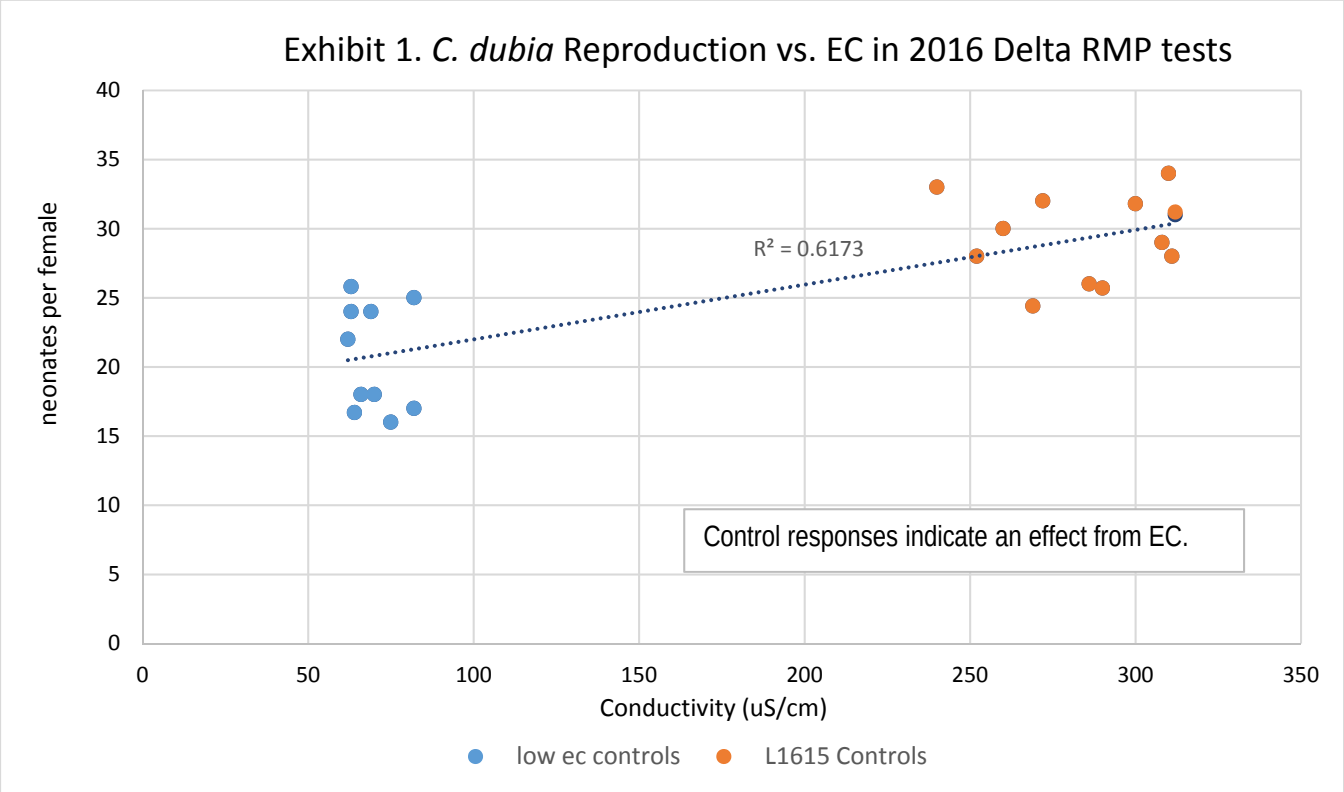
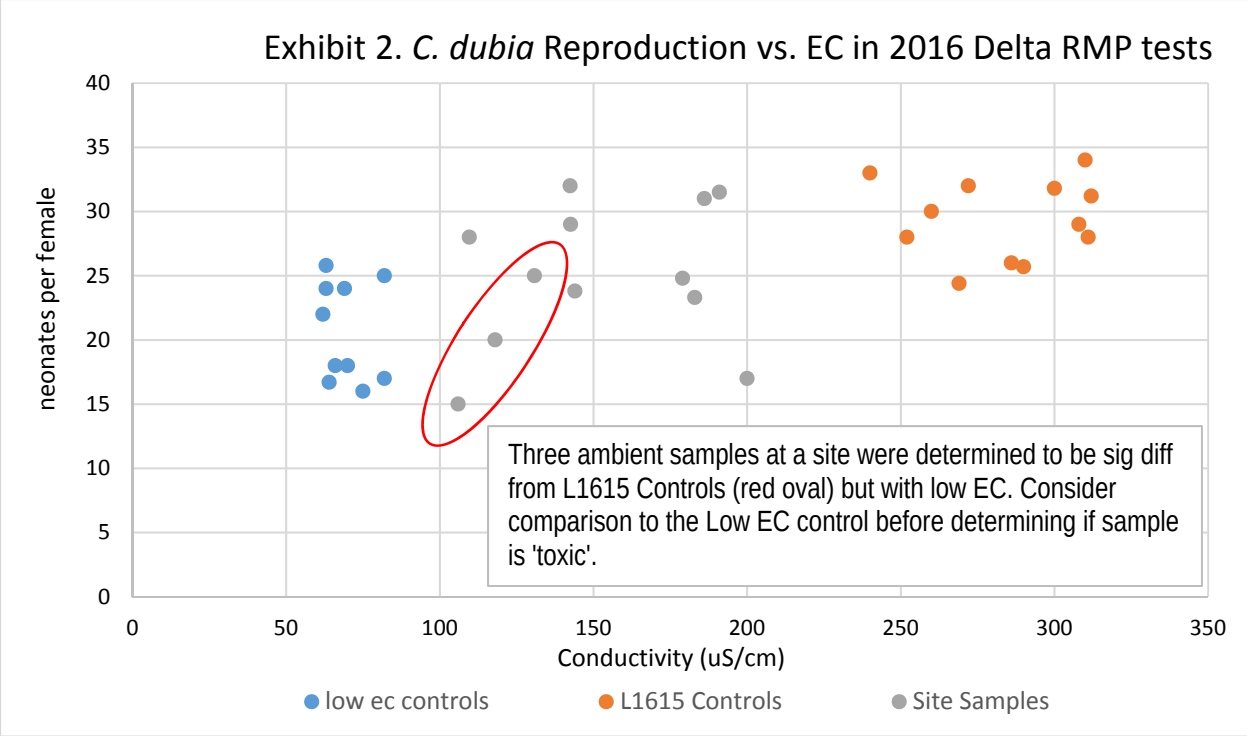
A discussion by the Delta RMP TAC Pesticide Subcommittee (and AHPL) is warranted, based on the *C. dubia* control results to date, about comparisons to the appropriate controls. It is good that AHPL evaluated low EC controls starting in November, 2015, as there appears to be an effect on *Ceriodaphnia* reproduction associated with low EC (Exhibit 1).




AHPL protocol is to compare samples with EC <100 uS/cm with the low EC control. Sample performance is otherwise compared with the L1615 control (EC 240-312 uS/cm). However, at least a few samples with low EC (but not <100) differed significantly from the L1516 control but may or may not differ from the low EC control (Exhibit 2). The current analysis and determination of toxicity does not consider an EC effect for these samples even though it seems warranted. All of the low EC controls in these tests were significantly lower than the associated L1615 controls.




The RMP TAC Pesticide Subcommittee (and AHPL) should give further consideration of how to appropriately evaluate effects relative to the appropriate controls so we are accurately characterizing sample toxicity with effect due to contaminants. Comparison to the Low EC control before concluding that a sample is exhibiting 'toxicity' would be appropriate for samples with EC closer to the low EC control than the culture water control. EPA (2000) provides guidance on dual controls and recommends culturing organisms that are acclimated to the expected ambient water quality conditions to avoid this artifact (see attached SWAMP discussion on salinity/conductivity control issues with reference to EPA guidance). The Delta RMP could follow these recommendations by adding a low EC culture for organisms acclimated to these water quality conditions.

The RMP pesticide report should indicate where samples are significantly different from both controls, and might want to avoid referring to "toxicity" for samples that have low EC and are not significantly lower than the low EC control while data interpretation is being considered. Low EC could be defined by a threshold other than 100 uS/cm that is agreed upon (e.g., 150 us/cm) or could apply when the site samples EC is closer to the low EC control than to the L1615 control.

**References**

U.S. EPA, 2000. Method guidance and recommendations for whole effluent toxicity (WET) testing (40 CFR Part 136). EPA/821/B-00/004. Office of Water, U.S. Environmental Protection Agency, Washington, D.C. 20460.

**Date:** July 8, 2013

**To:** Surface Water Ambient Monitoring Program (SWAMP) Round Table

**From:** SWAMP Toxicity Work Group

**Subject:** Salinity/Conductivity Control Issues

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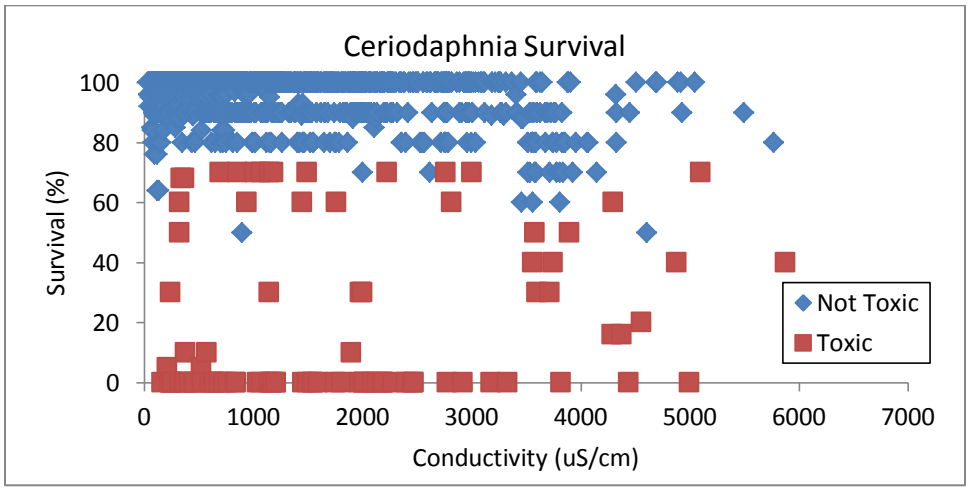
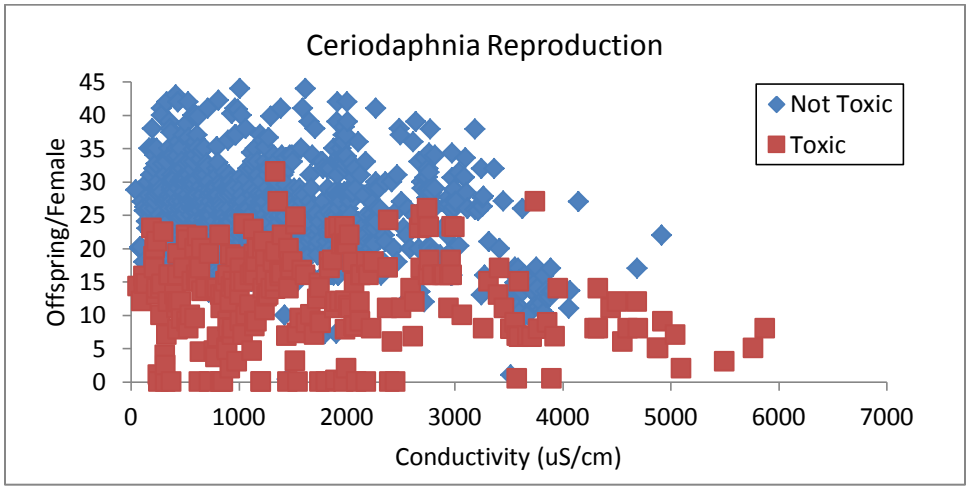
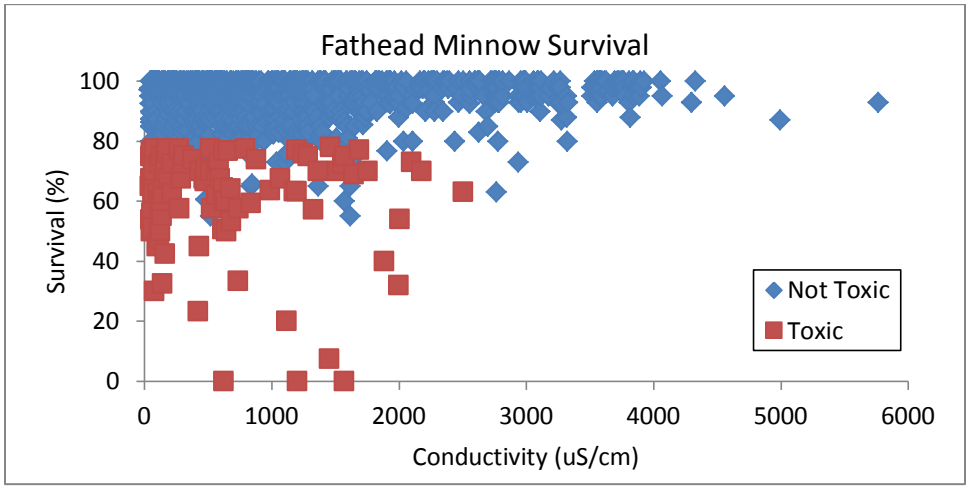
## Issue 1 - Provide specific guidance for organism selection based on conductivity

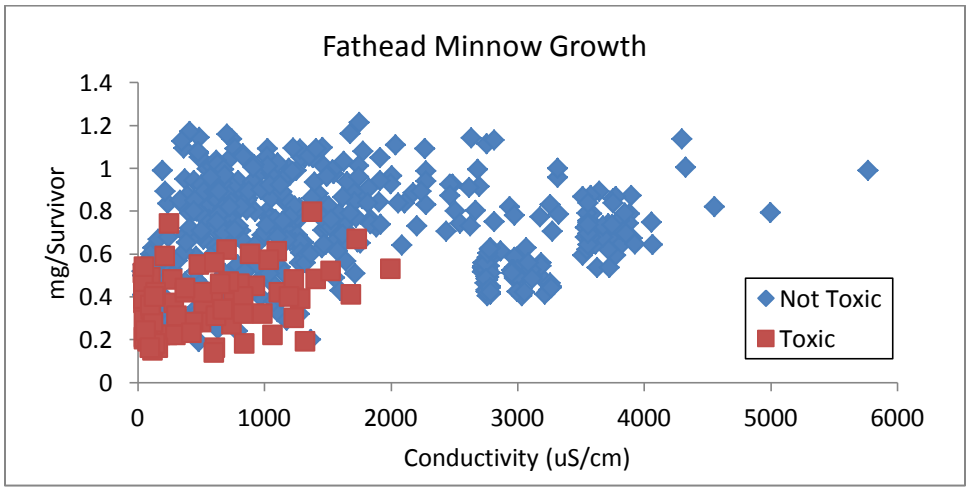
We are re-examining the conductivity tolerance of freshwater toxicity test species as part of the new SWAMP QAPrP. The SWAMP database was queried for paired toxicity test results and conductivity concentrations. The following tables represent the results of these queries.



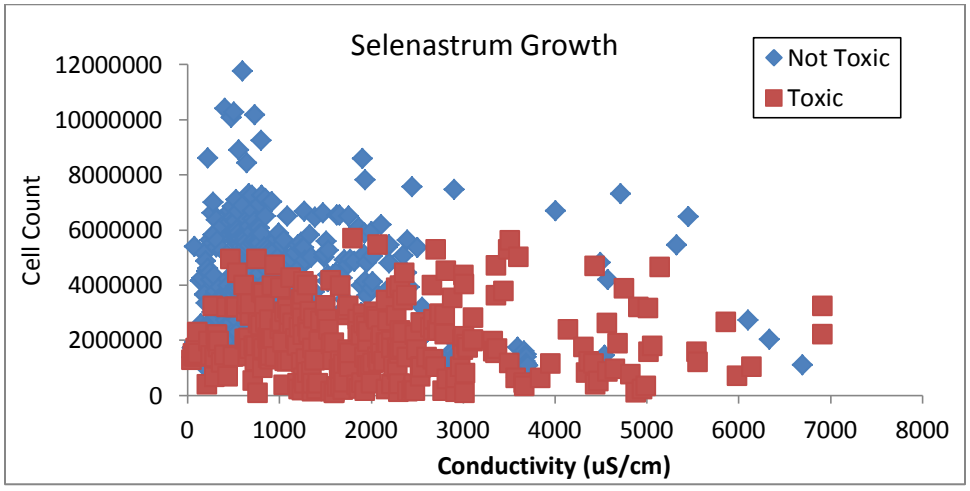




*Ceriodaphnia* survival is not adversely affected until conductivity exceeds 5000 µS/cm, but reproduction is likely affected at conductivities greater than 4000 µS/cm.




Fathead minnow survival and growth do not appear to be affected by elevated conductivity. All of the samples with conductivities greater than 3000 µS/cm were not toxic.



*Selenastrum* appears to be sensitive at higher conductivities, but because the results can be variable, lower growth responses can be considered not toxic.

## Recommendations

SWAMP provides some guidance for organism selection based on the conductivity or salinity of the sample. The following are excerpts from the Toxicity MQO Tables with additional recommended text in **bold**:

*Ceriodaphnia dubia* – Include appropriate controls when sample conductivities are 0 – 100, or >1900 µS/cm. Substitute with *Hyalella azteca* if conductivity is >2500 µS/cm.

*Pimephales promelas* – Include appropriate controls when sample conductivities are 0 – 100, or above 1900 µS/cm. **Consider an alternate species at conductivities greater than 6000 µS/cm (e.g., *Atherinops affinis*).**

*Selenastrum capricornutum* – Include appropriate controls when sample conductivities exceed 1500 uS/cm. **If conductivity exceeds 3000 uS/cm, use alternate species or forego testing.**

## Issue 2 – Incorporate statistical procedures for dual controls in the SWAMP database

The SWAMP database currently compares all sample responses to the corresponding response in the dilution control. As described above, SWAMP recommends the use of salinity/conductivity controls when the salinity or conductivity of the sample is near the organism's threshold of tolerance. There is currently no provision for using the additional control in the statistical comparison.

U.S. EPA provides guidance for using dual controls (U.S. EPA, 2000). In the excerpt below, guidance is provided for the use of dilution controls versus culture controls. Similar procedures could be used for salinity/conductivity controls. There are precedents for these procedures in the Marine Bioassay Project reports, and also the commonly used statistical software CETIS.

The following is from U.S. EPA (2000)

***When and how do I use dual controls?***

*When the dilution water used in a test differs from the water used to culture, hold, and maintain the test organisms, an additional set of dilution water controls should be evaluated in the WET test. This is generally the case when natural receiving water or adjusted synthetic water is used for dilution, but additional controls also may be necessary for standard synthetic dilution waters if organisms are cultured in alternative water. A culture water control should consist of 100% culture water, and a dilution water control should consist of 100% of the dilution water used in the test. These two controls*

*should be run concurrently in the test and undergo the same test conditions. Prior to the analysis of test treatment data, the two controls (dilution water control and culture water control) should be compared to determine if statistically significant differences exist. This comparison should be made using a t-test as described in Appendix H of the freshwater method manual (USEPA, 1994a) and Appendix G of the marine method manual (USEPA, 1994b). If there is no statistically significant difference between the two controls, the dilution water control should be used for further analysis and comparisons with the treatment groups. If a receiving water control is significantly different from the culture control, this may indicate ambient toxicity in the receiving water. In this case, the use of synthetic dilution water adjusted to approximate the receiving water may be more appropriate. If adjusted synthetic dilution water shows a significant difference from the culture control, this generally indicates that either the chemical adjustments of the dilution water were outside of the tolerance range of the test organism or acclimation of the test organisms to the dilution water is necessary. In this situation, the analyst should consider using organisms cultured in water more similar to the dilution water or consider acclimatizing the test organisms to the adjusted dilution water prior to the test. These options, however, may increase test cost and may be impractical for laboratories that test effluents from numerous dischargers, each with specific dilution water requirements. For this reason, local regulatory authorities may wish to reevaluate test objectives for this effluent and consider the use of standardized synthetic water (U.S. EPA, 2000).*

## Recommendation

Follow the guidance for use of dual controls provided in the MQO Tables. Both controls must meet test acceptability criteria, but if the secondary control is significantly different from the primary control, then the secondary control should be used for further statistical analysis in the determination of sample toxicity.

## Reference

U.S. EPA, 2000. Method guidance and recommendations for whole effluent toxicity (WET) testing (40 CFR Part 136). EPA/821/B-00/004. Office of Water, U.S. Environmental Protection Agency, Washington, D.C. 20460.