



Memorandum

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District
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CC: Linda Dorn, SRCSD
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SUBJECT: Comments Regarding the Regional Board
Staff Analysis of the 2009 Ammonia
Summit

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On August 18-19, 2009, the Central Valley Regional Water Quality Control Board (Regional Board) held a two-day workshop (“Ammonia Summit”) consisting of scientific presentations and panel discussions addressing the potential role of ammonia in the Bay-Delta ecosystem. A Regional Board staff analysis summarizing the Ammonia Summit was prepared by Chris Foe in late September, 2009¹. Mr. Foe’s review was intended to help answer two questions:

- Does ammonia cause beneficial use impairments in the Delta?
- Are additional studies needed to answer the above question?

Mr. Foe’s main conclusion was that no evidence has been collected to date demonstrating that ammonia concentrations are causing beneficial use impairments in the Sacramento River or Delta. Two issues were selected by Mr. Foe as key remaining uncertainties:

1. Does ammonia influence the nutritional quality of phytoplankton?

¹ The staff analysis is posted on the Regional Board’s Ammonia Summit web page:
http://www.swrcb.ca.gov/rwqcb5/water_issues/delta_water_quality/ambient_ammonia_concentrations/index.shtml.

2. How do current USEPA criteria and ambient ammonia levels compare to (as yet unknown) chronic effects thresholds for key taxa?

Selection of these issues was prompted, in part, by conclusions of several Ammonia Summit presenters which rely on assumptions or approaches which were not scrutinized in Mr. Foe's summary. Below, comments are provided which illustrate concerns about some of these approaches. Also, a few important findings from the Ammonia Summit are described that contradict hypotheses about ammonia's potential role at the base of the Delta food web, which were not included in Mr. Foe's summary. Comments fall into the following general workshop topics:

- Use of ACRs to infer chronic toxicity of ambient ammonia levels in the Delta
- Dave Fullerton's correlation approach
- Ammonium inhibition of primary production

Use of ACRs to infer chronic toxicity of ambient ammonia levels in the Delta

As acknowledged by Mr. Foe in his summary, acute-chronic ratios (ACRs) are being used by several investigators, in lieu of chronic toxicity test results, to postulate (1) that USEPA ammonia criteria are not sufficiently protective of sensitive species in the Delta, and (2) ambient concentrations of ammonia in the Delta may be causing chronic toxicity to sensitive species.

“At the Summit, three research groups lead by Drs. Michael Johnson, Inge Werner and Swee Teh independently concluded that U.S. EPA ammonia criteria are not sufficiently protective of sensitive resident species in the Delta. All will be completing reports within the next six months. Their conclusions rely upon ACR evaluations and there is always uncertainty about whether the correct ACR has been selected.” (Foe 2009a)

It is important that the applicability of the ACRs being used for extrapolation of test results between species is carefully examined. Review of the ACRs used by Ammonia Summit presenters was not part of Mr. Foe's summary. Below we provide comments about the ACRs that were used in the Ammonia Summit, and whether some of the inferences about ambient toxicity stemming from recent use of ACRs are supported by available monitoring data from the Delta.

Use of Hypothetical ACRs for Rainbow Trout to Infer Chronic Toxicity for Delta Smelt

Hypothetical ACRs for rainbow trout were used in Inge Werner's Ammonia Summit presentation (Werner 2009), and in recent reports to the Central Valley Regional Water Quality Control Board (Werner et al. 2009a,b), to support an argument that chronic exposure to ambient levels of ammonia in the Delta may cause toxicity for Delta smelt. This logic behind the argument can be summarized as follows:

- Chronic toxicity test results are lacking for Delta smelt.
- Delta smelt appear to be as acutely sensitive to ammonia as rainbow trout.

- Therefore, chronic toxicity values for Delta smelt are probably similar to those for rainbow trout.
- Hypothetical ACRs for rainbow trout are alleged to be in the range 14.6-23.5.
- Therefore, one can divide the LC50 for delta smelt (acute value) by hypothetical ACRs for rainbow trout to estimate the concentration of ammonia that would cause chronic toxicity to Delta smelt
- Some ambient ammonia concentrations in the Delta are higher than the numbers that result from this exercise.

Below we provide information which shows that the hypothetical ACRs for rainbow trout stated above (14.6 and 23.5) rely on information that was excluded by USEPA in 1999 and 2009 for use in developing the chronic criterion and are not based on evidence for chronic effects of ammonia effects on survival, reproduction, or growth of rainbow trout. Consequently, inferences about chronic toxicity for other species - such as Delta smelt - based on these ACRs are questionable and should be carefully qualified.

USEPA Position on Valid Chronic Endpoints and Chronic Test Design for Fish, and Interpretation of Chronic Data for Rainbow Trout

In 1999, USEPA used explicit criteria to re-evaluate the available chronic toxicity tests for fish and aquatic invertebrates (USEPA 1999). One result of this analysis was a list of acceptable chronic tests. This list appears as Table 5 (“EC20s from Acceptable Chronic Tests”) on page 65 of USEPA (1999), along with Species Mean Chronic Values (SMCV) and Genus Mean Chronic Values (GMCV) where it was appropriate to calculate them. Among the criteria for inclusion in this list were (1) the test had to be a flow-through test (except that static renewal is acceptable for daphnids), (2) test conditions had to include acceptable dissolved oxygen concentrations, and (3) the endpoint(s) of the test had to be survival, growth, and/or reproduction.² Where possible, regression analysis was used to generate EC20s for many of the acceptable studies.

In order for a chronic test to be used as part of the basis for a SMCV in USEPA (1999), it had to satisfy the definitions given in the USEPA (1985a) *Guidelines for Deriving Numerical Criteria* for a “life-cycle”, “partial-life-cycle”, or “early-life-stage” test. These criteria *as they apply to fish* are provided in Table 1 below.

² USEPA does not utilize concentrations associated with histopathologic or behavioral endpoints (e.g. swimming speed) for SMCV derivation because they have determined that there is “no justification for equating histopathological effects with effects on survival, growth, and reproduction” (USEPA 1999, p. 45). This position is more fully explained in Appendix 5 in USEPA (1999), and was maintained in the 2009 Draft Update, released on December 30, 2009 (USEPA 2009).

Table 1. USEPA Criteria for Life-Cycle, Partial-Life-Cycle, and Early-Life-Stage Chronic Toxicity Tests for Fish.

Test Type	Fish Test Criteria	Data should Include	Potentially used to Derive:
Life cycle	<ul style="list-style-type: none"> • Tests must begin with embryos or newly hatched young <48-hrs old • Test must continue through maturation and reproduction • Test should not end less than 90 days after hatching of the next generation (24-hrs for non-salmonids). 	<ul style="list-style-type: none"> • Survival and growth and adults and young • Maturation of males and females • Eggs spawned per female • Embryo viability (salmonids) • Hatchability 	Depending on results: <ul style="list-style-type: none"> • Upper limit for a CV • Lower limit for a CV • CV
Partial life cycle	<ul style="list-style-type: none"> • Allowed for use with fish that require more than a year to reach sexual maturity. • Test must begin with immature juveniles at least 2 months prior to active gonad development. • Test must continue through maturation and reproduction. • Test should not end less than 90 days after hatching of the next generation (24-hrs for non-salmonids). 	<ul style="list-style-type: none"> • Survival and growth and adults and young • Maturation of males and females • Eggs spawned per female • Embryo viability (salmonids) • Hatchability 	
Early life-stage	<ul style="list-style-type: none"> • Test must begin shortly after fertilization of eggs. • Test must continue through embryonic, larval, and early juvenile development. • Test must continue for 60 day post hatch for salmonids (28-32 days for non-salmonids). 	<ul style="list-style-type: none"> • Survival and growth 	

If not meeting the criteria for any of the three test categories in Table 1, USEPA guidelines allow for *potential limited* use of data from two alternative types of tests involving fish:

1. Seven-day tests of survival, reproduction, and/or hatchability, or
2. Ninety-day tests of growth

USEPA requires that such alternative tests using *growth* as an endpoint must last for at least 90 days because reductions in weight gain for fewer than 90 days can be temporary. Per the USEPA (1985a) guidelines, neither of the two alternative types of test above should be used as the basis for a discrete chronic value for a species. However, such tests can be used as evidence for an upper limit for a chronic value (in other words, determinations that the true chronic value is *likely less than* the threshold concentration observed in the test).

The list of acceptable chronic tests for fish and their associated EC20s, and SMCVs and GMCVs (standardized to pH=8 and T=25°C) that resulted from the 1999 vetting process are provided in Table 2 below. Not all of the acceptable chronic tests included in USEPA Table 5 resulted in specific EC20s, or SMCVs. When *none* of the concentrations used in an acceptable chronic test caused significant effects on survival, growth, or reproduction, the highest concentration from the test was entered in USEPA Table 5 as “>x” to indicate

that underlying (unknown) EC20 was not equivalent to the concentration in the table for that test, but higher than the concentration by an unknown amount. Conversely, if *all* of the concentrations used in an acceptable test caused significant effects on survival, growth, or reproduction (i.e., none of the concentrations were “no-effects concentrations”, or NOECs), the lowest concentration from the test was entered in the table as “<x” to indicate that the underlying (unknown) EC20 was not equivalent to the concentration in the table for that test, but less than the concentration by an unknown amount. “Less than” or “greater than” qualifiers were also applied to some of the SMCVs and GMCVs calculated by USEPA.

Table 2. EC20s and other Toxicity Parameters Accepted by USEPA (1999) from Chronic Tests Meeting USEPA Test Acceptability Criteria for Fish.

Species	EC20s	Species Mean Chronic Value at pH=8 & 25°C (mg N/L total ammonia)	Genus Mean Chronic Value (GMCV) at pH=8 & 25°C (mg N/L total ammonia)	Genus Mean Acute-Chronic Ratio (GMACR)
<i>Pimephales promelas</i> (fathead minnow)	1.97 2.92 5.12	3.09	3.09	10.9
<i>Catostomus commersoni</i> (white sucker)	>4.79	>4.79	>4.79	<8.4
<i>Ictalurus punctatus</i> (channel catfish)	8.38 9.33 <8.7 to <9.9	8.84	8.84	2.7
<i>Lepomis cyanellus</i> (green sunfish)	7.44 4.88	6.03	2.85	7.6
<i>Lepomis macrochirus</i> (bluegill)	1.35	1.35		
<i>Micropterus dolomieu</i> (smallmouth bass)	3.57 4.01 6.5 4.65	4.56	4.56	7.4
<i>Oncorhynchus clarki</i> (cutthroat trout)	<19.7	Not Available: USEPA determined it was inappropriate to calculate SMCVs for <i>Oncorhynchus</i> species (see text).	Not Available	Not Available
<i>Oncorhynchus mykiss</i> (rainbow trout)	>5.4(a) <18.7(b) <1.44(c) 1.34(d)			
<i>Oncorhynchus nerka</i> (sockeye salmon)	<4.16			

(a) based on the highest concentration tested by Thurston et al. (1984)

(b) based on LC50s obtained over 42-days by Burkhalter & Kaya (1977)

(c) based on 73-day LC20 obtained by Solbe & Shurben (1989)

(d) based on test results by Calamari et al. (1977, 1981), interpolated by USEPA to estimate a 72-day LC20

USEPA determined that EC20s from five tests using rainbow trout were from acceptable chronic tests. However, as a group, the EC20s for rainbow trout did not meet USEPA standards for further use in calculating SMAVs, or for use in calculating a GMCV for its genus *Oncorhynchus*:

“Because of the concerns about some of the tests, the differences among the results, and the fact that some of the results are either “greater than” or “less than” values, even though the various results are included in Table 5, a SMCV is not derived for rainbow trout; instead the results of the chronic tests will be used to assess the appropriateness of the CCC”. (USEPA 1999; p. 60)

No additional chronic test results for rainbow trout were included in the recently released USEPA Draft 2009 Update for the freshwater ammonia criteria (USEPA 2009), in which USEPA again declined to calculate a SMCV for rainbow trout.

“As noted in the 1999 AWQC document, five other studies have reported results of chronic tests conducted with ammonia and other salmonids including *Oncorhynchus mykiss* and *Oncorhynchus nerka*. There is a lack of consistency among the chronic values obtained from these tests, and several tests produced "greater than" and "less than" values (Table 5). Consequently, in keeping with the decision made in the 1999 AWQC document, a GMCV is not derived for *Oncorhynchus*. Instead, the results of the chronic tests were used to assess the appropriateness of the CCC.” (USEPA 2009, p. 21)

In Appendix 7 of USEPA (1999), Acute-Chronic Ratios (ACRs) were calculated for all EC20s that *were* used to generate SMCVs (from USEPA 1999, Table 5) *and* which could be paired with comparable acute values (LC50s; see more about pairing criteria below). Then, these ACRs were used to calculate Genus Mean Acute Chronic Ratios (GMACR). This analysis resulted in GMACRs for five genera of fish, which are included in Table 2. The USEPA-vetted GMACRs for fish occupy the range 2.7-10.9.

Origin of Postulated ACRs for Rainbow Trout Being Used to Infer Chronic Toxicity for Delta Smelt

In her Ammonia Summit presentation, Dr. Inge Werner provided two values as the upper and lower limits for the ACR for rainbow trout (14.6-23.5; Werner 2009a, slide 10). The derivation of these values was not a part of Werner’s talk at the Ammonia Summit. The same values were presented in the 2008 and 2009 annual reports for the UC Davis Aquatic Toxicology Lab’s Delta smelt ammonia toxicity tests (Werner et al. 2009a, b) as follows (language is from 2009 report; almost identical passage occurs in 2008 report):

“Exposure duration is an important factor influencing the toxicity of ammonia. Seven-day toxicity tests, as performed in this study, are unable to detect the potential chronic effects of ammonia/um exposure on delta smelt. Acute-to chronic ratios are one method that has traditionally been used to extrapolate between acute and chronic toxicity when procedures for chronic testing are not available. For fish, the US EPA (1999) reports mean acute-to-chronic ammonia/um ratios for warm water fish that range between 2.7 (channel catfish, *Ictalurus punctatus*) and 10.9 (fathead minnow, *P. promelas*). Cold water species such as rainbow trout, with acute ammonia/um sensitivity similar to delta smelt, have a ratio between 14.6 and 23.5, respectively (US EPA, 1999; Passell et al., 2007). If these safety factors were applied to acute effect concentrations for effluent and delta smelt larvae (7-d LC50:

3.92 mg/L)³ then the resulting threshold concentrations for total ammonia/um would be 0.27 and 0.17 mg/L for the above safety ratios of 14.6 and 23.6, respectively. These chronic effect thresholds are below long-term average concentrations in the Sacramento River below SRWTP.” (Werner et al. 2009b)

The passage above can be interpreted to mean that rainbow trout ACRs of 14.6 and 23.5 were derived by USEPA or by Passell et al. (2007). However, neither of these references provide ACRs for rainbow trout. As explained above, in 1999 and 2009, USEPA refused to calculate an ACR for rainbow trout - or for even for the genus *Oncorhynchus* - owing to inadequate data. Chronic toxicity tests were not a part of the original work reported in Passell et al. (2007). As clarification, Dr. Werner explained that she had calculated the ACRs for rainbow trout as follows:

“I used the chronic values for unionized ammonia provided in Table 3 of Passell et al. (0.031 mg/L and 0.05 mg/L), and the species mean acute value from EPA 1999 (given in total ammonia/um)⁴ to calculate the corresponding value for unionized ammonia (0.728 mg/L unionized ammonia), then calculated the ratio between them [which] results in 14.56 and 23.5.” (I. Werner, pers. comm., Dec. 22, 2009).

Table 3 in Passell et al. (2007) is a collection of acute and chronic values for several fish species from the literature that was included for discussion purposes in the article. In the table, Thurston et al. (1984) and Burkhalter & Kaya (1977) are cited as the original sources of the 0.031 and 0.05 mg/L un-ionized ammonia-N concentrations, respectively. The original sources of the values are not critically evaluated in the article. Below, we discuss the original studies, and associated information about them in USEPA (1999). The results indicate that the chronic concentrations Dr. Werner used to compute ACRs for rainbow trout did not meet USEPA criteria for such use.

Thurston et al. (1984). Thurston et al. (1984) was a 5-year life cycle test which exposed offspring from one pair of rainbow trout, and their F1 and F2 progeny, to the following mean concentrations of un-ionized ammonia in flow-through troughs: 0.001, 0.013, 0.022, 0.044, 0.063, and 0.074 mg N/L. Regarding this study, USEPA (1999) states that “the important data for each life stage are so variable that it is not possible to discern whether there is a concentration-effect curve” (USEPA 1999; p. 58). According to the original article, there was no significant relationship between ammonia concentration and (1) mortality of all three generations, (2) growth of F1 and F2 progeny⁵, or (3) egg production. Because none of the exposure levels used by Thurston et al. (1984) caused significant effects on survival, growth or reproduction, the results of this test fell under the “greater than” category of chronic test results in USEPA (1999). In other words, USEPA concluded that the underlying (unknown) chronic value for rainbow trout must be greater than the highest test concentration used in the study (5.4 mg/L total ammonia-N at pH=8, T=25°C).

³This appears to be a mistake in Werner et al. (2009b). 3.92 mg/L was the 7-day LOEC for this test. The LC50 was 5.40 mg/L (see Werner et al. 2009b, p. 15).

⁴ The species mean acute value for rainbow trout in USEPA (1999) is 11.23 mg/L total ammonia-N (standardized to pH=8, 25°C).

⁵ It was not possible to evaluate growth of the parental fish because they were not weighed at the start of the test.

Passell et al. (2007) do not explain why they identified 0.031 mg/L un-ionized ammonia-N as an appropriate chronic value from Thurston et al. (1984), or why it merited status as one of only two chronic concentrations for rainbow trout to include in their article. Because *none* of the test concentrations in Thurston et al. (1984) resulted in significant effects on survival, growth, or reproduction for 3 generations of fish, no EC20s (or other effects concentrations) are available from this test for approved endpoints. Earlier USEPA criteria documents (Table 2 in both USEPA 1985b, 1989) list 0.031 as a chronic value for Thurston et al. (1984) which - after comparison of the original article with associated text in USEPA 1984 - appears to have been calculated using a NOEC and LOEC related to epidermal cell changes. However, this interpretation of the results from Thurston et al. (1984), which depends on the use of a non-conventional endpoint, was rejected in both of the most recent USEPA criteria documents (1999, 2009).

Burkhalter & Kaya (1977). Burkhalter & Kaya (1977) did not report EC20s for rainbow trout. Instead, they reported LC50 results from a 42-day exposure of rainbow trout embryos and sac fry. Because the study did not provide LC20s, the results of this test fell under the “less than” category of chronic test values. In other words, USEPA concluded that the underlying (unknown) chronic value would have been less than the LC50 from their study (18.7 mg/L total ammonia-N at pH=8, T=25°C). However, the value of 0.05 mg/L unionized ammonia-N attributed to Burkhalter & Kaya (1977) in Passell et al.’s table (which was ultimately used by Dr. Werner to generate one of her ACRs for rainbow trout) is *not* that associated with the LC50 from their study (which was 0.25 mg/L unionized ammonia-N). The only available explanation for Passell et al.’s identification of 0.05 mg N/L as a chronic value from Burkhalter & Kaya is that 0.05 mg N/L was the lowest exposure concentration they used, which caused “some retardation of early growth and development” (quote from abstract of Burkhalter & Kaya). However differences in growth rate at this low test concentration (0.05) compared to the control were slight, and disappeared after two weeks of exposure. Because of the short duration of Burkhalter & Kaya’s test, it was not considered by USEPA in 1999 as an appropriate test to gauge the effects of ammonia on growth on early life stages of rainbow trout.

As indicated above, Thurston et al. (1984) and Burkhalter & Kaya (1977) are discussed in USEPA (1999) and were two of the rainbow trout studies included in the list of acceptable chronic studies (see EC20 values in Table 2 above). However, as explained above, after re-evaluation of these two studies, USEPA interpreted the results of these two studies as evidence for an EC20 *greater than* 5.4 mg/L total ammonia-N (Thurston et al. study) and *less than* 18.7 mg/L total ammonia-N (Burkhalter & Kaya study; both values standardized to pH=8, 25°C). Taken in isolation from other chronic tests, USEPA’s upper and lower limits from these two studies imply that the rainbow trout ACR falls somewhere within the range (0.60-2.08)⁶ - which is very different than the one proposed by Dr. Werner (14.6 - 23.5).

A recent 90-day chronic test measuring the hatching success of newly fertilized eggs from a wild strain of rainbow trout, and subsequent survival and growth of sac fry and swim-up fry (Brinkman et al. 2009), resulted in a chronic value (the geometric mean of

⁶ 11.23/18.7 = 0.60; 11.23/5.4=2.08

the LOEC and NOEC) of 8.06 mg/L total ammonia-N and a 90-day EC20 (based on biomass) of 5.56 mg/L total ammonia-N (standardized to pH 8). This test appears to meet the USEPA criteria for early-life-stage tests for salmonids outlined in Table 1; an ACR for rainbow trout based on the chronic value from this recent test would be about 1.4. However, even if Brinkman et al. (2009) was added to its list of acceptable chronic tests⁷, USEPA might still conclude that chronic test data for rainbow trout are too variable, or otherwise insufficient, to calculate SMCVs, or an ACR for the species or the genus.

In general, the approach of pairing acute values and chronic values from different investigations to compute ACRs is not necessarily in agreement with USEPA guidelines. USEPA (1985), outlines the following steps for producing an ACR from a chronic value:

1. The numerator for the ACR should be the geometric mean of the acute values for that species from all acceptable flow-through acute tests in the same dilution water.
2. For fish, the acute tests should have been conducted with juveniles.
3. The acute tests should have been (a) a part of the same study as the chronic tests, (b) from different studies but from the same laboratory and dilution water, or (c) from studies at different laboratories using the same dilution water.
4. If no such acute tests are available, an ACR should not be calculated.

In summary, *based on the most recent USEPA criteria for chronic test design and endpoints, derivation of ACRs, and interpretation of data from chronic tests for fish*, no information was presented at the Ammonia Summit, or in recent reports to the Regional Board, to support a proposal that the ACR for rainbow trout occupies the range 14.6-23.5. Derivation of hypothetical ACRs for rainbow trout as high as the ones presented at the Ammonia Summit is not possible using direct evidence for chronic effects of ammonia on survival, growth, or reproduction and represents a significant departure from current USEPA guidance concerning the use of data from chronic tests for the species. Assertions about chronic toxicity in the Delta that rely on these hypothetical ACRs for rainbow trout should be avoided. At a minimum, such assertions must be carefully qualified as not being based on evidence for population-level effects of ammonia on sensitive fish.

Use of ACRs to infer chronic toxicity for copepods

Dr. Swee Teh conducted static renewal acute toxicity tests with two copepods *Eurytemora affinis* (96-hr) and *Pseudodiaptomus forbesi* (72-hr) at three test pHs: 7.2, 7.6, and 8.1. As part of his Ammonia Summit presentation, Dr. Teh used ACRs to conclude that the USEPA CCC was potentially not protective of these two copepods (Teh et al. 2009). The data used for this exercise are summarized on slide 14 of Dr. Teh's powerpoint presentation. Regarding Dr. Teh's results, Mr. Foe observed in his summary:

⁷ Brinkman et al. (2009) was published after the Feb. 2009 cut-off for the literature review used for the development of the USEPA 2009 Draft Update of the freshwater ammonia criteria.

Dr. Swee Teh concluded, based on the 10 percent mortality and an ACR analysis, that ambient ammonia concentrations below the SRWTP may cause chronic toxicity to both species.” (Foe 2009b)

In order to predict a chronic value for each of the two copepod species, Dr. Teh divided the LC50s obtained using his lowest test pH (pH 7.2) by the arithmetic average of the discrete GMACRs for fish (listed in Table 2 above) and daphnids (*Daphnia*, 5.3; and *Ceriodaphnia*, 1.9) from Appendix 7 of USEPA (1999).

In his summary, Mr. Foe acknowledged that the test pH associated with toxicity in Dr. Teh’s experiments (7.2) was not representative of ambient pH levels in the Sacramento River:

“Ten percent mortality occurred to both species at ambient ammonia concentrations present in the river below the SRWTP. However, toxicity was only observed at a lower pH (7.2) than commonly occurs in the River (7.4 to 7.8). Toxicity was not observed when toxicity testing was done at higher pH levels.” (Foe 2009; p.2)

Available data indicate that this qualification also applies to the rest of the freshwater and brackish Delta. Evaluation of discrete pH measurements from long-term estuarine and freshwater monitoring stations during “POD” years (2000-2009; stations and sample sizes shown in Figure 1) confirms that the intermediate test pH in Dr. Teh’s experiment (pH 7.6) is more representative of pH in the Delta than the lower test pH (7.2). Median and mean values for pH from freshwater and brackish stations in the estuary are equal - or close - to pH 7.6 (Table 3, D. Engle unpub. data). In Dr. Teh’s experiment, both copepods showed similar acute sensitivity to un-ionized ammonia at the more representative test pH 7.6 (LC50 = 0.12 mg/L un-ionized ammonia-N for both species, see slide 9 in Teh's powerpoint presentation). Use of the more representative LC50 in Dr. Teh’s estimation procedure (division of an LC50 by the average GMACR for fish and daphnids) results in a hypothetical chronic value of 0.02 mg/L un-ionized ammonia-N for the copepods. This concentration is more than 2x higher than the highest un-ionized ammonia concentration sampled between 2000-2009 at estuarine stations illustrated in Figure 1 (0.009 mg N/L un-ionized ammonia) and equal to the 99.8th percentile value for pooled un-ionized ammonia concentrations measured during the same period at the freshwater stations (D. Engle, unpub. data). In fact, only 4 out of 2487 ambient water samples from the dataset described above had un-ionized ammonia concentrations higher than the 0.02 mg/L threshold predicted by the more representative LC50 derived from Dr. Teh's experiments.

In summary, when the LC50s for copepods presented at the Ammonia Summit are interpreted in light of ambient data for un-ionized ammonia and pH, there is little support for proposals that levels of ammonia in Delta are causing chronic toxicity for these two copepods. This conclusion is independent from calculations of the USEPA criterion, relying instead on examination of ambient data from almost 50 stations in the freshwater and brackish Delta - including many measurements from areas downstream from Hood (e.g. tip of Grand Island) where some speculate that higher pH might raise risk for ammonia toxicity compared to Sacramento River locations closer to the SRWTP. This outcome illustrates that comparisons of the USEPA chronic criterion with hypothetical chronic thresholds (for one species or another) should not be used as evidence of ambient ammonia toxicity.

Table 3. pH Measured during POD Years (2000-2009) at Long-Term Estuarine and Freshwater Monitoring Stations in the Upper San Francisco Estuary.

	Monitoring Program	Number of Stations	Number of Samples	Mean pH	Median pH	5th-95th Percentiles
Estuarine Stations ⁽¹⁾	DWR-MWQI	1	800	7.62	7.64	7.02 - 8.05
	IEP	5				
	UC Davis POD Investigation ⁽²⁾	13				
Freshwater Stations	DWR-MWQI	7	1687	7.63	7.60	6.98 - 8.34
	IEP	7				
	UC Davis POD Investigation	11				
	USGS	3				
	SRCSD CMP ⁽³⁾	2				

(1) Stations were classified as Estuarine or Freshwater based on procedures found in the California Toxics Rule. Details about the classification are found in D. Engle's Ammonia Summit presentation (Engle 2009b).

(2) Pelagic Organism Decline (POD): Acute and Chronic Invertebrate and Fish Toxicity Testing in the Sacramento-San Joaquin Delta, UC Davis Aquatic Toxicology Laboratory

(3) Sacramento Regional County Sanitation District Coordinated Monitoring Program

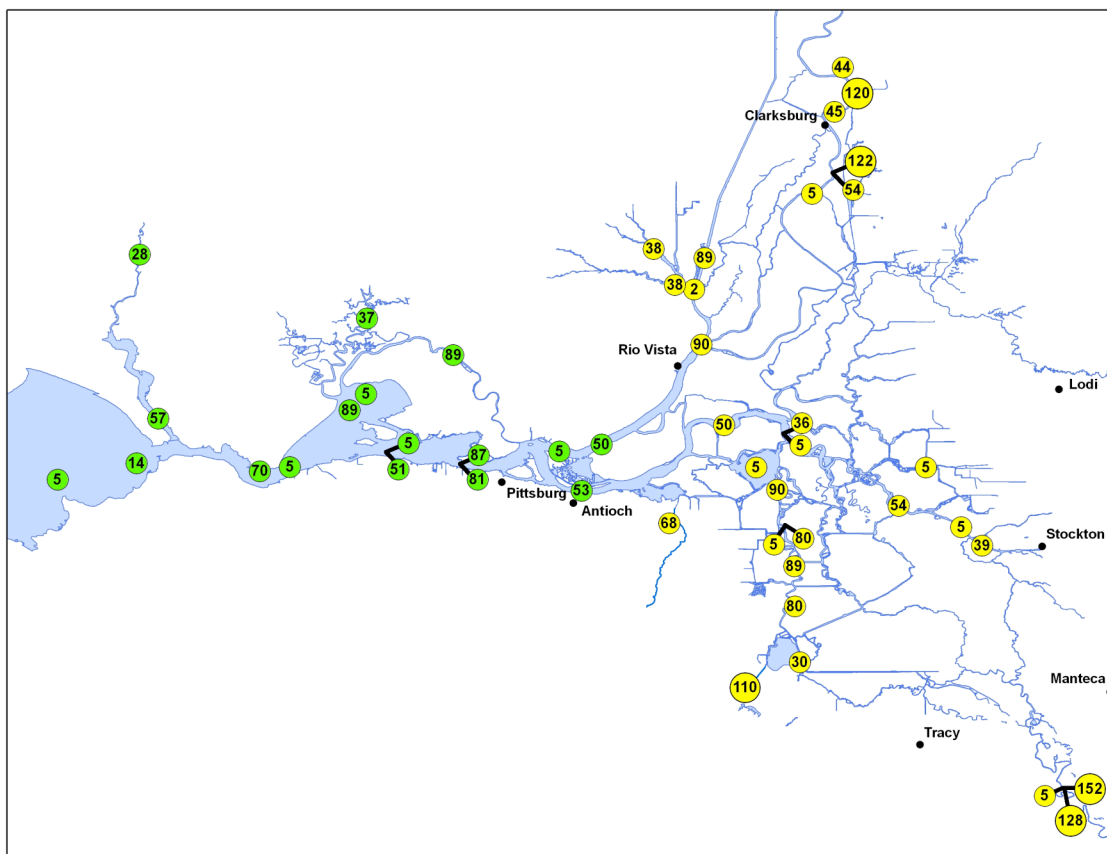


Figure 1. Long-term Estuarine (green symbols) and Freshwater (yellow symbols) Monitoring Stations in the Upper San Francisco Estuary Providing Data for Un-ionized Ammonia during POD Years (2000-2009). Numbers inside symbols are sample sizes. Monitoring stations are those of the IEP, DWR-MWQI, UC Davis POD Project, USGS, and SRCSD CMP (from Engle 2009a).

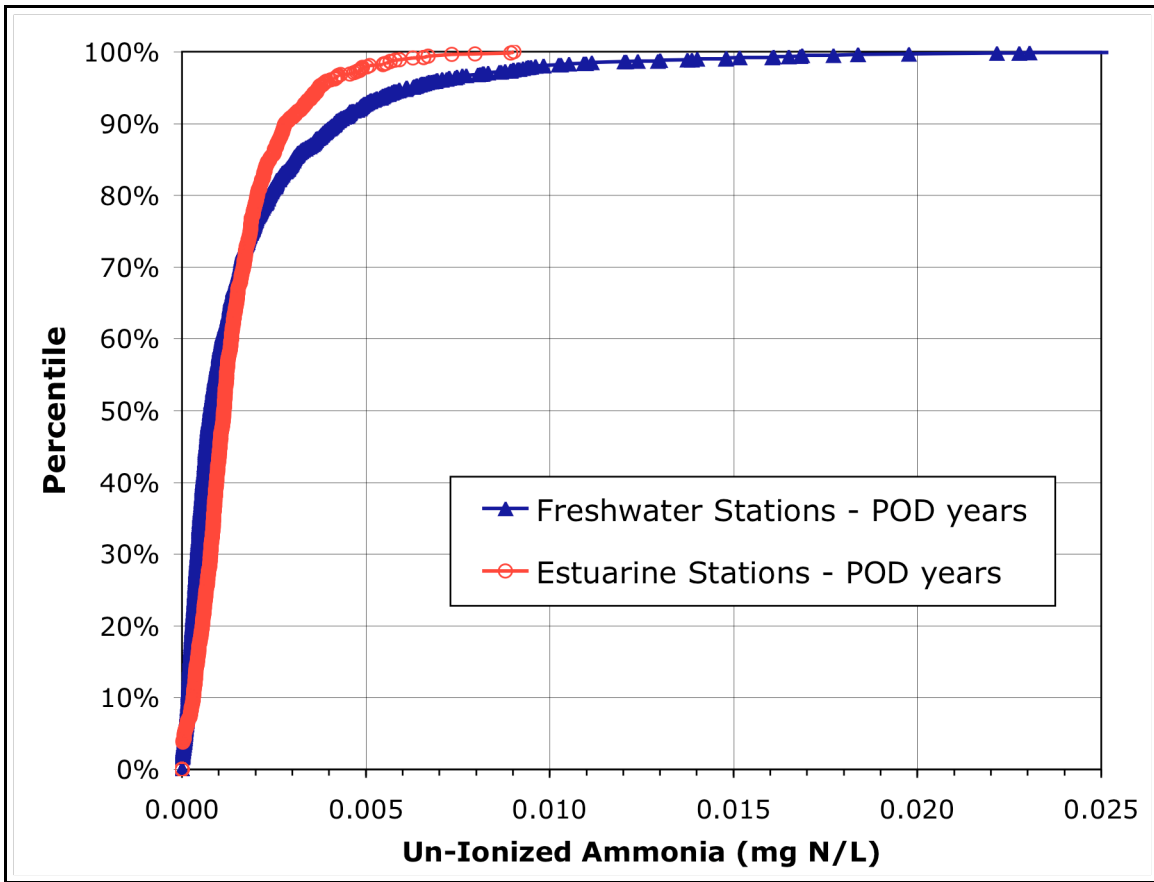


Figure 2. Ranked Distribution of Un-Ionized Ammonia Concentrations (mg N/L) from Long-Term Estuarine (red circles) and Freshwater (blue triangles) Monitoring Stations in the Upper San Francisco Estuary for POD Years (2000-2009). Station locations and sample sizes are shown in Figure 1. One freshwater value (0.04 mg/L) is not displayed on the graph.

Use of ACRs by Mike Johnson

For his Ammonia Summit presentation, Mike Johnson calculated his own acute values and ACRs for “vertebrates” and “invertebrates”, from which he derived chronic values for the two taxonomic groups (by simple division) (Johnson 2009). Through this exercise, it appears that Dr. Johnson was generating his own versions of the “USEPA” acute and chronic criteria as a basis for evaluating whether USEPA criteria are protective of Delta smelt or the copepods used in Dr. Teh’s tests. As of the date of this writing, no report was publicly available for this project, so it is unknown how the vetting procedures used by Dr. Johnson to build his database compare with those of the USEPA. Although Dr. Johnson referenced USEPA (1999) and “recent toxicity studies” as sources of data, it was not clear from his presentation how he selected genus mean ACRs of 3.17 and 5.59 for invertebrates and vertebrates, respectively, or what was meant by *genus* means for these broad taxonomic divisions. By taking the geometric mean of the GMACRs for *Daphnia* and *Ceriodaphnia* in Appendix 7 of USEPA (1999) it is possible to derive a value of 3.17. However, the same procedure using GMACRs for vertebrates (fish) in the

USEPA document yields a value of 6.38. More information would be needed to evaluate whether the data underlying the value of 5.59 resulted from combinations of acute or chronic tests for vertebrates that meet USEPA acceptability criteria. In addition, it is not clear which data he judged suitable for use in deriving his acute values for the taxonomic groups.

Dr. Johnson's alternative procedures seem a peculiar basis for determining whether current USEPA ammonia criteria (or future USEPA revisions) are adequate for Delta species. For example, USEPA does not derive separate criteria for invertebrates and vertebrates. Also, in 1999, USEPA stopped relying on ACRs to derive a chronic criterion for ammonia - opting instead to use chronic values from tests which met acceptability criteria for fish or invertebrates (USEPA 1999). The direct use of chronic test results was maintained during development of the 2009 USEPA Draft Update of the freshwater ammonia criteria (USEPA 2009). In addition, in order to draw conclusions about chronic toxicity for copepods, Dr. Johnson applied his "invertebrate ACR" to LC10s (instead of LC50s) from Dr. Teh's experiment -- which is not a conventional use of ACRs. Finally, Dr. Johnson's risk calculations, using monitoring data from two IEP stations, appear to impose a log-normal distribution on ambient data which clearly have a different underlying distribution. This step appears to inflate the probability associated with higher ambient concentrations.

Dave Fullerton Regressions

Dave Fullerton (2009) presented a suite of correlations in his Ammonia Summit presentation which he argues provide evidence that reductions in ammonium would boost the food chain all the way up to Delta smelt. However, these analyses, and previous correlation results presented by Mr. Fullerton at other venues, are sensitive to the averaging periods used for the independent and dependent variables. Non-significant relationships can be derived for many of Mr. Fullerton's pairings of x and y variables merely by altering the averaging periods - or using averaging periods that are more ecologically sensible (such as narrowing the averaging period for diatom bloom-related variables to April-May). For example, while Mr. Fullerton presents an inverse relationship between Suisun Bay *February-June* mean ammonia and an annual index of Delta smelt cohort success (slide 19), the analogous relationship disappears when a summertime averaging period for ammonia (June-August) is used (Figure 3).

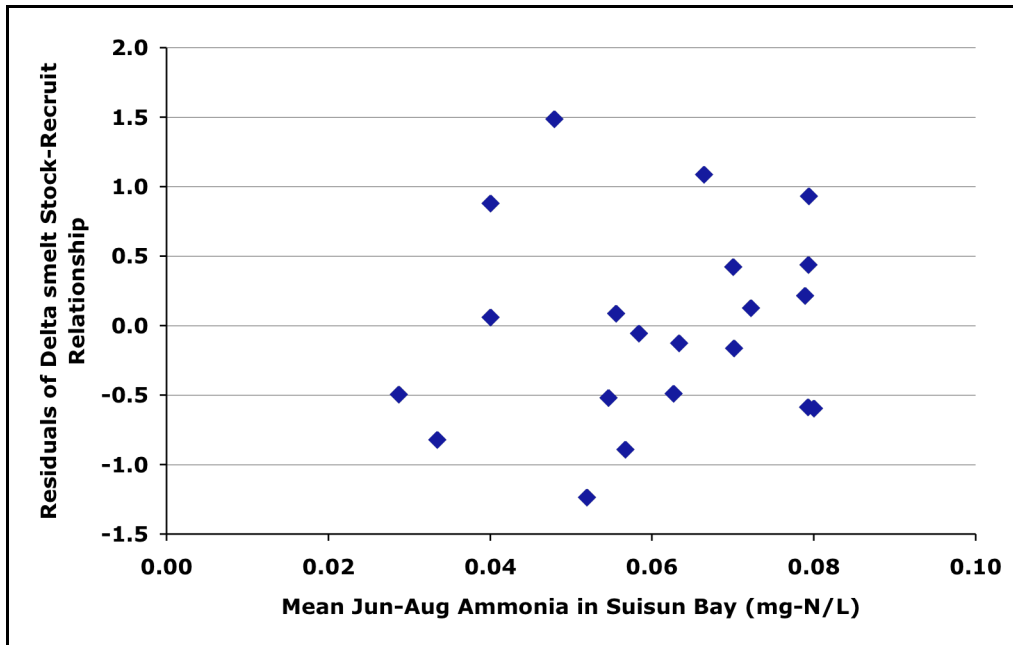


Figure 3. Mean June-August ammonia concentrations (mg N/L) in Suisun Bay and Carquinez Straight plotted against the residuals of the Delta smelt stock-recruit relationship for 1987-2008. Ammonia data are from DWR-MWQI, IEP, and USGS long-term monitoring stations. Annual residuals result from linear regression between current-year Summer Towntnet Survey and previous-year Fall Midwater Trawl abundance indices (log transformed). (D. Engle, unpub. data).

As another example, in order to achieve correlations between *Eurytemora* abundance and several parameters (ammonium, chl.a, diatoms), Mr. Fullerton uses a 12-month averaging period - although there is no ecological justification for doing so. Variation in the population size of short-lived organisms such as copepods is not logically linked to environmental conditions averaged over periods which greatly exceed generation time. Mr. Fullerton relies on a “daisy chain” of correlations for Suisun Bay ($\text{NH}_3 \rightarrow \text{chl.a}$; $\text{chl.a} \rightarrow \text{copepod}$; $\text{copepod} \rightarrow \text{delta smelt}$) to make the argument that Delta smelt abundance shall indirectly respond to ammonia levels through the food chain. However, Mr. Fullerton does not show that Delta smelt annual cohort success is related to chlorophyll-a. Mr. Fullerton’s argument relies on the assumption that ammonia reduction will result in more frequent diatom blooms in Suisun Bay. Owing to clam grazing pressure in summer months, April-May is the principal window available in Suisun Bay for postulated dividends in diatom abundance related to ammonia reductions. Significantly, for the last two decades, Delta smelt annual cohort success has not been related to chlorophyll-a levels in Suisun Bay *during the months when spring diatom blooms actually occur in the Bay* (April-May; Figure 4). A similar lack of correspondence between the abundance of Delta smelt and phytoplankton can be shown for other seasons and locations in the estuary.

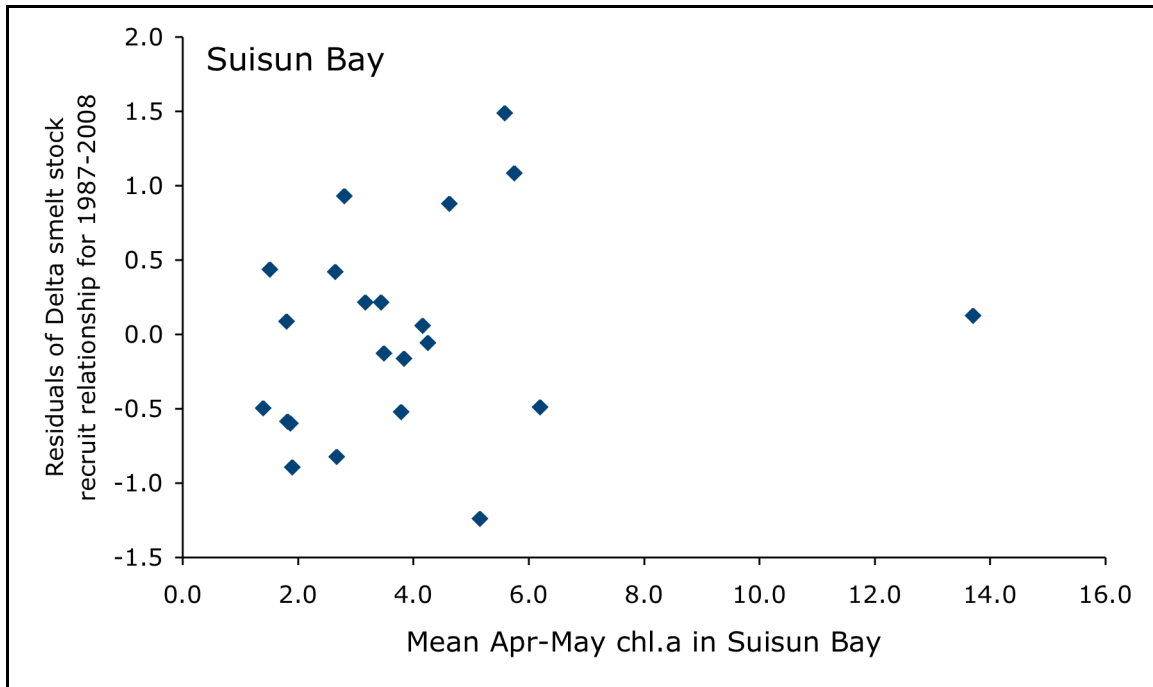


Figure 4. Annual Residuals of the Stock-Recruit Relationship for Delta smelt for 1987-2008 plotted against mean April-May chlorophyll-a ($\mu\text{g/L}$) in Suisun Bay. Chlorophyll-a data are from DWR-MWQI, IEP, and USGS long-term monitoring stations. Annual residuals as in Figure 3 (D. Engle, unpub. data).

Ammonium Inhibition of Primary Production

In the Ammonia Summit summary, Mr. Foe acknowledged that, based on transect sampling and associated container experiments during 2009, ammonia inhibition of primary production has not been demonstrated in the freshwater Sacramento River upstream of Rio Vista. However, based on “grow outs” conducted during, and prior to, 2009, Richard Dugdale and colleagues maintain that phytoplankton are unable to grow well in water collected at Rio Vista. This can be misinterpreted to mean that phytoplankton growth is hampered in the Sacramento River from Rio Vista downstream. However, the 2009 transect work reported at the Ammonia Summit indicates that ambient conditions in the Sacramento River below Rio Vista can allow significant increases in phytoplankton biomass before the river enters Suisun Bay. Another significant finding of the 2009 transect work that was not mentioned in the summary is that larger-scale processes - unrelated to ammonia but otherwise unexplained - *are* causing longitudinal trends in phytoplankton concentrations in the Sacramento River upstream from Rio Vista. Principal features of the Spring 2009 transects were:

- (1) algal biomass (diatoms and other taxa) decreased starting well above Sacramento and ending in the vicinity of Isleton/Rio Vista, *but*
- (2) conditions *downstream* from Rio Vista allowed for phytoplankton re-accumulation in the Sacramento River *well upstream from Suisun Bay*.

Transect results showing accumulation of algal biomass in the river between Rio Vista and Suisun Bay were included in Foe’s ammonia summit presentation (see slide 8 in Foe

2009b) and are supported by detailed transect results for March 2009 presented by the Dugdale/Parker research group at the September 2009 State of the San Francisco Estuary Conference (Parker et al., 2009; see Figure 5). The latter, more detailed transect results indicate that small-celled phytoplankton and green algae exhibit similar longitudinal trends as large diatoms between the Yolo/Sacramento County line and Suisun/San Pablo Bays. Significantly, the 2009 results showed no step-change in phytoplankton biomass (for several taxa) or carbon fixation associated with either (1) the location of the SRWTP discharge, or (2) a shift from primarily nitrate uptake by phytoplankton to primarily ammonia uptake below the discharge. These longitudinal trends are important because they contradict hypotheses that the SRWTP discharge influences the quantity or quality of phytoplankton that grow in response to *ambient* conditions in the Sacramento River.

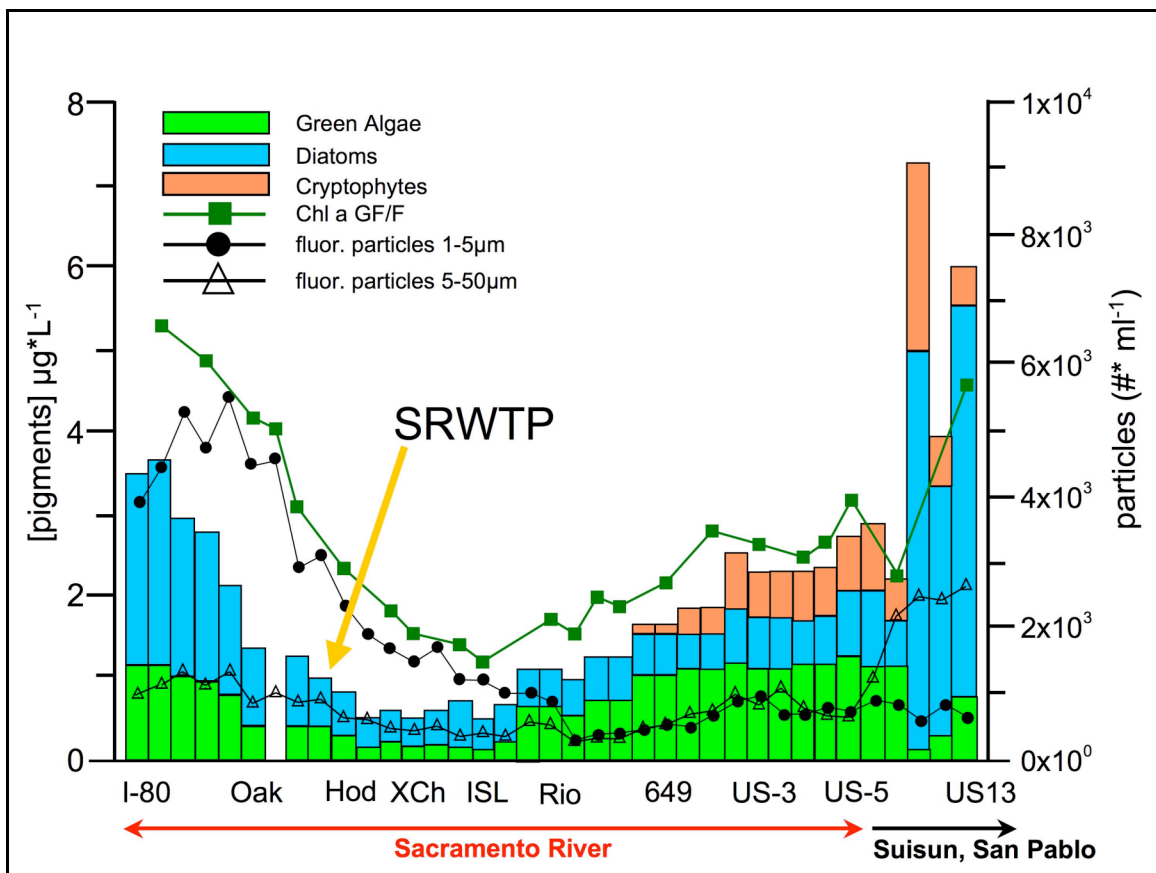


Figure 5. Longitudinal Pattern in Indices of Phytoplankton Biomass in the Sacramento River (starting at the Yolo/Sacramento County Border) to Suisun/San Pablo Bays (adapted from figure in Parker et al. 2009).

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