# Memorandum

LARRY WALKER ASSOCIATES

**Diana Engle, Ph.D.**
2151 Alessandro Drive, Suite 100
Ventura, CA 93001
805.585.1835

**Claus Suverkropp**
707 Fourth Street, Suite 200
Davis, CA 95616
530.753.6400

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TO: Stan Dean, District Engineer
Sacramento Regional County Sanitation District
10060 Goethe Road
Sacramento, CA 95827

Cc: Linda Dorn, SRCSD
Terrie Mitchell, SRCSD
Prabhakar Somavarapu, SRCSD

SUBJECT: Comments for Consideration by the State Water Resources Control Board Regarding the Scientific Article *Long-term Changes in Nutrient Loading and Stoichiometry and their Relationships with Changes in the Food Web and Dominant Pelagic Fish Species in the San Francisco Estuary, California* by Patricia Glibert

A pre-print of a scientific article authored by Patricia Glibert (University of Maryland Center for Environmental Sciences) entitled "*Long-term changes in nutrient loading and stoichiometry and their relationships with changes in the food web and dominant pelagic fish species in the San Francisco Estuary, California*", scheduled for publication in Reviews in Fisheries Science, was publicly released on May 17, 2010. In the article, Glibert uses a calculation termed *CUSUM* to transform long-term datasets for nutrient concentrations and abundances of phytoplankton, three copepod species, the invasive clam *Corbula amurensis*, and several fish species (including Delta smelt and longfin smelt). In brief, the CUSUM transformation converts time series of measured values into series of cumulative standardized deviations from a long-term mean (or other constant). The resulting time series of CUSUM values exhibit features and patterns which diverge in several important ways from those of the underlying measured data - some of which are useful for detecting change points in time series which contain a lot of seasonal or interannual variation.

In Glibert's study, the transformed data ("CUSUM values") were used in two ways: (1) displayed as time series to detect potential change points in underlying measured data, and (2) used in linear regressions by pairing CUSUM values for different environmental parameters. Based on visual inspection of CUSUM time series and linear regressions between CUSUM values for selected pairings of nutrient or biological parameters, Glibert concludes that changes in nutrient ratios (which she attributes primarily to changes in ammonia and phosphorus concentrations in the discharge from the Sacramento Regional Wastewater Treatment Plant) have driven changes in abundance and composition of organisms higher in the estuarine food web, such as phytoplankton, copepods, invasive clams, and pelagic and littoral zone fishes.

The route by which Glibert arrives at her conclusions raises concerns from both technical and ecological standpoints. The correlation approach used by Glibert (using CUSUM values instead of measured values) violates assumptions for linear regression, and can produce spurious relationships between variables that are unsupported by the underlying data. Although she analyzed chemical and plankton data from only one station in the freshwater Delta (Sacramento River at Hood), and two stations in Suisun Bay, Glibert generalizes her results to the whole of the upper San Francisco Estuary (SFE). Although they are not well articulated in the article, a number of problematic ecological assumptions are required to infer cause and effect from her correlation analysis. Key analyses that are necessary to support her conceptual model are missing from the publication. Many well-known alternative hypotheses for the observed changes in plankton composition and fish abundance in the SFE (and in estuaries, generally) - which would have been testable using her CUSUM methodology - were omitted from the analysis and from discussion in the article. Finally, owing to the peculiarity of the CUSUM transformation, it is likely that a wide variety of non-nutrient environmental factors (essentially any factors which have trended over time in the SFE in concert with changes in fish abundance) could be shown as highly correlated with pelagic fish abundance using CUSUM correlations. As an example included in Section 1 of this memo, it is shown that when subjected to the same analysis used in Glibert's paper, annual water exports perform as well as ammonia concentrations in explaining trends in the summertime abundance of Delta smelt.

At the end of the methods section of her paper, Glibert cautions against using CUSUM correlations as evidence of cause and effect, as follows:

> "Relationships between CUSUM trends for different nutrients or between different components of the food web, as shown herein, allow investigators to infer mechanistic relationships supported by known physiological or trophic relationships, or can lead to further testable hypotheses of the relationships between trophic components. <u>It is in this context that they are used here. As with all correlations, the variables may have a cause-and-effect relationship or both may be related to another variable.</u>" (emphasis added)

Unfortunately, the CUSUM correlations presented in Glibert's paper were not ultimately placed in such context. Instead, they were used to make unwarranted, overly simplistic conclusions regarding the food web of upper SFE.

The contents of this memo are organized as follows:

1. Concerns with the Statistical Analysis
2. Concerns with Underlying Ecological Assumptions
3. Alternative Hypotheses which are Unaddressed
4. Inconsistencies

## 1. Concerns with the Statistical Analysis

The type of correlation analysis used in Glibert's paper violates the underlying assumptions for linear regression and can produce misleading results. Other concerns include the limited geographic extent of the data, possible improper subsampling of CUSUM time series, nontransparent data reduction, and omissions of key analyses which would support alternative hypotheses.

***Geographic Coverage***. Sweeping generalizations are made regarding the estuarine food web and the Pelagic Organism Decline (POD) using data from only 1 station in the Freshwater Delta (Hood, IEP station C3) and 2 stations in Suisun Bay (IEP stations D8 and D7).

***Violation of Statistical Assumptions***. Glibert admits that the CUSUM approach mutes seasonal or other short-term variation in a time series. Also, Glibert admits that CUSUM series exaggerate shifts in the underlying raw data. These features of CUSUM are useful for pinpointing change points in inherently variable time series, or for exploring whether change points coincide for different factors. In the statistical literature, CUSUM is primarily used to create charts for single variables that allow the user to detect change points or determine whether deviations from control points are random or signal a trend. However, the characteristics of CUSUM that lend it to change-point analysis and quality control make it completely inappropriate to evaluate relationships between variables (such as CUSUM $NH_4^+$ *vs* CUSUM fish abundance) by conducting standard linear regression using CUSUM values (taken out of sequence from their time series) as independent or dependent variables.

The simple CUSUM correlations that represent the basis for the author's conclusions violate virtually every assumption of a standard correlation analysis. CUSUM series are inherently serially correlated, heteroscedastic and non-normally distributed, and the residuals of CUSUM correlations are non-independent. The CUSUM transformation is similar to a long-term moving average in that they both tend to smooth short-term variability and are inherently serially auto-correlated. However, CUSUM transformation is much more extreme in this regard and erases or obscures variability and relationships among data points in a series. Additionally, because they are *cumulative* sums of deviations, the variance structure of a CUSUM series changes throughout a time series. The values at the beginning of a CUSUM series are influenced by fewer preceding values than values at the end of the series. This means that values at the end of the series are more highly auto-correlated than values at the beginning of the series. The increasing serial correlation within CUSUM series increases the chances of violating the assumption of homoscedasticity (equal variance) for correlation analysis. Based on violations of several of the underlying assumptions of a traditional correlation analysis, the results and subsequent conclusions drawn by the author which rely on the CUSUM correlations presented should be considered invalid.

In addition to issues surrounding Glibert's uncustomary use of CUSUM values for correlation analysis, not all of the datasets used by Glibert were appropriate for customary CUSUM change point analysis. Autoregressive time series such as flow data are not appropriate for CUSUM change-point analysis. CUSUM change point analysis also assumes that underlying data are homoscedastic and often assumes that data are normally distributed. In the article, Glibert acknowledges that data were not tested for autocorrelation prior to the CUSUM transformation; tests regarding normality and equal variance were apparently also omitted.

***Artificial Relationships and Inflated $R^2$ Values***. Another problematic characteristic of the CUSUM transformation is that it results in a very limited range of serially correlated data structures. Owing to the dependence of CUSUM on the long-term mean of the time series, most CUSUM series of normally distributed data automatically begin near zero and end near zero (as is evident from many of Glibert's time series graphs of CUSUM values). Consequently, real trends or patterns in the data are obscured or inverted, and portions of a time series with no trend can produce a series of increasing or decreasing CUSUM values, depending on the relationship between the actual measurements and the population mean, and where they occur in the longer time series. For example, short-lived perturbations can cause trends in CUSUM values that persist for years (or decades) after underlying data return to normal ranges. This feature of CUSUM is well illustrated by the CUSUM series for $NO_3$:$NH_4$ in Glibert's Figure 4. Figure 4 (panels B and C) shows that $NO_3$:$NH_4$ ratios were intermittently anomalously high in Suisun Bay during about 5 years starting in 1987, but occupied similar ranges of lower values during most of the years prior and afterward. Inflections in the CUSUM charts in Figure 4 are useful for illustrating the beginning and end of this perturbation. However, owing to the perturbation in the longer time series, CUSUM values follow a conspicuous declining trajectory from 1994 onward, despite the fact that underlying data for that period are not trending up or down.

Such features of CUSUM generate artificial patterns in the data and can produce statistically significant relationships between independent and dependent variables for which no relationship can be derived using real-world concentrations or abundances. The result of the limitations of CUSUM data structure is that they generate "correlations" with impressively inflated $R^2$ values that are largely artificial and can't be interpreted in the same way as standard parametric correlation or regression analysis. Equally important, statistically significant relationships that *are* present in underlying data can be disguised when CUSUM time series are compared instead of real world measurements.

An example of a misleading result from CUSUM correlations is illustrated in Figure 1 below. For this figure, two contrasting synthetic time series were produced: one in which there was a step change in a time series (a doubling; red series, panel A) but no other trend, and another in which values gradually increased over time, with no step increase (blue series, panel B). The time series with the step change generates a CUSUM series that is nearly identical in structure to the CUSUM series for the monotonic increasing data (panel C). Although there is no abrupt change in the monotonic trend, the CUSUM calculation forces an inflection point midway through the time series of CUSUM values (the bottom of the blue trough in panel C). And although there is no feature of the monotonic trend which coincides with the step increase in the other parameter, the CUSUM series for both datasets are similar and highly "correlated" (Figure 1C, Figure 2). A similar misleading result would occur if the introduction of a non-native species was treated like a step change - with many years (or decades) of zero abundance included in the time series for an invasive species prior to its establishment. Such an approach appears to have been used by Glibert when comparing the CUSUM time series of ammonia and the invasive clam *Corbula amurensis*.

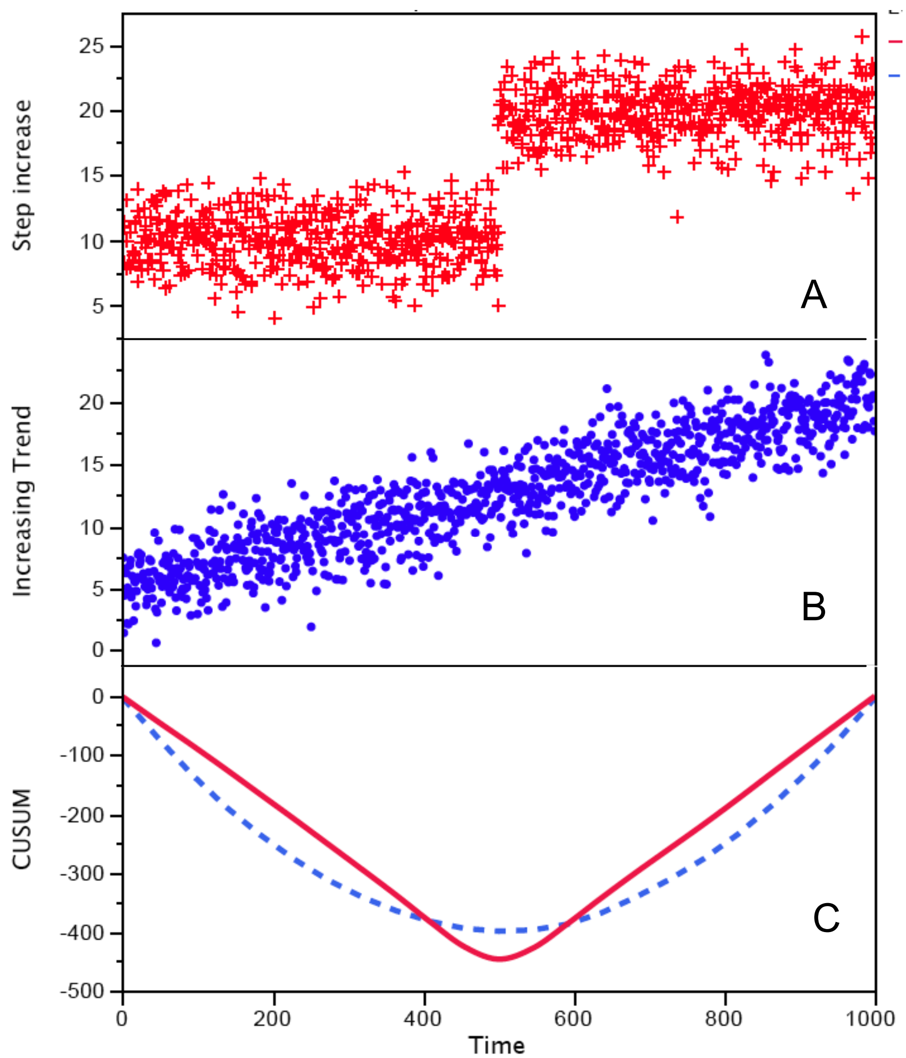


**Figure 1. Comparison of CUSUM charts for contrasting underlying time series. Upper panels show hypothetical time series featuring a step increase (A), and a monotonic increase over time (B). Lower panel compares the resulting CUSUM charts for the step increase (red line) and the monotonic increase (blue line).**

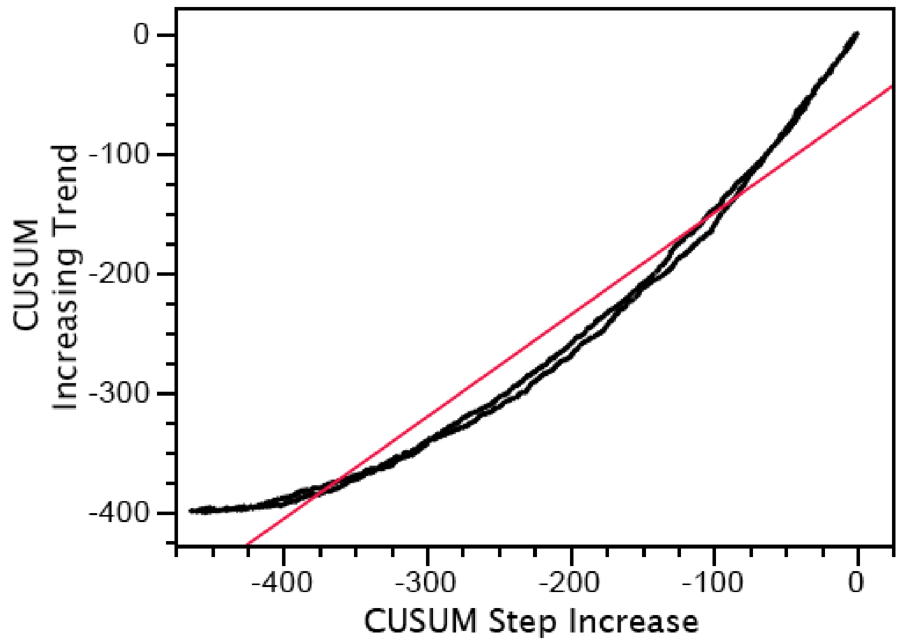


**Figure 2. Correlation between the CUSUM series shown in Figure 1C. The CUSUM series for the data with a step increase (red line in Figure 1C) was used as the independent variable; the CUSUM series for the data with a monotonic increase (blue line in Figure 1C) was used as the dependent variable. Regression line (red) was significant ($p < 0.0001$, $R^2 = 0.94$).**

***Issues Created by Breaking Up Time Series***. Another requirement of CUSUM analysis is that time series being compared using CUSUM must start and stop at the same point in time. Data series do not have to have the same number of observations, but the CUSUM series must be generated for the same time period for each variable. If variables cover two different but overlapping time periods, valid comparisons of trends must be made with CUSUM series calculated only for the common overlapping period. It is not clear from Glibert's methods section whether CUSUM series were appropriately recalculated for each correlation analysis. If this was not done, the comparisons are essentially meaningless. For example:

1. In Figure 13, Glibert included *Corbula* abundance values of zero from the period prior to its establishment (1975-1986) to generate the CUSUM series for the whole period 1975-2005. However, the caption for Table 1 states that correlation analysis between CUSUM-*Corbula* and CUSUM-X2 was performed using CUSUM values for 1987-2005 only. Were separate CUSUM time series (not presented in the article) generated for both variables using data only for 1987-2005 before running this correlation?
2. In Figure 8, were separate CUSUM series generated for flow and nutrient parameters for pre-POD and POD periods before $R^2$ values were generated for the partial time series?
3. In Figure 19, CUSUM values for *Pseudodiaptomus* and *Limnoithona* for 1987-2005 were apparently used as independent variables. Were these CUSUM values extracted from the CUSUM series generated using copepod data from all years (1974-2006, see Fig. 11), or

were CUSUM series *recalculated* for fish and copepods for the years 1987-2005 before linear regression was performed?

***Non-transparent Data Reduction.*** Glibert does not reveal how she aggregated raw data or CUSUM values for variables that were measured monthly (such as nutrients, phytoplankton, zooplankton) or daily (such as Delta outflow or X2) in order to pair them with annual fish abundance indices (such as Summer Townet survey or Fall Midwater Trawl indices). Were monthly or daily data aggregated prior to CUSUM transformation? If so, how did the averaging periods relate to timing of fish surveys? Summer Townet indices are derived from June-August fish catch; Fall Midwater Trawl indices are derived from Sept-Dec. fish catch. Were monthly nutrient or copepod data averaged for periods preceding or overlapping the fish catches? If so, how long were the averaging periods? Were they ecologically relevant averaging periods?

***Questionable Ecological Relevance of CUSUM Values***. CUSUM values at any single point in a time series are affected by all the values that precede them. The Delta time series used by Glibert extend over decades. This raises the question whether comparison of CUSUM values for nutrients and plankton are ecologically meaningful if the underlying ambient concentrations or organism counts don't covary in a meaningful way. Use of CUSUM values as independent or dependent variables in a regression analysis implies that the values have inherent meaning when taken out of sequence from their time series. Raw CUSUM values calculated using decades-long datasets might have some inherent meaning for long-lived organisms whose biological state (size, fecundity, physiological condition) can be affected by cumulative exposure to environmental conditions over years or decades. However, phytoplankton and zooplankton populations respond to short-term phenomena on scales of days to weeks. For short-lived organisms such as phytoplankton (days) and copepods (weeks) - or even Delta smelt and longfin smelt which live 1-2 years - it does not seem ecologically meaningful or valid to use CUSUM values derived over decades of observations for uses *other* than change point identification.

***Omitted CUSUM Correlations***. Several obvious pairings of environmental variables are omitted from Glibert's portfolio of CUSUM correlations. Some of the omitted analyses are needed to make the claim (using Glibert's approach) that the bottom two tiers of her conceptual model (nutrient ratios and phytoplankton taxa, Fig. 23) are statistically related. In addition, several widely hypothesized *non*-nutrient drivers of plankton composition or fish abundance (such as clam abundance, turbidity, or water exports) are not used as independent variables for CUSUM analysis in Glibert's study, although publicly available data would lend them easily to the same treatment. Because changes in ambient ammonia concentrations in Suisun Bay have coincided with changes in other environmental factors, it is likely that several non-nutrient factors would perform similarly as nutrients in CUSUM correlations with phytoplankton, copepods, or fish abundance. Examples of omitted analyses are discussed below.

<u>Fall abundance of Delta smelt is not compared to nutrient or copepod trends</u>. Glibert shows that the CUSUM values for the Summer Townet Index for Delta smelt are correlated with CUSUM values for copepods (Fig. 18) and $NH_4$ (Fig. 20). Why aren't analogous results presented using CUSUM values for the Fall Midwater Trawl index for Delta smelt?

_Trends in nutrient ratios were not directly compared to trends for organisms at the base of the planktonic food web_. Several of Glibert's conclusions rely on the premise that changes in nutrient *ratios* (TN:TP, $NO_3$:$NH_4$, DIN:DIP) have driven observed changes in phytoplankton composition. The only CUSUM correlations she presents for nutrient parameters *vs* phytoplankton taxa are in Figure 10: [$NH_4$] *vs*. five broad taxonomic categories of phytoplankton. CUSUM regressions between nutrient *ratios* (TN:TP, $NO_3$:$NH_4$, or DIN:DIP) and phytoplankton indices (chl.a or individual taxonomic groups) are omitted from the paper. CUSUM trends in nutrient ratios are also not directly compared to those for copepod abundance. $NO_3$:$NH_4$ trends are not compared to *any* of the biological trends (phytoplankton, copepods, clams, or fish); they are only compared to trends in Delta outflow. As a consequence, the current publication does not provide evidence that nutrient ratios and phytoplankton composition are statistically related. Thus it is debatable whether the findings presented by the author support the conclusion that "*...that nutrient _form_ is related to the "quality" of phytoplankton*," as is claimed on page 26.

_Corbula abundance is not tested as an independent variable_. Glibert does not use her statistical approach to test obvious top-down hypotheses regarding the widely acknowledged role of *Corbula* as an ecosystem engineer in the brackish Delta. The tight relationship between CUSUM for $NH_4$ and *Corbula* (Fig.14) strongly suggests that if *Corbula* were substituted for $NH_4$ as an independent variable (i.e., used as the x-axis), *Corbula* trends might be significantly correlated with those of the phytoplankton groups shown in Fig. 10, or those of fish species shown in Fig.s 20-21. In her summary, Glibert concludes that fish species could be divided into two groups: (1) those whose CUSUM trends were positively correlated with trends in abundance of *Eurytemora* and negatively correlated with *Pseudodiaptomus* and *Limnoithona*, and (2) those whose long-term CUSUM trends were negatively correlated with *Eurytemora* and positively correlated with *Pseudodiaptomus* and *Limnoithona*. Her thesis is that nutrient-driven changes in phytoplankton "quality" ultimately explain these patterns. However, the change point dividing these two copepod "regimes" (at least in the brackish Delta) coincides well with the establishment of *Corbula*, and the near elimination of (overall) phytoplankton biomass in Suisun Bay (see Fig. 9A).

_Turbidity is not tested as an independent variable_. A long-term decline in turbidity in the estuary has occurred since 1975, associated with several factors including upstream sediment trapping by dams, a gradual decrease in the sediment available downstream for resuspension and transport, washout during El Nino floods, and sediment trapping by submerged aquatic vegetation[1,2]. Because turbidity is required by Delta smelt for successful foraging and predator avoidance, this long-term trend is considered a credible contributor to population declines for the species. The decline in turbidity has occurred over the same time frame as increases in ammonia in Suisun Bay. Consequently, CUSUM correlations between Delta smelt abundance and turbidity are as likely to be as statistically significant (and equally invalid) as those using ambient ammonia concentrations as the independent variable.

_Trends in export volumes are not evaluated._ Although Glibert uses the results of her nutrient-related CUSUM analysis to argue that water management strategies have less influence than nutrients on population trends for fish, she does not acknowledge that water management strategies

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[1] Jassby A.D., J.E. Cloern, and B.E. Cole. 2002. Annual primary production: Patterns and mechanisms of change in a nutrient-rich tidal ecosystem. Limnol. Oceanogr. 47:698-712.

[2] Nobriga, M.L., T.R. Sommer, F. Feyrer, and K. Fleming. 2008. Long-term trends in summertime habitat suitability for Delta smelt (*Hypomesus transpacificus*). San Francisco Estuary & Watershed, Feb. 2008.

have allowed increases in seasonal and annual export volumes over the same time period that nutrient concentrations were evaluated. Glibert does not derive CUSUM scores for water exports in order to test alternative hypotheses related to the direct or indirect effects of exports on fish abundance. However, when subjected to the same analysis that Glibert uses in her paper, annual water exports perform as well as Suisun Bay ammonia concentrations in CUSUM correlations with summertime Delta smelt abundance. In Figure 3 below, CUSUM values for annual water exports volumes (summed for prior-year September through current-year August) are compared to CUSUM values for the Delta smelt Summer Townet index (STN). The overall relationship is statistically significant ($p < 0.0001$) and the $R^2$ value (0.42) is identical to that obtained when CUSUM for Suisun Bay ammonia was used as the independent variable (see Glibert Figure 18; also provided below as Figure 4).

Such omissions reveal the incomplete nature of the hypothesis testing provided by Glibert's study. CUSUM correlations are likely to be significant for any number of paired time series of environmental parameters from the Delta, provided the individual time series include an overall increasing or decreasing trend. However, all CUSUM correlations must be regarded as statistically flawed, and hypothesis testing using this approach is to be avoided. While CUSUM correlations between fish abundance and ammonia are convenient for focusing attention on ammonia (as opposed to other potential drivers of the food web or the POD), they ultimately signify little with respect to the relative importance of multiple environmental factors which have changed over recent decades in the San Francisco Estuary.

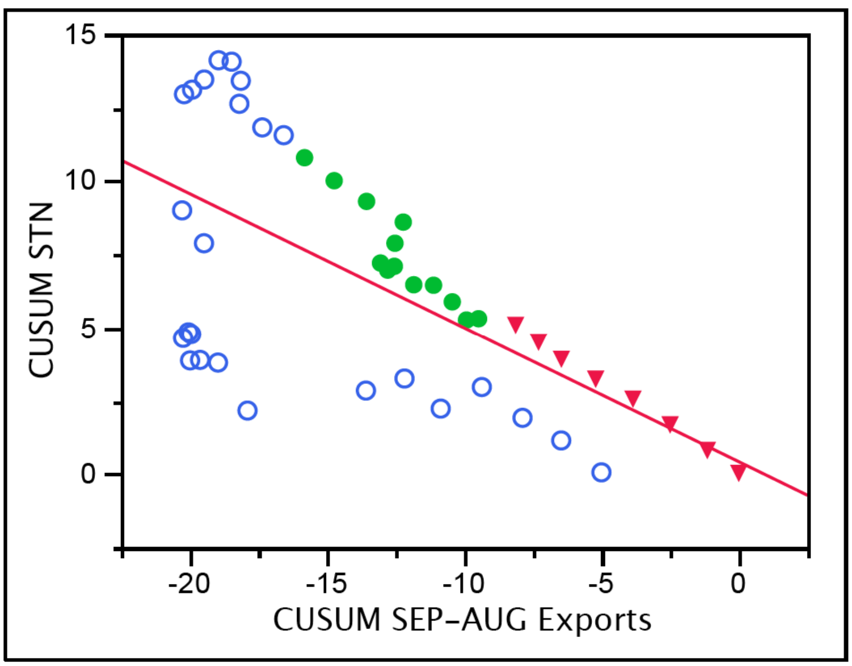


**Figure 3. Relationship between CUSUM values for annual water exports (x-axis) and CUSUM values for the Delta smelt Summer Townet Index (y-axis). The Summer Townet Index for each year (a proxy for June-August abundance) was paired with cumulative export volume for the 12-month period preceding the final summer townet (i.e. previous-year September through current-year August). Data for water exports were the combined export volumes (MAF) for the SWP, CVP and Contra Costa Canal computed from daily Dayflow model output. CUSUM series were calculated for the period 1959-2007 for both parameters. Regression line (red line) was significant ($P < 0.0001$; $R^2 = 0.42$). Color coding is as follows: open blue circles for pre-Corbula years (1956-1986), solid green circles for post-*Corbula* years 1987-1999, red triangles for POD years 2000-2007.**

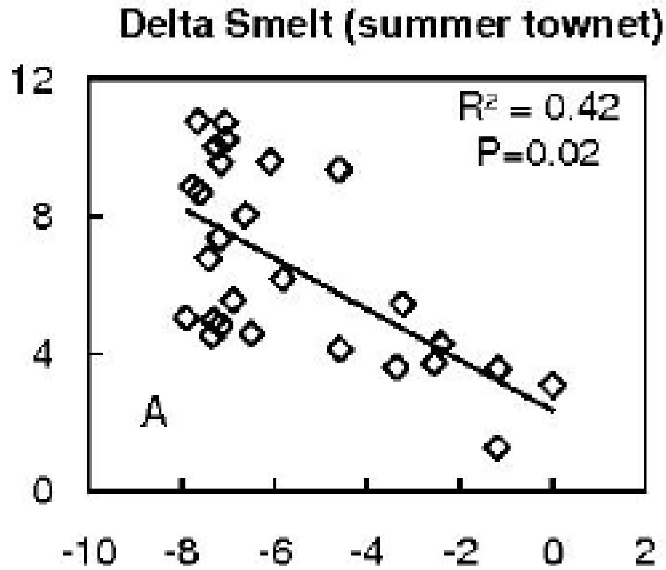


**Figure 4. Correlation between CUSUM for ammonium concentrations in Suisun Bay (IEP station D8) on the x-axis and CUSUM for the Delta smelt summer townet index on the y-axis, from Figure 20 in Glibert 2010. CUSUM series were calculated using data for 1975-2005.**

## 2. Problems with Underlying Ecological Assumptions

Many ecological assumptions are required to infer cause and effect from the correlations presented in Glibert's paper, or to support the conceptual model she developed. Several assumptions are problematic for reasons which include:

- Not New, but Not Well Tested. The assumption is a part of "prevailing wisdom" for the Delta, but may not be well tested or well supported by available research.
- New, but Unsupported. The assumption is not part of prevailing wisdom for the Delta, but no published or unpublished work is cited by Glibert which supports the assumption.
- Contradicted/Bounded. Other research contradicts the assumption, or places boundaries on its applicability.
- Logically Flawed. The assumption is not logical.

A few examples of problematic assumptions are provided below, with brief explanations.

***Assumption:*** *Ammonium places diatoms at a competitive disadvantage compared to other kinds of phytoplankton* (Contradicted/Bounded)

As revealed by SFSU research in 2008-2009,[3,4] phytoplankton biomass and growth rates in the Sacramento River do not respond to ammonium or $NO_3$:$NH_4$ ratios in the ways that were

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[3] Parker A.E., R.C. Dugdale, F.P. Wilkerson, A. Marchi, J. Davidson-Drexel, J. Fuller, and S. Blaser. 2009. *Transport and Fate of Ammonium Supply from a Major Urban Wastewater Treatment Facility in the Sacramento River, CA*. 9th Biennial State of the San Francisco Estuary Conference, Oakland, CA, September 29-October 1, 2009.

predicted by short-term bottle experiments conducted by R. Dugdale (SFSU) and colleagues using water from Suisun Bay and points westward in the estuary.[5,6] Based on the performance of the >5 µm size range of Sacramento River phytoplankton (a presumed proxy for diatoms) in a variety of nutrient addition experiments and several detailed longitudinal surveys of nitrogen and carbon uptake rates (from above Sacramento into Suisun Bay), it does not appear from the recent SFSU research that elevated ammonium or low $NO_3$:$NH_4$ ratios place diatoms at a competitive disadvantage compared to other phytoplankton taxa in the Sacramento River. Until analogous research is conducted in the interior Delta, the same caveat is reasonably applied to other freshwater Delta locations dominated by flows diverted from the Sacramento River. This means that Glibert cannot apply her conceptual model to the freshwater Delta without contradicting available research.

***Assumption:*** *Diatoms are vastly preferable as food for calanoid copepods, or at least for Eurytemora* (Not New, but Not Well Tested)

Non-diatom classes of phytoplankton include species which are perfectly good food for zooplankton - to such an extent that they are commonly used to culture zooplankton in the lab. Examples are *Cryptomonas* (a cryptophyte genus) and *Scenedesmus* spp. (a green genus), which are both used to rear zooplankton in laboratories. Both *E. affinis* and *P. forbesi* were more successfully cultured in the lab when fed the motile cryptophyte alga *Cryptomonas* than when fed the diatom *Skeletonema* or the green alga *Scenedesmus* suggesting these calanoid copepods might prefer motile prey.[7] Significant grazing on heterotrophic ciliates (non-phytoplankton) has been observed for both of the calanoid copepods included in Glibert's analyses: *P. forbesi* and *E. affinis.*[8] In feeding experiments using natural plankton assemblages from the SFE, another calanoid copepod (*Acartia*) grazed heterotrophic ciliates at higher rates than diatoms.[9] In addition the large diatom *Aulacoseira* (formerly *Melosira*) *granulata*, which is one of the more abundant taxa in blooms in the freshwater Delta, may not be very nutritious for zooplankton.[10] Ongoing research in the low salinity zone (LSZ) of the estuary indicates that bacteria and small-sized phytoplankton contribute to a complicated food web with many trophic levels between bacteria and the copepod prey favored by pelagic fish.[11]

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[4] Parker, A.E., A.M. Marchi, J. Drexel-Davidson, R.C. Dugdale, and F.P. Wilkerson. 2010. Effect of ammonium and wastewater effluent on riverine phytoplankton in the Sacramento River, CA. Draft Final Report, submitted to the Central Valley Regional Water Quality Control Board, March 17, 2010.

[5] Dugdale, R. C., F. P. Wilkerson, V. E. Hogue, and A. Marchi. 2007. The role of ammonium and nitrate in spring bloom development in San Francisco Bay. Estuar. Coast. Shelf Sci. 73: 17-29.

[6] Wilkerson, F.P., R.C. Dugdale, V.E. Hogue, and A. Marchi. 2006. Phytoplankton blooms and nitrogen productivity in San Francisco Bay. Estuaries Coasts 29: 401-416.

[7] Hall, C., and A. Mueller-Solger. 2005. Culturing delta copepods. IEP Newsletter., Vol. 18, No. 3, Summer 2005.

[8] Bouley, P., and W.J. Kimmerer. 2006. Ecology of a highly abundant, introduced cyclopoid copepod in a temperate estuary. Mar. Ecol. Prog. Ser. 324:219-228.

[9] Gifford, J.M., G. Rollwagen-Bollens, and S.M. Bollens. 2007. Mesozooplankton omnivory in the upper San Francisco Estuary. Mar. Ecol. Prog. Ser., 348:33-46.

[10] Orsi, J.J. 1995. Food habits of several abundant zooplankton species in the Sacramento-San Joaquin estuary. Interagency ecological program for the San Francisco Bay-Delta Estuary Technical Report 41.

[11] Kimmerer, W.J., SFSU, 2009, personal communication.

***Assumption:*** *Calanoid copepods have decreased in abundance in the Delta because they are food limited* (Not New, but Not Well Tested).

Kimmerer et al. (2005)[12] measured egg production by the calanoid copepod *Acartia* on several occasions during 1999-2002, and discovered that egg production during most of the year was below that observed during month-long spring phytoplankton blooms. However, similar data for the calanoid copepods *Eurytemora* and *Pseudodiaptomus* have not been reported. Direct mortality of copepod nauplii from entrainment by filtering clams was shown to be a better explanation than food limitation for declines in three species of estuarine copepods after the arrival of *Corbula amurensis.*[13] Chlorophyll-a levels below 10 µg/L are frequently cited as evidence that zooplankton in the Delta are food limited. However, this threshold is based on growth experiments conducted with a single cladoceran zooplankton species (*Daphnia magna*)[14] and it is unclear whether this threshold is appropriately applied to copepods in this system.

***Assumption:*** *Fish which have increased during the POD (largemouth bass, inland silversides, threadfin shad, sunfish) receive an advantage from the current nutrient/plankton regime*. (New, but Unsupported; Logically Flawed)

Glibert cites no ecological information from the Delta or other estuaries to support the hypothesis that largemouth bass, inland silversides, threadfin shad, or sunfish would thrive in an estuary populated with the copepod *Limnoithona* but not one populated with *Eurytemora* or *Pseudodiaptomus*. Also, largemouth bass and sunfish are associated with submerged aquatic vegetation (they aren't pelagic fish) and a credible hypothesis which has been advanced by Delta fisheries experts is that habitat changes (increasing lake-like conditions and proliferation of aquatic weeds) have allowed largemouth bass and sunfish to proliferate in the Delta.[15,16]

***Assumption:*** *Fish which have increased during the POD (largemouth bass, inland silversides, threadfin shad, sunfish) were disadvantaged by the previous nutrient/plankton regime* (New, but Unsupported; Logically Flawed)

As indicated above, Glibert implies that largemouth bass, inland silversides, threadfin shad, and sunfish have recently increased in the estuary because the current plankton regime favors them. To be logically consistent, one must conclude that the *previous* plankton regime was unfavorable for them. There is no reason to believe that the prior diatom/calanoid copepod regime would have provided a competitive *disadvantage* for these species prior to the POD.

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[12] Kimmerer, W.J., N. Ferm, M.H. Nicolini, and C. Penalva. 2005. Chronic food limitation of egg production in populations of copepods of the genus *Acartia* in the San Francisco Estuary. Estuaries 28:541-550.

[13] Kimmerer, W.J., E. Gartside, and J.J. Orsi. 1994. Predation by an introduced clam as the likely cause of substantial declines in zooplankton of San Francisco Bay. Mar. Ecol. Prog. Ser. 113:81-93.

[14] Müller-Solger, A.B., A.D. Jassby, and D. C. Müller-Navarra. 2002. Nutritional quality of food resources for zooplankton (*Daphnia*) in a tidal freshwater system (Sacramento-San Joaquin River Delta). Limnol. Oceanogr. 47: 1468-1476.

[15] Brown, L.R. and D. Michniuk. 2007. Littoral fish assemblages of the alien-dominated Sacramento-San Joaquin Delta, California, 1980-1983 and 2001-2003. Estuaries and Coasts 30:186-200.

[16] Moyle, P.B., W.A. Bennett, W.E. Fleenor, and J.R. Lund. 2010. Habitat variability and complexity in the upper San Francisco Estuary. Written Testimony submitted for the SWRCB Informational Proceeding to Develop Flow Criteria. Avail. at http://www.waterboards.ca.gov/waterrights/water_issues/programs/bay_delta/deltaflow/entity_index.shtml

***Assumption***: *Corbula are favored by the current phytoplankton assemblage*. (New, but Unsupported; Contradicted).

Glibert cites no evidence from the literature that would support her theory that *Corbula* fare better when filtering the current plankton assemblage, compared to prior assemblages. As explained below in one of the alternative hypotheses, there is evidence that the reverse is true: benthic grazing may influence phytoplankton composition in a top-down manner.

## 3. Alternative Hypotheses Regarding Trends in Plankton and Fish Abundance which are Unaddressed

Glibert's conclusions ultimately rely on the basic premises that (1) temporal shifts in the relative abundance of phytoplankton taxa were caused by changes in nutrient ratios, and (2) shifts in zooplankton abundance were caused by changes in the taxonomic composition of phytoplankton. There are credible alternative hypotheses for observed temporal shifts in the relative abundance of phytoplankton, zooplankton, and fish taxa which are not acknowledged in Glibert's article. In addition there is a credible alternative hypothesis for the timing of *Corbula's* establishment in the estuary (to contrast with Glibert's suggestion that the establishment of *Corbula* was ammonia driven). Below are several examples of alternative hypotheses related to community composition in the Delta which have been discussed in the literature and at recent Delta forums.

***Alternative Hypothesis 1:*** *Selective grazing by clams and copepods can influence the species composition of phytoplankton - and may contribute to the occurrence of Microcystis.*

Clam grazing selectively removes larger particles from the water column;[17] clams may consume a larger fraction of diatoms than smaller plankton taxa such as flagellates. Kimmerer (2005)[18] used long-term dissolved silica dynamics, corrected for mixing in the low salinity zone, as an indicator of diatom productivity in the northern SFE. He showed that there was a step decrease in annual silica uptake after 1986, which he attributed to efficient removal of diatoms by *Corbula amurensis* after its introduction in 1986. Grazing by *Corbicula fluminea* can cause shallow habitats in the freshwater Delta to serve as a net sink for phytoplankton;[19,20] it is possible that diatoms are differentially affected by benthic grazing (e.g., compared to motile or buoyant taxa) in both the brackish and freshwater Delta. In fact, benthic grazing has been implicated as a factor favoring *Microcystis* over other phytoplankton, as explained in the CalFed expert panel's "*Ammonia Framework:*"

"However, in places where filter-feeding mussels and clams overlap with habitat suitable for *Microcystis* (i.e., low salinity), the presence of these invertebrates might enhance bloom formation by selectively rejecting large *Microcystis* colonies. That grazer selectivity can give *Microcystis* a grazer-resistant, competitive advantage over

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[17] Werner, I., and J.T. Hollibaugh. 1993. *Potamocorbula amurensis*: Comparison of clearance rates and assimilation efficiencies for phytoplankton and bacterioplankton. Limnol. Oceanogr. 38: 949-964.

[18] Kimmerer, W.J. 2005. Long-term changes in apparent uptake of silica in the San Francisco Estuary. Limnol. Oceanogr. 50: 793-798.

[19] Lopez, C.B., J.E. Cloern, T.S. Shraga, A.J. Little, L.V. Lucas, J.K. Thompson, and J. R. Burau. 2006. Ecological values of shallow-water habitats: implications for the restoration of disturbed ecosystems. Ecosystems 9: 422-440.

[20] Parchaso F., and J. Thompson. 2008. *Corbicula fluminea* distribution and biomass response to hydrology and food: A model for CASCaDE scenarios of change. CALFED Science Conference, Sacramento, CA., October, 2008. Avail at http://cascade.wr.usgs.gov/CALFED2008.shtm

other phytoplankton, as Vanderploeg et al. (2001) reported for zebra mussels (*Dreissena polymorpha*) in the Great Lakes." (Meyer et al. 2009)[21]

In addition to mussels and clams, zooplankton can exert a top-down effect on phytoplankton composition; the literature regarding selective feeding by zooplankton is impractical to review herein. However, in a particularly pertinent example, selective grazing by the Delta copepod *Pseudodiaptomus forbesi* was recently demonstrated as a viable mechanism for encouraging *Microcystis* blooms.[22]

***Alternative Hypothesis 2:*** *Benthic grazing caused the crash in abundance of the copepod Eurytemora affinis in 1987.*

Direct mortality of copepod nauplii from entrainment by filtering clams was shown to be a better explanation than food limitation for declines in three species of estuarine copepods, including *Eurytemora affinis,* after the arrival of *Corbula*.[23]

***Alternative Hypothesis 3:*** *Residence time and other physical factors influence phytoplankton composition in estuaries.*

Physical factors (such as temperature, current speed, residence time, turbulent mixing, stratification, light penetration) may be strongly affecting competitive outcomes between diatoms and other phytoplankton taxa in the Delta, irrespective of (or in combination with) nutrient concentrations or ratios. The influence of flows and residence time on phytoplankton assemblages in estuaries is well-acknowledged in other regions. For example, hydrologic perturbations, such as droughts, floods, and storm-related deep mixing events, overwhelm nutrient controls on phytoplankton composition in the Chesapeake Bay; diatoms are favored during years of high discharge and short residence time.[24] The role of flow and residence time in regulating estuarine microfloral composition was summarized by the expert panel convened by CalFed in March 2009 in their final "*Ammonia Framework"* document:

"Diatoms have fast growth rates and may be particularly good competitors during high flows with concomitant short residence times, when their fast growth rates can offset high flushing rates. In moderate flows, chlorophytes and cryptophytes become more competitive, whereas low flows with concomitant longer residence times allow the slower-growing cyanobacteria, non-nuisance picoplankton, and dinoflagellates to contribute larger percentages of the community biomass. These spatially and temporally-variable patterns of phytoplankton composition are typical of many estuaries [e.g., Chesapeake Bay, Maryland; Neuse-Pamlico Sound, North Carolina; Narragansett Bay, Rhode Island; Delaware Bay, Delaware]." Meyer et al. (2009)

The idea that flows influence diatom abundance is not new in the Delta. P. Lehman (DWR) associated a multi-decadal decrease in the proportional biomass of diatoms in the Delta and

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[21] Meyer, J.S., P.J. Mulholland, H.W. Paerl, and A.K. Ward. 2009. A framework for research addressing the role of ammonia/ammonium in the Sacramento-San Joaquin Delta and the San Francisco Bay Estuary Ecosystem. Final report submitted to CalFed Science Program, Sacramento, CA, April 13, 2009.

[22] Ger, K.A., P. Arneson, C.R. Goldman, and S.J.Teh. 2010. Species specific differences in the ingestion of *Microcystis* cells by the calanoid copepods *Eurytemora affinis* and *Pseudodiaptomus forbesi*. Short Communication. J. Plankton Research. doi: 10.1093/plankt/fbq071

[23] Kimmerer, W.J., E. Gartside, and J.J. Orsi. 1994. Predation by an introduced clam as the likely cause of substantial declines in zooplankton of San Francisco Bay. Mar. Ecol. Prog. Ser. 113: 81-93.

[24] Pearl, H.W., L.M. Valdes, B.L. Peierls, J.E. Adolf, and L.W. Harding, Jr. 2006. Anthropogenic and climatic influences on the eutrophication of large estuarine ecosystems. Limnol. Oceanogr. 51(1, part 2): 448-462.

Suisun Bay to climatic influences on river flow.[25,26] The deep, pool-like bathymetry of the Stockton Deepwater Ship Channel is hypothesized by some investigators to function as a trap for diatoms in transport in the San Joaquin River.[27] The Central Valley Regional Board recently found that current speed in the Sacramento River was related to the difference in phytoplankton biomass between Freeport and Isleton.[28]

***Alternative Hypothesis 4:*** *Non-nutrient-related factors are drivers of Microcystis blooms and toxicity.*

Although Glibert did not analyze data for *Microcystis* using CUSUM, she advances the hypothesis that nitrogen enrichment underlies its recent proliferation in the Delta. Available research from the Delta argues against a simplistic association between *Microcystis* and nutrient form or concentration. Studies conducted by Peggy Lehman (DWR)[29,30] and Cecile Mioni (UCSC)[31] in the Delta have found no apparent association between ammonium concentrations or $NH_4^+$:P ratios and either *Microcystis* abundance or toxicity. Instead, it appears from these studies that water temperature is strongly positively correlated with *Microcystis* abundance and toxicity and that water transparency, flows, and specific conductivity are also potential drivers of *Microcystis* blooms in the Delta. Indeed, spring-summer mean water temperature trended upward in the freshwater Delta between 1996-2005.[32] An association between water temperature and *Microcystis* blooms in the Delta would be consistent with observations from other estuaries. Increased residence time (e.g., during drought) and warmer temperatures are acknowledged as factors stimulating cyanobacterial blooms in other estuaries.[33,34,35] In addition, as noted above, differential grazing by the currently dominant calanoid copepod in the Delta

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[25] Lehman, P.W. 1996. Changes in chlorophyll-a concentration and phytoplankton community composition with water-year type in the upper San Francisco Estuary. (pp. 351-374) In Hollibaugh, J.T, (ed.) San Francisco Bay: the ecosystem. San Francisco (California): Pacific Division, American Association for the Advancement of Science.

[26] Lehman, P.W. 2000. The influence of climate on phytoplankton community biomass in San Francisco Bay Estuary. Limnol. Oceanogr. 45: 580-590.

[27] P. Lehman, DWR, Feb. 2009, personal communication.

[28] Foe, C., A. Ballard, and S. Fong. 2010. Nutrient concentrations and biological effects in the Sacramento-San Joaquin Delta. Central Valley Regional Water Quality Control Board, Draft Report, May 2010.

[29] Lehman, P.W., G. Boyer, M. Satchwell, and S. Waller. 2008. The influence of environmental conditions on the seasonal variation of *Microcystis* cell density and microcystins concentration in the San Francisco Estuary. Hydrobiologia 600: 187-204.

[30] Lehman, P.W., S.J. Teh, G.L. Boyer, M.L. Nobriga, E. Bass, and C. Hogle. 2010. Initial impacts of *Microcystis aeruginosa* blooms on the aquatic food web in the San Francisco Estuary. Hydrobiologia 637: 229-248.

[31] Mioni, C.E., and A. Paytan. 2010. *What controls Microcystis bloom & toxicity in the San Francisco Estuary? (Summer/Fall 2008 & 2009)*. Delta Science Program Brownbag Series, Sacramento, CA. May 12, 2010.

[32] Jassby, A. 2008. Phytoplankton in the Upper San Francisco Estuary: recent biomass trends, their causes and their trophic significance. San Francisco Estuary & Watershed Science, Feb. 2008.

[33] Pearl, H.W., K.L. Rossignol, S. Nathan Hall, B.L. Peierls, and M.S. Wetz. 2009. Phytoplankton community indicators of short- and long-term ecological change in the anthropogenically and climatically impacted Neuse River Estuary, North Carolina, USA. Estuaries and Coasts. DOI 10.1007/s12237-009-9137-0

[34] Paerl, H.W., and J. Huisman. 2008. Blooms like it hot. Science 320: 57–58. doi:10.1126/science.1155398

[35] Fernald, S.H., N.F. Caraco, and J. J. Cole. 2007. Changes in cyanobacterial dominance following the invasion of the zebra mussel *Dreissena polymorpha*: long-term results from the Hudson River Estuary. Estuaries and Coasts 30: 163-170.

(*Pseudodiaptomus forbesi*) and by the non-native clams *Corbula amurensis* and *Corbicula fluminea* potentially confer a competitive advantage for *Microcystis* over other phytoplankton taxa.

***Alternative Hypothesis 5:*** *A period of low outfow and high X2 created favorable conditions for Corbula establishment in Suisun Bay after 1987.*

Salinity gradients are known to affect *Corbula* physiology, grazing rate, and settlement success.[36] A prolonged period of abnormally low Delta outflow from 1987-1992 is believed to have assisted the rapid establishment of *Corbula* in Suisun Bay after its introduction.[37] This seems a more plausible view of events than Glibert's hypothesis that *Corbula* was present in the estuary at undectable levels for years or decades and then received a sudden advantage of some kind beginning in 1987 from changes in the nutrient-phytoplankton regime.

***Alternative Hypothesis 6:*** *Physical characteristics of Liberty Island explain its current role as a Delta smelt refugium.*

On page 27, Glibert proposes that the apparent role of Liberty Island, in the Cache Slough area, as a recent year-round Delta smelt refugium is likely explained by lower $NH_4^+$ levels (compared to the Sacramento River) and more abundant diatoms (no data are presented to support this hypothesis in her article). However, other features of this habitat are seriously considered by Delta scientists as explanations for its above-average suitability for Delta smelt: (1) it is the remaining area within the Delta containing numerous dead-end sloughs (which were characteristic of the pre-settled Delta and which are believed to create opportunities for tide-induced retention of organic matter and suspended sediment),[38] (2) it has not become choked with invasive aquatic weeds, and (3) owing to wind-driven resuspension of sediment in shallow water and enhanced flood tide currents, it has higher levels of suspended sediment (needed by Delta smelt for predator avoidance and successful foraging) compared to the adjacent Delta.[39]

***Alternative Hypothesis 7.*** *Water exports affect the food web.*

Water exports affect residence time in the interior Delta and directly remove phytoplankton and zooplankton biomass from productive areas of the freshwater Delta. A carbon budget for the Delta indicates that water exports remove more phytoplankton carbon from the freshwater Delta than is contained in net Delta outflow.[40] Consequently, water exports effect the quantity - and likely the quality - of planktonic food resources transported through the Delta to Suisun Bay.[41]

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[36] Stillman, J. 2010. Metabolic responses to environmental salinity in the invasive clam *Corbula amurensis*. Interagency Ecological Program (IEP) Annual Workshop, Sacramento, CA, May 25-26, 2010.

[37] Moyle, P.B., W.A. Bennett, W.E. Fleenor, and J.R. Lund. 2010. Habitat variability and complexity in the upper San Francisco Estuary. Written Testimony submitted for the SWRCB Informational Proceeding to Develop Flow Criteria. Avail. at http://www.waterboards.ca.gov/waterrights/water_issues/programs/bay_delta/deltaflow/entity_index.shtml

[38] Whipple, A. 2010. Historical ecology of the Delta: Habitat characteristics of a fluvial-tidal landscape. Interagency Ecological Program (IEP) Annual Workshop, Sacramento, CA, May 25-26, 2010.

[39] King, T. 2010. Sedimentation processes and turbidities favoring endangered fish in the northern Sacramento-San Joaquin River Delta. Interagency Ecological Program (IEP) Annual Workshop, Sacramento, CA, May 25-26, 2010.

[40] Jassby, A.D., J.E. Cloern, and B.E. Cole. 2002. Annual primary production: patterns and mechanisms of change in a nutrient-rich tidal ecosystem. Limnol. Oceanogr. 47: 698-712.

[41] Baxter, R., R. Breuer, L. Brown, M. Chotkowski, F. Freyer, M. Gringas, B. Herbold, A. Müller-Solger, M. Nobriga, T. Sommer, and K. Souza. 2008. Pelagic organism decline progress report: 2007 synthesis of results. Interagency Ecological Program for the San Francisco Estuary.

Glibert suggests that water management strategies have not prevented pelagic fish declines because they are over-riden by nutrient-related processes. Unfortunately, Glibert does not subject data for water exports to CUSUM analysis, nor does she acknowledge that water management strategies have been accompanied by increases over time in seasonal and annual export volumes from the Delta. As shown above in Section 1, water exports perform as well as ambient ammonia concentrations in CUSUM correlations with Delta smelt summer abundance data.

***Alternative Hypothesis 8.*** *Aquatic weeds encourage non-native fishes.*

Glibert does not acknowledge that the rise of non-native, non-pelagic fishes (such as largemouth bass, sunfish species, and Mississippi silversides) is attributed in large part to the proliferation of invasive submerged aquatic vegetation (SAV) in the Delta.[42]

## 4. Inconsistencies

Pseudodiaptomus as prey. *Pseudodiaptomus forbesi* is widely touted as an important prey item for Delta smelt and other pelagic fish. Enough so that (1) the water agencies have gone to lengths to argue at recent venues that ammonia must be behind the recent decline in *Pseudodiaptomus*[43] and (2) the Central Valley Regional Board is funding ammonia toxicity tests using *Pseudodiaptomus*. However in Fig. 18, Glibert's CUSUM analysis suggests that Delta smelt and longfin smelt trend in opposite directions from *Pseudodiaptomus*. This is an example where a CUSUM correlation produces a result which contradicts our understanding of fish feeding ecology. In an environment of food scarcity, Delta smelt should be closely tracking their remaining food resources, unless sources of mortality other than food shortages are driving population trends.

Flows effect on $NO_3$:$NH_4$. Figure 8E in Glibert's paper shows an inverse relationship between outflow and $NO_3$:$NH_4$ in Suisun Bay prior to 2000, but a positive relationship after 2000. Glibert contends that $NH_4$ in Suisun Bay is controlled by effluent-$NH_4$ delivered to Suisun Bay via the Sacramento River (Fig. 7B). Higher levels of $NH_4$ would result in lower $NO_3$:$NH_4$ ratios, and vice versa (provided nitrate inputs are reasonably constant, see Fig. 6B). If so, why would flow (which presumably diluted effluent ammonia throughout the time record) have an opposite relationship with $NO_3$:$NH_4$ during two different portions of the time series? The relationship prior to 2000 is inconsistent with a premise that ammonium from the SRWTP, diluted and delivered via the Sacramento River, controls the $NO_3$:$NH_4$ ratio in Suisun Bay. The results suggest an incomplete understanding about the extent to which ammonium from SRWTP influences the $NO_3$:$NH_4$ ratio downstream in the brackish estuary.

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[42] Brown, L.R. and D. Michniuk. 2007. Littoral fish assemblages of the alien-dominated Sacramento-San Joaquin Delta, California, 1980-1983 and 2001-2003. Estuaries and Coasts 30: 186-200.

[43] Written Testimony submitted for the SWRCB Informational Proceeding to Develop Flow Criteria for the Delta Ecosystem on behalf of the San Luis & Delta-Mendota Water Authority, State Water Contractors, Westlands Water District, Santa Clara Valley Water District, Kern County Water Agency, and Metropolitan Water District of Southern California. Avail. at http://www.waterboards.ca.gov/waterrights/water_issues/programs/bay_delta/deltaflow/entity_index.shtml