changes in the Delta-Suisun Bay ecosystem should be given appropriate research preference to provide a body of work that allows comparison among the drivers.

Response: Our assigned task was to recommend a research framework for addressing the role of ammonia/ammonium in the Sacramento-San Joaquin Delta and the San Francisco Bay Estuary Ecosystem. Although in our report we have identified the need to consider other potential drivers of the POD and the food web changes in the Bay-Delta ecosystem, we have chosen to maintain the assigned focus on ammonia/um in the title of our report and in most of our recommended research topics. We believe our primary recommendation for an overarching, integrative model of processes affecting POD species and the food web in the Bay-Delta ecosystem sends a clear and strong signal to readers, that more stressors than just ammonia/um must be considered in integrative research and management programs.

While one reason for this major research body of work is the decline of the pelagic organisms, only two of the thirteen research needs focus on those organisms. Topic 11 looks at the sensitivity of those organisms to ammonia/um and topic 13 calls for field observations of those organisms for physiological status and their responses to the waters.

Many of the suggested research needs address nutrients in the Delta-Suisun Bay ecosystem, and none of the suggested research needs specifically address the impacts from the large-scale water exports and potential they have to negatively affect POD populations. According to research CCCSD has seen, this driver may be the primary cause for the POD. In the CALFED State of the Bay-Delta Science-2008 report, export pumping is cited as a potentially significant contributor to declining fish populations. This preliminary conclusion needs to be quantified with new research. The existing research on water exports is insufficient and inadequate to be useful for input to the conceptual model proposed in this framework. The conceptual framework as diagrammed should be augmented with the item "Delta water exports". Other areas of study that would fall within this research include the effects of screens in SWP and CVP pumping areas and likely increased predation of pelagic fish as they enter SWP and CVP facilities that do not provide the protection of their natural habitat

Response: Although only Research Topics 11 (now Topic 10) and 13 (now Topic 12) focus directly on POD species, almost all of the other research topics have potential indirect implications for POD species via the sources fate of N (including the potentially toxic ammonia and nitrite) and via food web effects.

We now explicitly mention water exports as a potential driver in the Bay-Delta ecosystem, and we added a box for water exports in Figure 1.

Despite the presence of an alternative in the research framework that hypothesizes that ammonia/um may have no detrimental effects to the receiving water body, the current title and suggested research direction of the framework builds on an underlying assumption that the ammonia/um issue may be the primary driver, and uses it as a starting point. CCCSD agrees that the health of the Delta-Suisun Bay water body is important, and that the two major stated concerns need to be addressed scientifically. However, the focus points of this research framework, while large in scope, remain too limited and focus too specifically on the ammonia driver without scientific justification.
Response: The emphasis on ammonia/um in our report and its title reflect what appeared to be a major impetus for the ammonia workshop. Whether that scientific and public concern with ammonia in the Bay-Delta system is justified remains uncertain to us. However, we believe we have struck an appropriate balance between recommending N-focused research (as we were charged to do) and concurrently calling attention to the need for an overarching, integrative analysis and model of all the potentially important drivers in the Bay-Delta ecosystem.

Project Schedule
The streamlined schedule for tasks and deliverables for this CALFED Science Program does not allow enough time to ensure that the panel and all stakeholders have adequate time to thoroughly consider the wide-ranging impacts of moving forward with the proposed research necessary to fill in the gaps of the over-arching model.

If the research remains primarily limited to ammonia and nutrient drivers, the research results will expectedly be disproportionately skewed and the real cause(s) of the POD may go unacknowledged and unresolved. The health of the Delta-Suisun Bay ecosystem depends on accurate research focused on all contributing factors. Ultimately, this could lead to misinformed and inappropriate regulatory decisions that do not contribute to the health of the Delta-Suisun Bay. More time must be provided to allow for thoughtful, thorough responses by stakeholders, and thoughtful, thorough consideration and incorporation of these responses by the panel in revising the research framework.

The anticipated finalization of the research framework and reply to comments just seven days after comments are received is unrealistic and inadequate. The final research framework should be based on sound scientific decisions that are not unreasonably rushed because of a short schedule.
Response: The timeline for preparation, review and revision of our proposed research framework was not determined by us. However, the content of the proposed research framework has solely been written by us. Recognizing the importance of many other potential drivers of the POD and the Bay-Delta food web, we expanded our discussion and recommendations well beyond the initial focus of the workshop on ammonia/um. We believe our primary recommendation for development of an overarching, integrative model conveys the need to consider all potential drivers, not just ammonia/um. However, to adhere to our assigned task of

recommending a research framework for addressing the role of ammonia/ammonium in the Sacramento-San Joaquin Delta and the San Francisco Bay Estuary Ecosystem, we focused mainly on ammonia/um, other N species of potential importance, and potential food web consequences of N enrichment on POD species and the phytoplankton community.

Peer Review

The potential drivers of the concerns facing the Delta-Suisun Bay ecosystem are many and diverse. In the presentation given at the workshop on March 10-1 1, these potential drivers were listed as hydrologic changes, nutrient loading and types, food-web effects, climate change (temperature and hydrology), toxins, and light. The panel members selected for this workshop are primarily experts in issues related to riverine and estuarine nutrient dynamics, food web processes and ecotoxicology. Additional expertise and review are necessary to fully grasp the potential of the drivers that do not fall into the expertise of the current panel members. CCCSD recommends that a full peer review of the expanded research framework (as suggested above under project scope) be conducted. Without this peer review, the final research will be disproportionately directed toward nutrient and ammonia related research, as is evident in the majority of the 13 suggested research topics in the draft research framework, while other potentially more culpable drivers remain stated, but understudied.

A peer review conducted by experts with appropriate backgrounds of each of the potential drivers will give balance to the final research direction. This course of action will ultimately yield a more complete understanding of the complexity of issues facing the concerns addressed in this framework, and will enable proper, scientifically justified actions to begin mitigation of the factors driving the two major concerns.

Response: Although we welcome review of our recommended research framework, the most important review will come at the stage in which the recommended research framework is fleshed-out into an enacted proposed research program. We recognize that our proposal is merely a suggestion to help shed light on needed research from perhaps new, outside perspectives, and that researchers and program managers will most likely drastically modify and/or reject at least some of our recommendations, based on more detailed knowledge of the Bay-Delta system. It is important to understand that a proposed research framework is merely a starting point, not the end of the development of a research program.

Specific Comments on Research Topics 2 and 3

While the major focus of research topic 2 is sources and sinks of N and P species, measurement of turbidity and salinity when constructing transects would be a low-cost augmentation of the data and could provide valuable insights. Therefore, CCCSD recommends adding these two parameters to the list of ancillary parameters to be measured. Research topic 3 deals with nutrient dynamics in stands of aquatic macrophytes. This topic should be expanded to investigate the role of benthic filter-feeding clams in the stands of aquatic macrophytes.

Response: We have added turbidity and salinity to the list of parameters to be measured under Research Topic 2; and under Research Topic 3, we have added a sentence recommending investigation of the role of benthic filter-feeding clams in the stands of aquatic macrophytes.

In closing, CCCSD appreciates the effort CALFED has put into beginning this study of the POD and phytoplankton issues facing the Delta-Suisun Bay water body. The draft research framework represents significant thought and leadership, and we applaud the panel of scientists for tackling important and controversial issues. Protecting the environment has long been a part of CCCSD's mission statement. Our vision and hope for this CALFED Science Program is that the research will yield scientifically sound information, allowing the appropriate response and mitigation measures to begin and for all the Delta-Suisun Bay citizenry to partner intelligently to protect the natural resource we share.

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Reviewer: Dr. Suzanne van Drunick, Cooperative Institute for Research in Environmental Sciences (CIRES), University of Colorado, Boulder

Page 1, pgh. 1: Expand introduction to briefly distinguish between ionized and unionized ammonia speciation with respect to toxicity and the relationship to pH, temperature, and salinity (see more detailed comments below). Basic information on the standard analytical techniques for ammonia that quantify total ammonia (the sum of NH_3 and NH_4^+) and report measurements in molar concentrations or as ammonia-N (e.g., 1.0 µM ammonia = 14µg/L ammonia-N) would also be helpful.
Response: We have not included the suggested primer on ammonia speciation and toxicity because we assume readers will be adequately conversant with the topic, as we also have assumed they will be adequately conversant with the biogeochemical and ecological concepts underlying our discussions of nutrient dynamics, food web processes, and phytoplankton communities (including diatoms and cyanobacteria).

Page 1, pgh. 4, last sentence: The reference to human activities here should be better clarified before its introduction as major driver in the Conceptual Framework. There are myriad human influences on the Bay-Delta other than the two quite obvious activities listed. In fact, one could argue practically every driver (pesticides, N/P, invasive species, and even climate change) has an anthropogenic component. So it would be helpful to cite several human-attributed activities associated land-use change from urbanization and agriculture (e.g., change in permeable surfaces, stormwater runoff, residential and agricultural pesticide use, nutrient inputs, channelization and dredging, etc).
Response: We have added some of those items to the list of human activities in that sentence.

Page 3, pgh. 5: The possibility that in wetter / high-flow years, dilution of the ammonium pool to concentrations that favor utilization of nitrate by diatoms should be considered as a likely mechanism to explain why blooms are more prominent in high-flow years and less so in low-flow years.
Response: In the last paragraph of that section, we have added two sentences acknowledging this possibility.

Research Topic 11 pages 9-10

Page 9, section on Research Topic 11, pgh 2, and page 10, pgh 1: Instead of an LC50 endpoint, EC20 values would be useful as lower exposure concentrations are more likely to occur in the Bay-Delta and addresses the panel's concerns that disagreement about "the relevance and interpretation of any laboratory toxicity test result" if the "exposure concentrations exceeds the effects concentration considerably".

Response: We added two sentences following that sentence, to acknowledge that other endpoints (e.g., LC20s) could be calculated and to explain the major uses of the acute-toxicity data.

Page 10, section on Research Topic 11, pgh 1: "Although salinity, temperature, feeding level and/or other types of stressors besides swimming could be varied in a more extensive ammonia/um toxicity testing matrix, the expenditure of resources needed to complete such a large testing program would not be justified".

Yes, but careful selection and standardization of these parameters are essential. In addition to values being representative of the Bay-Delta, the shift in equilibrium to NH3 (the unionized form that is more toxic to aquatic organisms) is primarily dependent on pH – a parameter not included in the proposed toxicity testing. For example, an increase 1 pH unit results in approximately a 10-fold increase in NH3. Temperature also plays a role. A 5˚C increase in temperature (between 0-30˚C @pH 7) results in about a 40-50% increase in NH3. In freshwater, salinity is less of a factor than pH and temperature and could be a fixed parameter to minimize the expenditure of resources.

Response: Those statements are correct, and we have added a sentence at the end of that section, stating the importance of carefully selecting and standardizing the water quality parameters in the toxicity tests. However, because the effects of temperature, pH, and salinity on ammonia speciation are well documented, we do not believe a large matrix of toxicity tests in which those parameters are varied would be justified unless preliminary tests like those we have recommended demonstrate a need for more detailed studies.

In addition to increased sensitivity to NH3 while swimming, cyclical exposure to lower ammonia concentrations may be a factor to consider. Thurston et al. (1981) compared acute toxicity of ammonia in steelhead exposed to cyclical fluctuations (6 hrs on/off, 12 hrs on/off) versus exposure at constant concentrations. Lethal concentrations (LC_{50}) values of total ammonia were 25 to 42% less in the cyclical bioassays and ranged from 7.2 to 25.5 mg N/L. These findings are important for evaluating the toxicity of ambient ammonia concentrations from sewage treatment facilities because daily cycles, related to household water use patterns, often have at least two peaks, so aquatic organisms in receiving streams are subjected to the more harmful cyclical fluctuations of ammonia. Under these more typical exposure scenarios, EPA's CMC (acute) values for pH of 7.8 or less would not be protective of salmonids.

Response: Yes, the LC50s for time-weighted ammonia concentrations in the fluctuating exposures in Thurston et al. (1981) were lower than the LC50s in constant exposures; however,

we do not believe the usually <50% differences are a first-order consideration at this stage in ammonia toxicity testing. Additionally, fish exposed to sublethal ammonia concentrations in the same study appeared to "acclimate" and thus subsequently tolerated higher ammonia concentrations than naïve fish. As a consequence, the effects of exposure to fluctuating concentrations of ammonia are probably more complicated than a straight-forward exposure × time analysis might indicate. Therefore, exploring the impacts on POD species during time-varying exposures to ammonia probably would only be justified after preliminary toxicity tests that we have recommended demonstrate a need for more in-depth studies. But because the point about fluctuating exposure to ammonia in many aquatic systems (whether or not downstream from a WWTP) is valid, we have added toxicity tests with time-varying exposures as a possible consideration for a more extensive ammonia/um toxicity testing matrix, in the last paragraph under Research Topic 11 (now Topic 10).

Minor editorial note: Toxicant is a broader term which includes all chemical and physical agents including toxins, which are chemicals of biological origin (e.g., microcystin).
Response: That is correct. We have changed "toxin" to "contaminant" where appropriate in the text, although we have retained "toxin" when specifically discussing toxicants of biological origin.

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Reviewer: Dr. Richard Dugdale, Romberg Tiburon Center, San Francisco State University

General comments:

The panel has relied too heavily on published literature (especially Jassby 2008) on primary productivity in the bay system that is based almost entirely on biomass multiplied by factors (i.e. the Cole and Cloern 1984 relationship using Secchi depth and PAR) established in the 1970's which have been shown to be overestimating current productivity by a factor of 2, as this biomass/physical factor relationship has apparently changed in the last 30 years. Three separate studies show this (summarized by Parker, Kimmerer and Lidstrom, in prep. and presented recently at the EET meeting 3/26/09).
Response: We believe this difference in perspectives among peer-reviewed, published articles demonstrates the need for the integrative analysis and overarching model we proposed in the draft research framework.

The panel appears to have ignored almost all new but unpublished information, which of course is not of the same reliability at the moment as reviewed publications. However, the information is available is several CALFED funded proposals that are in the public domain and were described in the CALFED background document for the workshop and could have been considered in their report. We had previously discussed these relatively recent studies with one of the panel members, Hans Paerl and so he was aware of these new results. NH_4 suppression of NO_3 reductase and NO_3 uptake is a well documented phenomenon (Refs can be provided and are

easily found in Nitrogen in the Sea, Carpenter and Capone, 1985; and Nitrogen in the Marine Environment, Capone et al. 2008) and in the reviewed ocean upwelling literature dating back to the 1970's and more recently in studies off the California coast (e.g. Dugdale, R.C., F.P. Wilkerson, A.Marchi and V.Hogue.2006. Nutrient controls on new production in the Bodega Bay, California, coastal upwelling plume. Deep-Sea Research II 53: 3049-3062.).
Response: We do not question the excellent research that has been conducted on NH_4 inhibition of nitrate reductase and NO_3 uptake in ocean systems. However, we do not believe the information provided to us has conclusively demonstrated that the phenomenon is the dominant driver (i.e., to the exclusion of other factors including grazing and hydrologic variability) of phytoplankton production and composition in the Bay-Delta ecosystem.

Detailed comments:

p.1, L15 from bottom, "including 1) increasing upstream discharges"
Response: It is not clear what change/comment is being made here.

p2. Figure 1 and caption. The arrow from N dynamics to primary producers is dashed and caption indicates it is "proposed" inhibitory /competitive effect of NH_4 on uptake of NO_3 by diatoms. I believe we have shown in 2 reviewed publications and in many presentations that NO_3 uptake in SF Bay water and Suisun Bay water happens only when NH_4 levels are low. Further that chlorophyll only accumulates in the whole system at low NH_4 concentrations when NO_3 is taken up. Further that chlorophyll blooms strongly in Suisun when NH_4 has been diluted by strong flow. I don't understand why this pathway is degraded to "proposed" when that could be said for many other pathways for which there is virtually no evidence, e.g. other toxins on primary producers. We know from experience in publishing these two papers that there is a reluctance by the estuarine community to embrace this changing paradigm and are disappointed to see it again in this document even after publication in reviewed journals.
Response: We have removed the offensive "proposed" from the caption (Meyer is to blame for that wording) and reworded the clause to attribute the ammonium-inhibition concept to Dugdale et al. (2007). We used a dashed arrow to distinguish that process from the parallel solid arrow and still believe that is a helpful visual aid.

p.3, L.9. Lastly, light availability, and the proportions of N and P loading play roles. I only know of one paper supporting the role of P in this system. The proportions of NH_4 to NO_3 do clearly play an important role (for both diatoms and *Microsystis*). I suggest change to " proportions of NH_4 , NO_3, and P loading play roles. These drivers ----production, biomass and composition (Jassby 2008, Wilkerson et al. 2006, Dugdale et al. 2007- add some other references besides Jassby).
Response: We have made the suggested changes.

P3, 12 from bottom. "High flow delivers high nutrient (N and P) loads. A little confused, since the anthropogenic loads are not changed by flow conditions, but only what concentrations are

experienced at different locations. But when diatoms are unable to access the NO_3 pool in the SFE system, they will not be able to compensate for high flushing rates.
Response: We agree that N and P loadings from wastewater treatment plants might remain relatively constant on a daily basis during high flows, but N and P loading from urban stormwater runoff and agricultural runoff usually increase somewhat exponentially as stream/river discharge increases. Additionally, NO_3^- loadings usually increase more than NH_4^+ loadings during high flows.

P4. L 8 beginning "Although ----dominant controls on productivity and biomass appear to be freshwater discharge (residence time) and grazing (Jassby, 2008). I strongly disagree with this sentence in two ways. First, the NH_4 effect is in reducing primary productivity and biomass. Secondly on the grazing control. The clams are present only in the deep channel and then only towards fall in Suisun Bay in numbers that could significantly affect phytoplankton by grazing l. Clams are not a factor in the lack of spring blooms. I believe there is no longer any disagreement on these facts.. Amy Kleckner (a masters student of Frances Wilkerson) sampled clams from Sept 2006-Dec 2007 in Suisun Bay and showed 1) low clam populations in shoals at all times, and in channels at all seasons except late summer, and 2) chlorophyll biomass increases in spring when NH_4 concentrations fell below 4 □M .The thesis has just been completed but the data were reported at ERF 2008 and CALFED Science 2009 and the data have been available. The conclusion in the sentence in regards to grazing is overstated and unfortunately restates the conventional dogma set in stone 20 years ago and difficult to shake even with modern data.
Response: We have revised that sentence to reflect the potential interaction of NH_4 inhibition, freshwater discharge, and grazing. However, despite the reviewer's confidence that clams are not a factor in the lack of spring blooms, we do not believe the information available to us conclusively substantiates that contention. This uncertainty is why we recommended an integrative analysis of available information and subsequent development of an overarching model that incorporates all the major drivers in the Bay-Delta ecosystem.

P4, L13 State Changes ----The most notable changes----and the conversion of the Sacramento WWTP to secondary treatment in 1983, along with consolidation of many regional treatment plants, and the discharge of NH_4 , initially from about 5 mT/day to present day 15 tons N/day. Our current view, based on the crash of chlorophyll concentrations, that progressed down river after this date, is that the NH_4 suppression of NO_3 uptake most likely arrived in Suisun Bay at the same time as the arrival of the clams, but was unrecognized until the recent work of the Dugdale/Wilkerson Laboratory at SFSU.
Response: We believe that making this conclusion is premature and does not take interactions of co-occurring drivers into consideration. This again demonstrates the need for an integrative, overarching model of the Bay-Delta ecosystem.

P5, L8 frombottom, "However, some of the ----on the right side of the model---". We don't understand this statement as it seems that even now, it will be easier to quantify the right side that includes primary producers. This seems to negate the call in the previous paragraph for inclusion of "the biogeochemistry of N and P?

Response: The key word in that sentence is “some”, which does not mean “all”. We believe the modelers will be best positioned to determine the levels of detail, quantitation, and complexity that are justified for each component and interactions among components in the model(s). However, as an example, it is likely that phytoplankton populations can be modeled much more easily and accurately than stands of aquatic macrophytes and populations of some of the POD species and their prey.

Modeling

P6.L17 "Which is (are)-----" Something must be missing here since the main drivers of phytoplankton productivity are environmental. Biomass and species composition are not drivers in the normal sense.,

Response: The potential drivers in question are (1) delivery of ammonia/um and other N species and P, and (2) water residence time – not biomass and species composition, which are structural responses of the phytoplankton. To clarify this apparently confusing sentence, we have rewritten it as three separate questions that specifically state what the potential drivers and combination of drivers in question are.

Food Web Effects

P8, L13 from bottom "Recent state changes in chlorophyll a---than to more gradual increases in NH4----". Recognition of recent research would suggest modification of this sentence. We suggest " Recent state changes in chlorophyll a concentrations and primary productivity in Suisun Bay have been attributed to the rapid expansion of invasive filter feeders (i.e. clams) and relatively rapid changes in freshwater discharge. However, threshold changes in response to increasing nutrient loading have been proposed to have occurred concurrently with the arrival of the clams. A thorough examination of existing NH_4 and chlorophyll data for the period from late 1970's to the 1990's should be made to sharpen the focus on this mechanism. It should be pointed out that there is a dearth of rate measurements of primary productivity during this period and calculations of primary productivity from chlorophyll concentrations may be overestimated by a factor of two beginning at some time during this two decades.

Response: We have adopted some of the suggested sentences in a revision of the text for this research topic.

Research topic 9 Inhibition versus preferential uptake of NH_4

P9, L7

"How can NH_4 inhibition of NO_3 uptake be distinguished from preference?" Preference is a very ambiguous and anthropomorphic term. Used in the sense of McCarthy (McCarthy, J. J., Taylor, W. R., and Taft, J. L. (1977). Nitrogenous nutrition of the plankton in the Chesapeake Bay. I. Nutrient availability and phytoplankton preferences. Limnol. Oceanogr. 22, 996–1011) preference is determined by the ratio of uptakes to ambient concentrations in the environment and has no physiological meaning. We see no reason to worry about the ambiguous "preference" compared to demonstrated inhibition, well know and observed elsewhere. In both phytoplankton and higher plants, NH_4 has been shown to inhibit NO_3 reductase without which NO_3 assimilation does not occur.. On the contrary, our observation that peak specific NO_3 uptake (equivalent to a

growth rate) exceeds that of NH_4 by a factor of 3 or 4 would suggest a "preference" for NO_3. We suggest eliminating the first two questions and going on to the next few that really matter.
Response: We disagree that "preference" is ambiguous. In addition to McCarthy et al. (1977), a vast amount of literature discusses this phenomenon (based on energetics among other things) under natural light conditions and in the laboratory.

P9L15. "If this phenomenon only affects the spring diatom bloom----." We have said that the spring diatom blooms have been disrupted in both occurrence and time of occurrence and NH_4 suppression appears to occur throughout the summer as well***. Please remove the "only" from this sentence.*** Then the rest poses a very interesting problem with regards to the food chain and early life history of fishes.
Response: We have removed "only" from that sentence.

Conclusions

This section is great and hits the nail on the head, recognizing the lack of system directed research in this ecosystem.

Continuing on the theme of the paucity of direct or even valid indirect measures of primary productivity in the system, we suggest adding "primary production" to the list of field measurements in the 3rd paragraph of this section.
Response: We have added "measurements of primary production" to that list. We have also recommended measurement of primary production under Research Topic 4.

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Reviewer: Dr. Inge Werner, Dept. of Anatomy, Physiology and Cell Biology, University of California-Davis

First, I would like to commend the panel for its insightful analysis and recommendations.

I would like to comment on the following statement, page 11, first paragraph: "Therefore, like other biomarkers, genomic arrays will be most useful in ruling-out potential adverse effects of ammonia/um (or other toxins).

Additionally, they can add to the weight-of-evidence about a given toxin if a genome-level response is detected. However, a positive genomic biomarker response alone is not sufficient cause-effect evidence for higher-level toxicological responses."

Comment: Although the panel is correct in stating that data is currently lacking to draw conclusions for higher-level responses, genomic biomarkers have the potential for providing strong evidence for higher-level toxicological responses, both in human as well as in eco-toxicology. As is common with novel approaches, research is needed to develop such biomarker-based tools for ecotoxicological field research.

Response: We agree and have added "currently" after "alone", to not preclude the possibility of future advances in this field.

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Reviewer: Frances Brewster, Santa Clara Valley Water District

The undersigned water agencies thank you for sharing your expertise on ammonia/um processes and effects and for producing the subject document. The Panel correctly identified the two major concerns related to ammonia/um in the Bay-Delta ecosystem: (1) the Pelagic Organism Decline (POD) might be caused directly by toxicity of ammonia/um to the four fish species, and (2) the Bay-Delta ecosystem is rapidly progressing through major food web shifts that might be caused by ammonia/um. In general, the Research Framework does a good job identifying the questions that need to be answered in order to determine the degree to which ammonia/um contributes to those concerns. The Research Framework also provides adequate guidance on approaches to answer those questions. However, given the time critical nature of the major concerns identified and the perpetual limitation on available resources, we hope the Panel can provide additional guidance on the priority of each identified research need. If all the identified research cannot be initiated immediately, in what order would the Panel recommend addressing the identified needs? Are any research topics more important than others to answering the two major concerns identified?
Response: We have inserted a "Research Prioritization" subsection at the end of the "Major Research Needs" section.

Following are specific questions and comments on the Research Framework:
1. The Panel states that Bay-Delta research is "breaking new ground" (page 1, ¶3). What does the Panel believe is so unique about the Bay-Delta system that "paths trodden by others" cannot be used as a template for this system?
Response: We have reworded that sentence as follows: "In many ways, Bay-Delta research is breaking new ground by tackling a large-scale, complex freshwater-estuarine ecosystem affected by multiple, interactive stressors. Consequently, the Bay-Delta research program will establish a template for future research and management decisions in other hydrologically, biogeochemically, and trophically-similar systems, rather than being able to rely on paths trodden by others." Perhaps the only other large-scale estuarine system of this type that is being studied extensively is Chesapeake Bay; however, its climate and associated hydrology (including lack of water exports) differs considerably from the Bay-Delta system. Therefore, although paths trodden by others might be useful for addressing individual pieces of the Bay-Delta puzzle, we do not believe an overarching template for Bay-Delta research exists elsewhere.

2. We strongly disagree with the panel's decision to call out two underlying human activities: (1) upstream discharge of ammonia/um containing municipal wastewater and (2) large-scale pumping of water from the Delta (page1, ¶4). There are numerous human activities that underlie the current state of the Delta ecosystem including extensive dredging, diking and channelization,

rapid urbanization, agricultural activities, point and nonpoint pollution, and intentional and accidental introduction of alien and invasive species to name only a few. These activities all potentially contribute to the POD and the observed shifts in the food web. Many of these activities, and pumping especially, have already been, or are currently being researched extensively. While it is important to understand the role of ammonia/um relative to other stressors on the POD, calling out pumping specifically in this document places undue emphasis on a single factor unrelated to the specific questions posed to the Panel.
Response: Frankly, we specifically identified municipal wastewater discharges and water exports because they were the two invisible gorillas lurking in the room during the ammonia workshop. However, we agree that many other stressors affect the Bay-Delta ecosystem. Therefore, we revised the sentence in question to state that municipal wastewater discharges and water exports are activities that have received considerable attention; and we added a sentence after that, stating that other potential stressors include urbanization, stormwater runoff, residential and agricultural pesticide use, nutrient inputs, channelization, dredging, diking, and invasive species.

3. The Panel states that "phytoplankton composition, productivity, standing crop and bloom dynamics appear to be closely controlled by freshwater discharge and residence time" (page 3, 5). This inference appears to be based on observations in Jassby (2008) that spring diatom productivity and blooms are low in years with low discharge and more prominent in high-flow years. However, an alternative hypothesis is that in high-flow years, ammonium concentrations are diluted, allowing diatoms to access the large nitrate pool as described in Wilkerson et al (2006) and Dugdale et al (2007) and vice versa in low flow years. In other words, if ammonium concentrations were reduced, freshwater discharge rates may not appear to be as important to phytoplankton production. What approach would the Panel recommend to distinguish between these two hypotheses?
Response: Because of the difficulty in conducting ecosystem-level manipulation in the Bay-Delta system, this would have to fall within the realm of the data analysis recommended under Research Topic 1. The ability to discriminate between the two processes will depend on whether the historical record is cooperative or conditions in coming years allow such a discrimination.

4. The Panel minimizes the importance of ammonium inhibition of nitrate utilization as a mechanism suppressing spring diatom blooms as described by Dugdale et al. (2007) based on analyses presented by Jassby (2008) (page 4, ¶1). However, Jassby (2008) only looks at long-term trends based on annual averages and would not likely be able to distinguish the seasonal effect described by Dugdale et al. (2007).
Response: We have reworded those sentences to not minimize the role ammonia inhibition might play.

5. The Panel describes invasive benthic filter-feeding species and hydrologic modifications (i.e. water withdrawal and channelization) as the most notable recent anthropogenically imposed changes that have had "dramatic impacts on production and composition of phytoplankton and macrophyte communities" (page 4, ¶2). What recent hydrologic modifications is the Panel

referring to? Channelization of the Delta was predominantly done by the 1920s. Water withdrawals from the Delta have been increasing on a gradual basis since the 1950s, and include upstream depletions, in-Delta water use and water project operations.
Response: We deleted "recent" from that sentence. There is no doubt that human hydrologic modifications *vis a vis* chanellization and water withdrawal have occurred over a quite long time span.

6. Modeling. We believe the Panel has placed an appropriate emphasis on the research and development needs in the area of modeling. Under Research Topic 1, what approach would the Panel recommend for distinguishing between the influence of changes in nutrient loading from changes in freshwater discharge/flow that is closely correlated with changes in nutrient concentration?
Response: Until the historical data are analyzed, we will not know whether that distinction can be made.

7. Research Topic 2. There has been considerable long term ammonium discharge into western Suisun Bay from the Central Contra Costa Sanitation District WWTP outfall. As this could be as important as, or more so than, the Sacramento Regional Wastewater Treatment Plant (SRWTP), the Panel should identify this second WWTP by name if the Panel feels the Central Contra Costa Sanitation District WWTP merits investigation. Further, the Panel provides approaches for biogeochemical models. Can the Panel elaborate on what metrics or quantifications within these models would identify whether or not the WWTPs are contributing to elevated ammonium in specific locations such as Suisun Bay? Do we have to wait until the modeling is complete to answer this question?
Response: We have added Central Contra Costa Sanitation District WWTP as a named WWTP in that section. The future research described in the fifth paragraph of that section is a guide to the types of information that could be used to estimate the contributions of N from WWTPs to the ammonia pool in locations such as Suisun Bay. Because this is a puzzle for which we aren't sure what the final picture looks like, it is difficult to specify beforehand exactly what information will be conclusive and at what stage the conclusions can be made. This is a case where clever researchers will have to immerse themselves in the problem and figure their way to a conclusion with good detective work.

8. Research Topic 3. UC-Davis and USGS have been using remote sensing to track areal coverage of submerged aquatic vegetation (SAV) in the Delta since 2004 and estimate that SAV currently covers about 5-10% of Delta waterways in total, a large percent of which occurred in one area, Franks Tract. They also have observed a decline in aerial coverage in the last two years which they attribute to herbicide applications in Franks Tract. Given this additional information, does the Panel still believe that Research Topic 3 is important to pursue? How would the Panel rate the relative importance of Research Topic 2 versus Research Topic 3?
Response: We believe the future of SAV in the Bay-Delta ecosystem is difficult to predict. However, we still believe it is important to track location and areal extent of macrophyte beds, and their impact on retention/export of nutrients. Nevertheless, we also believe Research Topic

2 is a higher priority, as we discuss in the "Research Prioritization" subsection we have added at the end of the "Major Research Needs" section.

9. Food Web Effects. The food web issues of whether ammonium inhibits phytoplankton productivity, and if so, is the ammonium from the two WWTPs, are key questions. However, there is no obvious priority or sequencing of the research topics. What sequence(s) of research topics does the Panel recommend to address these questions in the shortest amount of time?
Response: See the "Research Prioritization" subsection we have added at the end of the "Major Research Needs" section.

10. Research Topic 6. What methods would the Panel recommend to experimentally test the effects of climate change on phytoplankton?
Response: There is no established methodology to examine effects of climate change on phytoplankton communities. However, examining the interactive influences that temperature increases and hydraulic residence times have on phytoplankton community structure and function are worthwhile experimental approaches, and that interaction should also be included in the interactive model.

11. Research topic 7. The Panel describes "relatively rapid changes in freshwater discharge" as being more closely related to "state changes" in chlorophyll a than is the more gradual increase in ammonium concentrations/loading during the past few decades (concentrations and loading have more than doubled over the past few decades). What rapid changes in freshwater discharge is the Panel referring to? What methods would the Panel recommend to answer whether threshold responses to changes in nutrient loading plays a role in "recent 'state changes'-- instead of or in addition to the roles of invasive filter feeders and hydrologic changes"?
Response: We have deleted "relatively rapid" from that sentence. Additionally, we have deleted Research Topic 7 and incorporated some of the sentences into Research Topic 1, where it appropriately belongs as a data-analysis need.

12. Research Topics 8 and 9. The Panel's question about ammonium inhibition or preference seems the key question to answer prior to addressing whether the WWTPs inhibit phytoplankton productivity. However, the framework does not appear to describe how to address this specifically beyond the nutrient addition bioassays. What methods does the Panel recommend to identify whether the delayed growth response is caused by "container effects", inherent lag times, or by ammonium inhibition of (or competition with) nitrate use? What methods does the Panel recommend for answering the other important questions identified under Research Topic 9?
Response: We believe our task is to provide a framework for research, not a detailed how-to manual. Although discriminating among the several possible explanations for the delayed growth response will not be trivial, we suspect the challenge is not insurmountable. The details

of how to address this challenge should be left to clever researchers who know these systems better than us.

13. Toxicity. The issue of whether ammonia/um causes direct toxicity to the four POD species or their food organisms, and if so, is the ammonia/um from the two WWTPs, are key questions. However, there is no obvious priority or sequencing of the research topics. What sequence(s) of research topics does the Panel recommend to address these questions in the shortest amount of time?
Response: See the "Research Prioritization" subsection we have added at the end of the "Major Research Needs" section.

14. Research Topic 13. We appreciate the Panel's acknowledgment that exclusive reliance on laboratory toxicity tests will not likely be productive and its recommendation to initiate routine fish health monitoring protocols. With respect to developing genomic arrays for the resident POD species, we agree that this could be an important tool for future use. However, it is our understanding that this effort takes many years to develop to a level that could reliably be used to rule in or to rule out specific contaminant effects on the POD species. For Delta smelt, handling alone may cause certain genes to express. In addition, even if you don't see any gene expression when exposed to specific contaminants at environmentally relevant levels compared to controls, how can you rule out that gene response would not occur after a longer exposure time, or under different conditions? Please explain how genomic arrays can reliably rule out potential adverse effects of ammonia/um or other toxins?
Response: In concept, genomic responses that are manifested only after long-term exposures should be identifiable in the resident POD fish that we recommended be analyzed in conjunction with naïve fish that would be assayed after *in situ* exposure to Bay-Delta waters. Of course, there will still be a greater-than-zero probability of a Type II error (i.e., not identifying an adverse response that actually is occurring in that population), but we believe genomic arrays offer the possibility of decreasing that Type II error more than other currently available approaches (e.g., much lower Type II error probability than standard fish health surveys).

Again, thank you for your thoughtful recommendations for additional research to answer the critical questions of whether ammonia/um is causing direct toxicity to the four fish species or their food organisms, and/or contributing to major food web shifts. Given the time critical nature of the major concerns and the perpetual limitation on available resources, we hope the Panel can provide additional guidance on the relative priority of identified research.
Response: See the "Research Prioritization" subsection we have added at the end of the "Major Research Needs" section.

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Reviewer: Stan R. Dean, District Manager, Sacramento Regional County Sanitation District

SRCSD found that this framework for research provides a clear and concise overview of the current state of the Delta in relation to ammonia/ammonium and related factors potentially affecting the POD. The recommended research focuses on important issues and key data gaps and recognizes that factors other than ammonia/ammonium (e.g., flow and invasive species) affect the Delta ecosystem and must be considered in concert with nutrients. It is clear that many research questions need to be resolved in order to allow scientifically informed decisions for managing nutrients in the Delta.

The framework recognizes the overarching importance of hydrology, and human impacts on hydrology, as a key driver of ecosystem structure and function in the Delta. Our primary concern related to the framework is that direct effects of water exports on POD species (entrainment and increased predation in the Clifton Court Forebay) and their food supply (entrainment of high quality seston) are not acknowledged as potential key drivers of the POD. This comment and a few other comments are provided in Attachment 1.
Response: We have added those effects of water exports to the stressors in the Bay-Delta system that we list in our rewording of the first paragraph in the "Major Concerns Related to Ammonia/um" section.

SRCSD is also pleased to provide supplemental information to the expert panel resulting from a comparison of historic ambient ammonia/ammonium concentrations in the Delta with the acute and chronic National Recommended Water Quality Criteria for Ammonia (USEPA 2006) (Attachment 2). This information was only briefly discussed at the workshop, and separately with Dr. Joseph Meyer during breaks. The supplemental analysis supports conclusions by the expert panel at the workshop that ambient ammonia concentrations in the Delta do not exceed, and are in fact, much lower than these conservative toxicity benchmarks.
Response: We are impressed by the extensive analyses that have been conducted and pleased that the ambient ammonia concentrations were directly compared to the ammonia criteria. However, the ammonia criteria are not necessarily conservative toxicity benchmarks, because they are only intended to protect at least 95% of the species. Therefore, if at least one of the POD organisms (or their major prey) is more sensitive that the 5th percentile sensitivity that is used to derive the criteria, adverse effects could be occurring even though the criteria are not exceeded. Because threatened and endangered species, species of special concern, and commercially or recreationally important species can trump aquatic life criteria, it will be important to also compare ambient ammonia concentrations in the Bay-Delta waters to the sensitivities of the POD organisms and their major prey when appropriate toxicity data (recommended by us under Research Topic 11 (now Topic 10)) become available.

Thanks for your participation in our discussions and investigations to determine the potential role of ammonia/ammonium in the Delta ecosystem. SRCSD is available to discuss or clarify any of these comments if you have questions. We look forward to working with all interested parties on answering research questions that allow scientifically informed decisions for managing nutrients in the Delta.

Comments and Recommendations for the Draft Framework

page 1, paragraph 4: "Alternatively, ammonia/um enrichment might have no detrimental effects, and other drivers are responsible for the *biogeochemical* changes and trophic changes." (emphasis added)

Comment: Although concentrations and loadings have unarguably been altered by human activity upstream from, and within, the estuary, we are aware of no published studies that indicate that *biogeochemical processes*, per se, have changed in the ecosystem.
Response: This appears to be a matter of semantics. We interpret "biogeochemical changes" to include changes in concentrations and not be limited to changes in processes.

page 2, paragraph 1: "Finally, export of Delta water altered hydrologic conditions,…"

Comment: Alteration of net outflows and other hydrologic characteristics is one important ecosystem impact of water exports. However, two other major impacts of water exports are not acknowledged in the framework document:

1. Direct mortality of fish owing to entrainment (undisputed) and increased predation of POD fishes by predators in Clifton Court Forebay (under investigation).

2. Indirect effects of reduced food supplies for estaurine consumers owing to export of high quality seston from the south Delta.

Response: We have added those effects of water exports to the stressors in the Bay-Delta system that we list in our rewording of the first paragraph in the "Major Concerns Related to Ammonia/um" section.

page 2, Figure 1.

Comment: We recommend acknowledgement of 4 additional drivers of ecosystem structure and function in the conceptual diagram.

1. Add arrows directly connecting Human Activity to Primary Producers and the POD organisms, to acknowledge (a) the direct effects of water exports on phytoplankton and zooplankton supplies in the estuary (via export of seston) and (b) the direct effects of water exports on POD fishes (via mortality through entrainment and increased predation in the conveyance infrastructure). These drivers are distinct from indirect effects of water exports on the POD through changes to estuarine hydrology (net outflow, salinity gradients, etc.).

2. Add an arrow connecting Human Activity to Optical Properties to acknowledge that upstream retention of sediment in reservoirs has caused a decrease in mineral turbidity in the estuary.

3. Add an arrow connecting Optical Properties to POD organisms to recognize that turbidity directly influences foraging success of Delta smelt.

4. Add an arrow connecting Climate/Hydrology to Human Activity to recognize that climate/flows influence the volume of water exported for irrigated agriculture and municipal use.

Response: We have made the suggested changes to Figure 1, with the exception that the arrow from the Human Activity box now goes to a new Water Exports box, from which arrows then go to the Primary Producers and the POD Organism boxes.

page 3, paragraph 2. "These cyanobacterial blooms have increased in recent years, in part due to persistent drought conditions (poor flushing, long residence time), a recent warming trend, and increasing nutrient (especially N) loads."

Comment: This observation should be couched as a hypothesis, not a statement of fact. Identification of environmental correlates for *Microcystis* occurrence, abundance, and toxin production is currently under investigation in the San Francisco Estuary, but published work from the Delta, so far, has indicated that *Microcystis* abundance and toxicity is not explained by nutrient (or N) loads. Lehman et al. (2008) performed canonical analysis on data from a Delta-wide sampling program for 17 environmental factors, *Microcystis aeruginosa* cell abundance, and microcystin cell content. East side tributary flow, Contra Costa Canal pumping, and water temperature were the primary factors explaining the abundance and microcystin content of *Microcystis* in the brackish and freshwater reaches of the Delta. Total dissolved solids and nutrient concentrations were of secondary importance. Ammonia and nitrate concentrations were weakly *negatively* correlated with *Microcystis* abundance, meaning that higher ammonia and nitrate concentrations were associated with fewer *Microcystis*. Sacramento and San Joaquin River flows were strongly negatively correlated to *Microcystis* abundance, while East Side stream flow was strongly positively correlated with *Microcystis* abundance.
Response: We have reworded that sentence to couch it as a possibility instead of a certainty.

page 4, paragraph 2: "For example, increased loading of N (and specifically NH_4^+) that has impacted this region could have caused a shift in the Delta phytoplankton community toward *Microcystis* in a relatively short period (~5 years). This shift, in turn, has led to major modification in C and nutrient cycling as well as trophodynamics."

Comments:

- We recommend qualifying the first sentence of the passage, based on the observation that increased loading of N has occurred gradually over several decades, so it is not clear how well N loading can explain the recent phenomenon of *Microcystis* blooms starting in 1999.
  Response: We believe the words "could have" provide appropriate qualification. Deletion of those words would imply certainty, which we agree is not warranted here.

- This passage may exaggerate the potential role of *Microcystis* as an environmental factor in the ecosystem. *Microcystis* blooms are not ubiquitous in space or time in the estuary. They are a summertime occurrence in part of the Delta.
  Response: We do not believe "could have caused a shift … toward *Microcyctis*" implies dominance by *Microcyctis* in space and time. However, we believe the summertime occurrence of *Microcyctis* in part of the delta is a harbinger of major change that should not be ignored.

- The latter statement should be presented as a hypothesis, not a statement of fact. To our knowledge, there is no evidence from the San Francisco Estuary that *Microcystis* blooms have led to major modification of C and nutrient cycling, or trophodynamics, although this may prove to be true locally during portions of the year.
  Response: We have reworded that sentence to the following: "Consequently, this shift likely has led to major modification in C and nutrient cycling (toward the benthos) as well as trophodynamics, especially during summer bloom periods in the parts of the Delta where *Microcystis* occur (and possibly downstream if and when toxic cells are transported there)."

page 9, research topic 11-Sensitivity of POD organisms to ammonia/um.

Comment: The environmental relevance of a chronic test involving continuous swimming at ~1 body length/sec as a stressor (especially for younger, or seaward migrating life stages) should be considered in light of the fact that many estuarine fish have evolved behaviors to passively utilize tidally induced currents and other hydrodynamic processes in the estuary, during some or all of their life stages, for retention within the estuary, maintenance of position in appropriate habitat, or for migration.
Response: We agree and have deleted the words "and relevant" from that sentence, and we have replaced "at, for example, ~1 body length/sec" at the end of that sentence with "although other more relevant stressors might identified". However, we still believe swimming is an easy, generic stressor to use in laboratory toxicity tests and is probably no more fraught with controversy about relevance than any other easily imposed stressor.

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Reviewer: Dr. Frances Wilkerson, Romberg Tiburon Center, San Francisco State University

I thank the Panel for taking on this challenging task and I think they did a great job in the time they had available. The draft document gave a fair assessment of the Delta-Bay situation regarding aspects of the ammonia/ammonium situation although it seemed somewhat focused on a) the Delta especially the San Joaquin region with and consequently bb) *Microcystis*. This also links with the emphasis on including P as well as N-since the interplay of these two is really

important for cyanobacterial success. As it was pointed out *Microcystis* doesn't grown in > ~2 psu. It would have been more complete to have more about the western delta and the Rio Vista/Suisun Marsh, Suisun Bay region and the role of diatoms contributing to blooms or chlorophyll biomass. Unlike the east coast estuaries where silicate (an obligate requirement for diatoms) can be a limiting resource, concentrations are abundant in the SF Bay/Delta (often > 150-200 µM). The panel would have benefited from Cloern and Dufford (2005-MEPS) which is a great review of the phytoplankton community structure-especially the diatoms. The occurrence of large numbers of dinoflagellates is uncommon in SF Bay/Delta. Since the panel was working with relatively old publications and not unpublished but recent data there were some other things missing- for example the role of gelatinous zooplankton and the increase in jellyfish (voracious predators) on the food web and grazing potential for phytoplankton. There has been more work and data collected by Jan Thompson (USGS) on Corbula in Suisun Bay and my student Amy Kleckner and its role in a) excreting NH4 and b) grazing and c) use of DON. This may help address Research Topic 10. The CALFED IRWM project also information regarding productivity and abundance of macrophytes in Delta wetland areas.
Response: We acknowledge not having had access to all the information about the Bay-Delta ecosystem that is available in published and unpublished forms. In fact, an adequate review of that vast amount of information will require considerable time and effort, well beyond the scope of our task of recommending a research framework. That is why we recommended in our report that the most important gap to be filled in the Bay-Delta research program is the development of an overarching, integrative model of the major drivers controlling the Bay-Delta ecosystem, which would be based on an in-depth analysis of the available information.

Regarding the paragraph on State Changes (P4) - the massive increase in Bay area population seems to be a significant anthropogenic imposed change but not really addressed here.
Response: To more directly address concerns about the human-population increases in the Bay area, we added the following sentence at the end of the first paragraph in the Major Concerns Related to Ammonia/um section (on page 1): "However, many other potential stressors also are present in the Bay-Delta system, including urbanization, stormwater runoff, residential and agricultural pesticide use, nutrient inputs, channelization, dredging, diking, and invasive species." Although that type of statement could just as well have been inserted into the State Changes in the Bay-Delta Ecosystem section, we believe it is more effective earlier in the report [i.e., on page 1 (where we placed it) instead of page 4 (as suggested here)].

The statement about benthic filter feeders altering cycling of C and nutrients- this should be referenced specifically for Corbula –I would like to read the reference.
Response: We inserted "probably" into that clause, which now states: "…the presence of these benthic filter feeders probably has led to altered flowpaths, cycling, and fates of C and nutrients." That removes the apparent certitude that the reviewer questioned.

I liked the Major Research Needs and would agree to most of the panels comments. However on Page 5 they say that only the left hand side of the model can really be addressed and yet the first research topic is aimed at modeling the biological/biogeochemical parameters on the right side. A little confusing.
Response: The key word in that sentence is "some", as follows: "…some of the biological processes represented mostly on the right side of Figure 1 might have to be modeled only

qualitatively…" We believe some of the processes (e.g., controls on phytoplankton populations) will be more tractable than others (e.g., effects of changes in the community of primary producers on POD organisms), and therefore this statement does not contradict our recommendation in Research Topic 1.

It should also be remembered that SacWTP (P.6) is not the only WTP and that Contra Costa County and other smaller plants may also play a role and should be surveyed and included in any future work.
Response: In addition to SACWWTP, we have added Central Contra Costa WWTP as another wastewater treatment plant that should be included when investigating the sources and fates of N and P in the Bay-Delta ecosystem (under Research Topics 2 and 3).

I really believe that we need to know more about the multitude of biogeochemical reactions of N in the ecosystem but these need to be measure as rates, maybe also with molecular approaches but stable isotopic signatures should not be used a the only resource- as thee can be misleading without supplementary data.
Response: We agree and have added words in several places in the report, to emphasize the need to measure rates of N transformations in addition to just identifying which transformations occur.

Research Topic 7 suggests that recent state changes are due to Corbula- which arrives 20 years ago. Has it been increasing in numbers, densities, extent into the Delta since then? In fact does it occur in the Delta or only up to northern reach of Suisun. Future reports might want to clarify the distinct locations of Corbula, Microcystis and macrophytes. Will Corbula impact phytoplankton processes in the western Delta?
Response: We agree that those questions should be addressed. In our revised report, we combined the former Research Topic 7 into an expanded Research Topic 1 and inserted the following question: "During the period when Dugdale et al. (2007) suggested NH_4^+ inhibited NO_3^- uptake by diatoms, were benthic clam filtration rates high enough to depress the phytoplankton (including diatom) biomass -- as suggested in Cloern (1982), Thompson and Nichols (1996) and Jassby (2008)?" We believe that question even more directly strikes to the heart of the overriding concern; and to fully answer it, the questions posed above will implicitly have to be answered.

Research Topic 8 – we have found the lag time to be quite variable, with lag times extended beyond 1-2 days that seems to be related to ambient nutrient conditions. The lag time can be changed also with experimental manipulations. This suggest that it is unlikely to be an experimental container effect.
Response: This is part of the information that will have to be incorporated into the data analysis needed to help construct a model of controls on phytoplankton populations, which we recommended under Research Topic 1.