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April 25, 2012

Ms. Jeanine Townsend, Clerk to the Board
State Water Resources Control Board
P.O. Box 100
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Submitted electronically to commentletters@waterboards.ca.gov

Subject: SRCSD Comment Letter on the Water Quality Control Plan for the San Francisco Bay/Sacramento-San Joaquin Delta Estuary (Bay-Delta Plan) Supplemental Notice of Preparation – Comprehensive Review

Attention Ms. Townsend:

The Sacramento Regional County Sanitation District (SRCSD) appreciates the opportunity to comment on the Bay-Delta Plan Supplemental Notice of Preparation and Comprehensive Review (Bay-Delta Plan). SRCSD provides wastewater collection and treatment services to over 1.3 million residents of the greater Sacramento area. Our mission is to protect human health and keep the Sacramento River clean and safe. We take our mission seriously and work on a daily basis to meet our obligations to protect water quality and beneficial uses in the Delta. Our excellent compliance record with our NPDES permit speaks to this commitment and performance.

SRCSD was involved in the review of the State Water Resources Control Board's (State Water Board) 2006 Bay-Delta Plan and the 2009 Periodic Review of the Bay-Delta Plan. Many of the issues discussed in the previous versions of the Bay-Delta Plan are still relevant today, and our comments submitted on the 2009 Periodic Review still apply. In addition, we would also like to point the State Water Board to our comments on the 2010 Delta Flow Criteria hearings, specifically our closing comments.

SRCSD is committed to ensuring that sound science is the basis for policy decisions regarding ecosystem protection and water supply in the Delta. Additional research to address evolving hypotheses related to water quality, including ammonia/um, is appropriate, and SRCSD is supporting that ongoing research. However, the potential effects of water quality constituents on the Delta are being addressed in other scientific and regulatory venues, including basin planning activities, and as a result, do not need to be included in the State Water Board's Bay-Delta Plan. SRCSD recommends that the State Water Board continue to focus its efforts on identifying flow criteria that addresses the magnitude, frequency, and duration of Delta flows for public trust resources.

We are providing some additional technical documents (attached) that pertain to ammonia in the Bay-Delta. Following is a discussion on recent studies and publications that we would like the State Water Board to be aware of as you complete the comprehensive review of the Bay-Delta Plan.

Ammonia Comments:

In the 2009 Periodic Review, State Water Board Staff recommended no further review of Ammonia Objectives and that the State Water Board coordinate with the San Francisco Bay and Central Valley Regional Water Boards on ammonia and toxicity related issues. We agreed with this decision in 2009 and encourage the State Water Board to continue coordinating with the Regional Water Boards.

There has been ongoing work on ammonia in the Delta since the 2009 Periodic Review and the 2010 Delta Flow Criteria hearings. Two important documents are discussed below and attached for your convenience.

In 2011, Lancelot et al. wrote a paper entitled *Rejoinder to "Perils of correlating CUSUM-transformed variables to infer ecological relationships" (Breton et al. 2006; Glibert 2010).* The Lancelot et al. (2011) document is important because it addresses criticisms of Glibert (2010) – a document that is often cited in Delta planning documents. In brief, Lancelot et al. (2010) states:

> In their comment, Cloern et al. (2011) develop theoretical evidence that cumulative sum of variability (CUSUM)-transformed variables should not be used to lead to inference due to the increase of auto-correlation. Indeed the use of statistical tools based on the independency between variables is misleading. The p-value associated to the tests described in Breton et al. (2006) and Glibert (2010) as well as in earlier papers (Ibanez et al. 1993; Le Fevre-Lehoerff et al. 1995; Choe et al. 2003) should be disregarded.

Another paper that has been cited frequently by the Regional Water Boards is Teh et al. (2011). SRCSD provided comments on Teh et al. (2011), most of which were addressed. However, more recently, Pacific EcoRisk (PER) provided an independent review of the paper. PER found flaws and erroneous calculations in the report. For example, using the same statistical software as Teh et al., PER's independent analysis of 31-day reproduction toxicity data resulted in lowest observed effect levels of 1.62 mg/L total ammonia nitrogen (TAN) when the article reported 0.79 mg/L TAN for juveniles. Likewise, independent analyses found a LOEC of >3.23 mg/L TAN for adults when the study reported a LOEL of 0.79 and 0.36 mg/L TAN.

Also, there was high variability within many of the test results that leads to great uncertainty on the reported results. This is especially true when significant results are reported despite the lack of clear dose-response relationships.

PER concluded that:

> The reviewer is troubled by the absence of any discussion by Teh et al. regarding the variability in their test response data, either between tests or within tests (i.e., inter-replicate variability). Without such acknowledgement, it is left for the non-scientist to assume that the data as presented are definitive. Moreover, it raises the question of whether the data from this study are adequate (or 'ready') for use in regulatory decision-making. However, it is important to note that this critical review is not intended to negate Teh et al.'s general observations that ammonia is toxic to

> naupliar, juvenile, and/or adult P. forbesi at elevated concentrations and that this toxicity is strongly influenced by pH. Indeed, the primary question of 'what are the effects of ammonia on P. forbesi' is relevant and Teh et al.'s study results certainly compel a more thorough examination of this. However, the problems associated with Teh et al.'s experimental methodology for Subtasks 3-3 and 3-4-1 and significant questions regarding the analysis of the resulting data do indicate that the quality of the work should preclude the resulting "critical threshold" data (i.e., NOECs, LOECs, and point estimates [e.g., ECx, LCx, and ICx values]) from being used for regulatory purposes.

Summary:

In conjunction with the record of comments, letters, and other material included in past Bay-Delta Plan efforts and the 2010 Delta Flow Criteria, please include the two attached scientific papers to the Bay-Delta record.

We recognize the hard work needed to update the Bay-Delta Plan, and we appreciate the opportunity to participate. We look forward to participating in the next workshop. Please contact me at mitchellt@sacsewer.com or (916) 876-6092 if you have any questions before then.

Sincerely,

Terrie Mitchell
Manager, Legislative and Regulatory Affairs

cc: Stan Dean, District Engineer
Prabhakar Somavarapu, Director of Policy and Planning

Attachments: Lancelot, et al. (2011)
Pacific EcoRisk, Inc. (2011)

L&O 11-252 - November 28, 2011 - 2nd revision

Rejoinder to "Perils of correlating CUSUM-transformed variables to infer ecological relationships (Breton et al. 2006; Glibert 2010)."

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In their comment, Cloern et al. (2011) develop theoretical evidence that cumulative sum of variability (CUSUM)-transformed variables should not be used to lead to inferences due to the increase of auto-correlation. Indeed the use of statistical tools based on the independency between variables is misleading. The *p*-value associated to the tests described in Breton et al. (2006) and Glibert (2010) as well as in earlier papers (Ibanez et al. 1993; Le Fevre-Lehoerff et al. 1995; Choe et al. 2003) should be disregarded.

We however, do not support the concluding remark of the paper that advises against any comparison of CUSUM-transformed variables. Indeed, such comparisons are useful as they visually accentuate transitions in time between independent variables, a task for which the CUSUM transformation is particularly efficient (Ibanez et al. 1993; Nichols 2001; Breaker and Flora 2009). If CUSUM-transformations of two independent series show transitions at the same time periods, there is a basis for assuming a direct or indirect relationship between those variables; there is most likely a common underlying mechanism (or mechanisms) that is (are) responsible for the similar transitions in the two series. As with any correlative approach, hypotheses resulting from such relations ultimately must be demonstrated by alternate methods.

For instance, the synchronism between CUSUM of diatom biomass and of the North Atlantic Oscillation (NAO) suggested in fig.3A, B of Breton et al. (2006) is supported by a large set of observational (Lancelot et al. 1987, 1995) and modeling (Gypens et al. 2007; Lancelot et al. 2007) papers all showing the importance of meteorological conditions and human activity on the watershed in driving the interannual variations of diatom and *Phaeocystis* colonies in the central Belgian coastal zone.

Similarly, long-term trends between nutrient concentrations and nutrient ratios and changes in abundances of multiple trophic levels, including fish, inferred from CUSUM analysis by Glibert (2010) in San Francisco Estuary, have been further shown using bivariate

analyses with original data as well as data adjusted for autocorrelation (Glibert et al. 2011). Glibert (2010) interpreted the change in delta smelt abundance, as well as changes in other fish species, along with other trends in nutrients, phytoplankton, and zooplankton, as an indirect effect due to multiple changes in the food web over time driven by bottom-up changes in both nitrogen and phosphorus loading, not as a singular or as a direct effect of ammonium on delta smelt.

In ecology, the application of CUSUM transformations for identifying links between meteorological, hydrological and ecological patterns has been recently increasing (Adrian et al. 2006; Molinero et al. 2008; Breaker and Flora 2009; Briceño et al. 2010) and the combination of CUSUM charts and bootstrapping has been identified as an important tool in regime shift analysis (Andersen et al. 2008). Therefore, while supporting the Cloern et al. (2011)'s cautious comment, we agree with those who have previously used CUSUM in ecological analysis, that comparisons of transitions in time, using CUSUM transformations, are useful for the identification of synchrony between time series.

Acknowledgements

The helpful comments of M. Auffhammer were appreciated in the preparation of this rejoinder. We also like to thank the L&O editor and three anonymous reviewers for their constructive comments.

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Pacific EcoRisk, Inc. (2011)

FINDINGS REPORT

From A Critical Review of:

Full Life-Cycle Bioassay Approach to Assess Chronic Exposure of *Pseudodiaptomus forbesi* to Ammonia/Ammonium - Final Report

Dated August 31, 2011

Prepared by: Teh S, Flores I, Kawaguchi M, Lesmeister S, Teh C
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Submittal Date:
December 26, 2011

1. INTRODUCTION

On behalf of the Central Contra Costa Sanitary District (CCCSD), Larry Walker Associates has contracted Pacific EcoRisk, Inc. (PER) to perform a critical review of the "Final Report: *Full Life-Cycle Bioassay Approach to Assess Chronic Exposure of Pseudodiaptomus forbesi to Ammonia/Ammonium*" authored by Teh S, Flores I, Kawaguchi M, Lesmeister S, and Teh C (dated August 31, 2011).). As requested by CCCSD, the primary focus of this review were the experiments described as Subtasks 3-3 and 3-4-1 in the Teh *et al.* report. Additional comments on study methodology and data analysis were developed and can be provided to interested parties on request as evidence that additional study is needed.

2. COMMENTS ON SUB-TASK 3-3 (CHRONIC [31-DAY] LIFE CYCLE TOXICITY TESTING)

Comment #1. Teh *et al.*'s analysis of the number of nauplii and number of juveniles produced during the chronic (31-day) exposure is believed to be flawed at a very fundamental level. It is apparent in Teh et al.'s derivation of 'mean number of nauplii, juveniles, and adult *P. forbesi* produced per female' (in Teh *et al.*'s Table 11) and in the 'sum total number of nauplii, juvenile, and adult *P. forbesi* produced' (in Teh *et al.*'s Appendix III table) that they summed the counts of nauplii and juveniles that were counted on the progressive 2-3 day intervals (the raw data for these counts were provided in Teh *et al.*'s Appendix I) as if each new progressive count was of new individuals that had not been counted on the previous count day. So when 17 nauplii were counted in Control replicate A on Day 5 of the test, and 20 nauplii were counted on Day 7, and 17 were counted on Day 10, and so on, Teh *et al.* summed these up as if they were different nauplii that had been produced during the progressive 'count days'.

This would be correct had the nauplii and juveniles that were counted on each 'count day' been removed from the original replicate container and transferred to a new replicate container such that any nauplii or juveniles observed and counted in the original replicate containers on subsequent days would have been new organisms separate and distinct from the organisms that had been counted during the previous count day(s). Note that this approach would have created a logistical challenge, with a doubling of the number of experimental replicate beakers on Day 3 of the test (going from the original n=20 to n=40), a tripling of the beakers on Day 5 (n=60), a quadrupling of beakers on Day 7 (n=80), and so on and so on. This would then be compounded as nauplii that had transformed into juveniles would again need to be transferred to new replicates so as to allow observation of new juveniles produced by the remaining nauplii. The number of necessary beakers rapidly becomes logistically improbable.

However, it is not believed that this is what happened. Unfortunately, their report's inadequate description of test methodology is not explicit on this. However, it can be deduced from the nature of the study that the neonates were left in place in each replicate, as these were the source of the subsequent juveniles, which were similarly left in place to serve as the source for the

subsequent adults. This was confirmed by inquiry made with one of the other authors of the report (M Kawaguchi, pers. comm.). As a result, when 20 nauplii were counted in Control replicate A on Day 7, some (if not most) of these organism were the very same organisms that had been counted on the earlier Day 5 count, and the nauplii that were counted on Day 10 were some of the same as had been counted on Days 7 and Day 5.

This conclusion is also supported by the following observations made for closely-related congener *Pseudodiaptomus annandalei* (Golez et al. 2004):

1. hatching of the first brood of nauplii occurs within 24-hrs of spawning;
2. females produced new ovisacs at ~ 1 day intervals, again with hatching occurring within that 24-hrs;
3. "females that were isolated from males produced only two clutches of viable eggs". Additional ovisacs were produced (making it appear that the female is reproductive), but the "succeeding clutches of eggs were aborted or shed off within 48 hrs and never hatched out".

Of course, the reproductive biology of *P. forbesi* may differ from that of the congener *P. annandalei*; however, in the absence of contradictory empirical evidence, Occam's razor would dictate otherwise.

We are left to conclude that **Teh *et al.*'s reported results for 'total number' and 'mean number per female' for the nauplii and juveniles are incorrect, and that their analyses of that data are similarly incorrect**.

Interestingly, in Teh *et al.*'s analyses of the 'total number' and 'mean number per female' of adults produced during the study, the number of adults counted on each progressive 'count day' were **NOT** summed in similar fashion, with Teh *et al.* instead evaluating on the count data from a single 'count day' (Day 31).

Comment #2. While it is believed that Teh *et al.*'s count data are incorrect, let us assume for a moment that they are in fact correct. The organism counts using Teh *et al.*'s summation method are summarized in Table 1 below. When their juvenile count data are analyzed using CETIS (a statistical software specifically designed to analyze aquatic toxicity data), the NOEC and LOEC are shown to be 0.79 mg/L TAN and 1.62 mg/L TAN (Table 2 below), NOT the lower concentrations reported by Teh *et al.*

It should noted that CETIS is the statistical software most commonly used by toxicity testing labs to analyze toxicity test data, and is believed to be the statistical software used at the UC Davis Aquatic Toxicology Lab; indeed, Teh *et al.* used CETIS to analyze their Subtask 3-4-1 and Subtask 3-4-2 experimental data as evidenced in Appendices IV and V of their report.

It should also be noted that our assessment of problems with Teh *et al.*'s statistical analyses should not be interpreted as indicating that there was no effect resulting from the ammonia, but

simply that the experimental data do not support any differences that were observed as being statistically significant.

Table 1. Production on *Pseudodiaptomus forbesi* nauplii, juveniles, and adults (from Appendix I in Teh *et al.* report)

Test Treatment (mg/L TAN)	Test Replicate	Total # of *Pseudodiaptomus forbesi* Life Stage Counted			
		Nauplii[A]	Juveniles[A]	Adults[A] (counts made only on Day 31)	Adults[B] (counts made as for nauplii & juveniles)
Control	A	86	38	11	93
	B	100	73	26	178
	C	68	45	7	122
	D	75	52	3	52
0.36	A	60	27	0	1
	B	62	57	3	36
	C	83	79	18	167
	D	71	43	7	77
0.79	A	24	48	10	77
	B	64	31	4	45
	C	41	17	1	17
	D	52	22	8	77
1.62	A	47	1	0	0
	B	32	0	0	0
	C	46	14	5	28
	D	54	23	19	108
3.23	A	15	1	1	4
	B	39	1	1	6
	C	42	18	13	83
	D	30	13	5	34

A - For the nauplii and juveniles, Teh et al. summed the progressive counts on successive days as separate individuals; as explained in our review, this is believed to be erroneous, and is inconsistent with the counts of the "produced" adults which consist of the number of adults that were alive on Day 31 of the test.

B - Counts of "produced" adults using the summation of the progressive counts on successive days as separate individuals (as used by Teh et al. for the nauplii and juveniles); as explained in our review, this is believed to be erroneous.

Table 2. Comparative analyses of juvenile and adult production in the 31-day test (from CETIS analysis of juvenile data using Teh et al. summation method)

Statistical Endpoint	Juveniles		Adults	
	Teh et al. Analyses	CETIS Analyses	Teh et al. Analyses	CETIS Analyses
NOEC =	0.36 mg/L TAN	0.79 mg/L TAN	**<0.36 mg/L TAN**	**3.23 mg/L TAN**
LOEC =	0.79 mg/L TAN	1.62 mg/L TAN	**0.36 mg/L TAN**	**>3.23 mg/L TAN**
Chronic Value =	1.13 mg/L TAN	1.13 mg/L TAN	<0.36 mg/L TAN	>3.23 mg/L TAN

Chronic Value = geometric mean of NOEC and LOEC.

Comment #3. Teh *et al.*'s apparently erroneous statistical analysis of the adult data is even more significant (Table 2). Teh *et al.* reported that the NOEC and LOEC for adults were <0.36 mg/L TAN and 0.36 mg/L TAN, respectively. However, their inter-replicate variability for that endpoint is so high (CVs ranged from 70% to 150%) that even qualitative evaluation suggests otherwise. CETIS analysis indicates that the NOEC and LOEC are 3.23 mg/L TAN and >3.23 mg/L TAN.

Again, it should be noted that our assessment of problems with Teh *et al.*'s statistical analyses should not be interpreted as indicating that there was no effect resulting from the ammonia, but simply that the experimental data do not support any differences that were observed as being statistically significant. Certainly, the NOECs and LOECs resulting from this experiment should not be considered suitable for use in a regulatory framework.

3. COMMENTS ON SUBTASK 3-4-1 (EFFECTS OF AMMONIA ON NAUPLII PRODUCTION OVER 3 DAYS)

Comment #4. In this test, Teh *et al.* exposed individual gravid female copepods to TAN concentrations of 0 (control treatment), 0.38, and 0.79 mg/L for 3 days after which the number of nauplii produced were counted. The results of this test have been summarized in the Table 3 below.

From data reported in Teh et al.'s Table 12 and Appendix V:

Table 3. Effects of ammonia on *P. forbesi* production of nauplii over 3 days (Teh *et al.* Subtask 3-4-1).

TAN Concentration (mg/L)	Mean # of Nauplii per Female
Control	7.6
0.38	5.5
0.79	5.4

The results from this test are somewhat troubling in that, while technically monotonically increasing as the ammonia concentration increases, no apparent concentration-response relationship is observed between the 0.38 mg/L treatment and the 0.79 mg/L treatment. One would expect that as the TAN concentration increases from 0.38 mg/L (a presumably toxic concentration) to 0.79 mg/L (a two-fold greater concentration), there should be an increase in the toxic response – this is a fundamental paradigm of toxicology.

We have already seen in the data evaluations presented above that there is variability in toxic responses made by these organisms. Indeed, in some cases, the variability has been so extreme as to preclude a meaningful statistical analysis (as in the case of the adult data from the 31-day test). The absence of the expected concentration-response in the current test (Table 3) suggests that variability in organism response is occurring (the CV was 48% in the 0.38 mg/L treatment) such that the treatment means may be deviating from the true population mean (in statistical terms, this is referred to as a "false positive" or a "false negative").

In the present case, it is impossible to determine which of the two test responses is deviating most from the true population mean response. However, it is worth noting that:

1. there were two replicates at the 0.38 mg/L treatment that had 10 nauplii (the highest number observed in ANY replicate) whereas there was only one replicate at the control treatment that had 10 nauplii, and
2. the CV at the 0.38 mg/L treatment was 48%, which was markedly higher than at the Control or 0.78 mg/L treatment.

This is suggestive that the variability at the 0.38 mg/L treatment was elevated and may have resulted in a false positive, such that the observed mean response of 5.5 nauplii per female was lower than the true population mean. If correct, then the conclusion(s) drawn from the test data may not reflect true conditions, and the true LOEC could be 0.79 mg/L, and not 0.38 mg/L. At a

minimum, the absence of the expected concentration-response should cast enough uncertainty on the test results as to make them inappropriate for regulatory decision-making.

Comment #5. It is fortunate that multiple sets of test data from the study allow comparison of results between tests; for instance, the results of Subtask 3-4-1 can be compared to those generated in the earlier Subtask 3-3 (31-day) test in which gravid females were exposed to varying concentrations of TAN and counts of nauplii produced after 3 days were counted, but were also counted after 5 days and 7 days (recall that counts made on progressive count days are not believed to be all new organisms). The Subtask 3-3 data are summarized in Table 4 below, along with the data from Task 3-4-1.

If one were to "cherry-pick" the Day 3 data and exclude the additional data, then Teh *et al.*'s conclusion for the Subtask 3-4-1 might stand. However, by extending the observation period beyond 3 days, it becomes evident that not only is there no reduction in nauplii production at 0.36 mg/L TAN, but nauplii production actually appears to be *increased* relative to the control treatment (the maximum mean # of nauplii on Day 5 at the 0.36 mg/L TAN treatment is **31% greater** than the highest mean # of nauplii produced in the Control treatment on any of the count days). Furthermore, CETIS analysis indicates that there were no statistically significant reductions in nauplii production at the 0.36 mg/L (Table 5). Even if we use the count summation used by Teh et al., by extending the counts beyond 3 days, it becomes apparent that there is no statistically significant difference between the response at 0.36 mg/L TAN and the Control treatment. This certainly creates a very significant uncertainty over the results of the Subtask 3-4-1 test of the effects of ammonia on nauplii production over 3 days.

It could be argued that this phenomenon is the result of ammonia having caused a delay in egg hatching, and the 31-day data are certainly suggestive of that. However, the only way to address that would have been to have some information from the scientific literature on the egg gestation period for this species, coupled with testing being performed under the current test conditions using females with egg sacs of the same age.

Table 4. Effects of ammonia on *P. forbesi* nauplii produced over 3 and 5 days.

Teh *et al.* Study Task	TAN Treatment (mg/L)	Mean Number of Nauplii per Female		
		Day 3	Day 5	Sum through Day 5 (Day 3 + Day 5)[A]
Subtask 3-4-1	Control	**7.6**	not counted	not counted
	0.38	**5.5**	not counted	not counted
	0.79	**5.4**	not counted	not counted
Subtask 3-3	Control-A	5.67	6.67	12.33
	Control-B	6.67	6.67	13.33
	Control-C	5	5	10
	Control-D	5	5	10
	treatment mean	**5.6**	**5.8**	**11.4**
	0.36-A	3	5	8
	0.36-B	2.33	8.33	10.67
	0.36-C	3.33	8.33	11.67
	0.36-D	3.33	3.33	6.67
	treatment mean	**3.0**	**6.3**	**9.3**
	0.79-A	0.33	1.67	2
	0.79-B	6.67	3.33	10
	0.79-C	2.67	2.67	5.33
	0.79-D	6.67	4	10.67
	treatment mean	**4.1**	2.9	**7.0**

A – These counts are made using method of Teh *et al.*, which assumes that the progressive counts on successive days are separate individuals; as explained in our review, this is believed to be erroneous.

Table 5. Comparison of nauplii production test results (all results expressed as mg/L TAN) (from CETIS analysis of data)

Statistical Endpoint	**Subtask 3-4-1**	**Subtask 3-3**				
	Day 3	Day 3	Day 5	Day 3 + Day 5[A]	Total (31 days)[A]	Total (31 days)[B]
NOEC =	<0.38	3.23	0.36	0.36	0.36	0.79
LOEC =	0.38	>3.23	0.79	0.79	0.79	1.62
Chronic Value =	<0.38	>3.23	0.53	0.53	0.53	1.13

Chronic Value = geometric mean of NOEC and LOEC.

A – These counts are made using method of Teh *et al.*, which assumes that the progressive counts on successive days are separate individuals; as explained in PER's review, this is believed to be erroneous.

B – These counts are made using what is believed to be the best remaining method: identifying the maximum number of nauplii observed on any given day for each replicate (this assumes that the individuals were left in the replicate beakers and were counted again and again on progressive days [i.e. repeated measures]).

4. FINAL COMMENT

The reviewer is troubled by the absence of any discussion by Teh et al. regarding the variability in their test response data, either between tests or within tests (i.e., inter-replicate variability). Without such acknowledgement, it is left for the non-scientist to assume that the data as presented are definitive. Moreover, it raises the question of whether the data from this study are adequate (or 'ready') for use in regulatory decision-making. However, it is important to note that this critical review is not intended to negate Teh *et al.*'s general observations that ammonia is toxic to naupliar, juvenile, and/or adult *P. forbesi* at elevated concentrations and that this toxicity is strongly influenced by pH. Indeed, the primary question of 'what are the effects of ammonia on *P. forbesi*' is relevant and Teh *et al.*'s study results certainly compel a more thorough examination of this. However, the problems associated with Teh et al.'s experimental methodology for Subtasks 3-3 and 3-4-1 and significant questions regarding the analysis of the resulting data do indicate that the quality of the work should preclude the resulting 'critical threshold' data (i.e., NOECs, LOECs, and point estimates [e.g., ECx, LCx, and ICx values]) from being used for regulatory purposes.

References Cited:

Golez MSN, Takahashi T, Ishimaru T, Ohnoa A (2004) Post-embryonic development and reproduction of *Diaptomus annandalei* (Copepoda: Calanoida). Plankton Biology & Ecology 51(1):15-25.