**Hg OBJECTIVES**
Deadline: 2/28/07 5pm

SRCSD

Wastewater Treatment

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February 28, 2007

Tam Doduc, Chair and Members
State Water Resources Control Board
1001 I Street
Sacramento, CA 95814

**Re: Comments on the December 2006 Informational Document for the Public Scoping Meeting for Proposed Methylmercury Objectives for Inland Surface Waters, Enclosed Bays, and Estuaries in California.**

Dear Chairwoman Doduc & Members:

The Sacramento Regional County Sanitation District (District) is pleased to provide comments regarding the CEQA scoping for proposed methylmercury objectives for inland surface waters, enclosed bays, and estuaries in California. From a historical perspective, the District has been actively involved with mercury issues and the associated regulatory and scientific efforts that have been evolving in the Central Valley. Our efforts have been extensive, ranging from supporting mercury monitoring in the Sacramento River Watershed through in-kind services and securing federal grant funding for the Sacramento Regional Toxic Pollutant Control Program for over 12 years, to participation in the development of new analytical methods for detecting low levels of mercury (methods 1630, 1631). More recently, we have evaluated the feasibility of mercury offsets as a viable regulatory compliance tool. As a result, we have a thorough understanding of the current challenges that confront the wastewater industry in meeting increasingly stringent mercury and methylmercury goals.

We support, and strongly urge the State to include an additional alternative where individual watershed conditions are considered in establishing regional mercury fish tissue objectives, based on region-specific fish and fish consumption. Fish Tissue objectives are acceptable under *U.S. EPA Guidance for Implementing the January 2001 Methylmercury Water Quality Criteria (Section 3.1.2.1).* Fish tissue objectives are a better surrogate for beneficial use protection because they best reflect the risk to human and wildlife consumers of fish. They also keep the management focus on important mass loadings and in-system processes, which are key to broad-based watershed solutions.

In contrast, we believe strongly that a single objective for the entire state is inappropriate because environmental conditions, fish species, and food web structures vary greatly throughout the State and greatly alter the bioavailability of mercury. Fishing practices and consumption rates vary greatly as well. USEPA rules, regulation and guidance provide support for the notion of setting different water quality objectives for different parts of the state based on varying water quality conditions (e.g. different site-specific objectives for copper in different sub-regions of San Francisco Bay). This approach is embodied in the California Toxics Rule and the USEPA Water Quality Standards Handbook.

Sacramento Regional County Sanitation District

We recognize that the State Board should consider Options 1 through 5 and conduct a thorough CEQA review of their impacts. However, we strongly urge the State Board to reject Options 1 through 5, due to their reliance on total mercury water column objectives. The State Board's comprehensive CEQA review should recognize that the science behind the conversion of a fish tissue objective to an ambient water quality objective using BAFs is the subject of significant controversy. It is well established that total mercury levels in water bear no reliable relationship to levels in fish (see May 2003 LWA memo attached). The proposed total mercury water column objectives (Page 6, Implementation Procedures, paragraph 1) go against the national body of evidence and USEPA's action to not adopt a total mercury water column objective (sections 3.1.2.1 and 7.5.2 of USEPA Guidance).

We fail to see any value in employing mercury water column objectives in addition to fish tissue objectives. The focus on total or methyl mercury concentrations in water unnecessarily complicates the regulatory process and doesn't offer any gains in mercury management.

For certain substances that may lead to localized toxicity, concentration controls clearly make sense (for example, ammonia, copper, et. al.). For other substances that exert a regional effect, and particularly bioaccumulative substances like mercury, controlling mass is the most important and effective mechanism known at this time. Although we believe the actions are well intended, we find the State's, as well as the Central Valley Regional Board's attempts to control methylmercury discharges a distraction at best, and at worst, an impediment to meaningful mercury reductions (*e.g.*, offsets) in our region. The likely parties to perform offsets will be dischargers operating under NPDES permits. If such dischargers are required to install additional treatment to meet methyl mercury aqueous water quality objectives (AWQOs), (a) the financial resources for offsets will be diminished or eliminated and (b) the need for offsets will be similarly reduced. This will distract from any meaningful mercury load reduction efforts in the watershed.

Another, significant, disadvantage of Options 1-5 is the SWRCB staff's own acknowledgement that NPDES dischargers will need variances if water column objectives are adopted. Because reliance on the variance procedure is not a meaningful ***mercury management*** strategy, and will only provide additional, limited time for compliance, the State Board should instead turn its efforts and limited resources on developing a mercury management strategy that achieves ***real*** reductions in mercury loadings to the state's waters. The focus of mercury regulation statewide should remain on the outcome, specifically reductions in either mercury loads or in-system methylation processes as ways to seek attainment of fish tissue objectives.

We have provided additional specific comments by page reference following this letter. The District appreciates the opportunity to participate at this time and urges the State Board to continue to involve the wastewater community and other interested stakeholders in this important process. Please feel free to contact myself (876-6115) or Vicki Fry (876-6113) if you have any questions or wish to discuss any of these issues further.

Sincerely,

Terrie L. Mitchell
for Wendell H. Kido
District Manager

Attachment: Mercury BAF in Sacramento River Watershed Fish, LWA, May 2003

cc: Mary Snyder, Terrie Mitchell, Vicki Fry - SRCSD
Pamela Creedon, Executive Officer - CVRWQCB
Tom Kimball, Joanna Cox - SWRCB

**Specific Comments on the December 2006 Informational Document for the Public Scoping Meeting for Proposed Methylmercury Objectives for Inland Surface Waters, Enclosed Bays, and Estuaries in California.**

The order of these comments corresponds to the organization of the informational scoping document. Page and paragraph references are provided. Direct quotes from the document are *italicized*; in some cases emphasis has been added by using ***bold italics***.

1. **Page 1, paragraph 3:** CTR criteria replace mercury objectives in Basin Plans except in Region 2. The statement that "If there is a CTR criterion and an applicable objective for a water body, the more stringent of the two values applies" is incorrect, as applied to other Regions.

2. **Page 4, paragraph 1:** Clarify that the consumption rate refers to a small percentage of the overall population (anglers only). We do not believe there is sufficient justification to extrapolate the SFEI results for SF Bay anglers to the entire state. Alternatives should incorporate local or regionally relevant consumption rates.

3. **Page 6, Exhibit 2:** The use of a margin of safety in the determination of reasonable potential is not required and is not consistent with ongoing NPDES permit practice. Moreover, we suggest that the reasonable potential analysis is not required since the process of listing mercury impairment and pursuing mercury TMDL's already fulfills the purpose of establishing NPDES permit, as well as non-point source discharge requirements. This alternative procedure is unnecessary and should be eliminated from any proposed objective, if the State chooses to move forward with this effort.

4. **Page 6, Exhibit 2:** The use of an AWQO value to determine reasonable potential suffers the same lack of scientific basis as stated in our cover letter, and should not be encouraged or recommended under any alternative.

5. **Page 6, paragraph 2:** The fact that a variance procedure would be required as part of the implementation strategy for a methylmercury water column objective provides a strong reason to not pursue a methylmercury water column objective. Each alternative that proposes a variance procedure must address the benefit derived from a methylmercury water column objective that would offset the burden to all parties of a variance program.

6. **Page 7, possible implementation requirements:** Offset Projects that provide for both total and methyl mercury discharge compliance should be added to the list as a possible implementation option.

7. **Page 7, last paragraph:** We recommend the use of the following aspects of the USEPA guidance:
   - ☐ Use of the methylmercury fish tissue criterion in a non-traditional approach in the development of water quality-based effluent limits (WQBELs) in NPDES permitting (Section 7.5.2, pages 91 through 97) and TMDLs. As stated in the guidance, a fish tissue criterion is the preferred approach (Section 3.1.2.1, page 12). It is more closely tied to the fishing use, is easier to measure, provides a more direct measure of methylmercury effects, since fish consumption is recognized to be the primary route of exposure, and it avoids the need for use of bioaccumulation factors (BAFs). NPDES permitting and TMDL determinations that do not rely on the use of BAFs avoid introduction of the unnecessary uncertainty and assumptions associated with these factors into these important regulatory processes.

- Considerations to be made in the determination of whether a discharge is a significant source of mercury (section 7.5.2.1, p. 92 and 93).
- Collection of low detection limit mercury data to better understand water column concentrations in effluents and ambient waters as the foundation for watershed based approached to mercury management (Section 7.5.1.1.1, page 87).
- Collection of local fish tissue data to better understand methylmercury levels in various specifies and trophic levels for compliance determinations with the methylmercury criterion (Section 7.5.1.2.1, page 89).
- Mercury WQBELs for NPDES dischargers that emphasize performance of mercury minimization activities (source control and pollution prevention studies) (Section 7.5.2).
- Use of voluntary mercury offset programs that reduce mercury loadings to a watershed to provide NPDES permit relief to new sources or new dischargers (Section 7.6, Page 99). {Note: An important provision that should be added is that projects to reduce total mercury loads to a watershed must be credited as an offset to both total and methylmercury loadings from a discharger. Without this provision, the offset approach will not be a useful tool.}
- Development of mercury use attainability analyses (UAAs) as a tool to define subcategories of fishing uses and to understand and address the attainability of specific methylmercury targets and criteria (Section 3.2.3).

LARRY WALKER ASSOCIATES

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# TECHNICAL MEMORANDUM

| | |
|---|---|
| DATE: | May 2003 |
| TO: | Sacramento Regional County Sanitation District, c/o Vicki Fry and Mary James |
| CC: | Claus Suverkropp, LWA |
| SUBJECT: | Mercury BAF in Sacramento River Watershed Fish |

## INTRODUCTION

The U.S. Environmental Protection Agency (USEPA) has developed a criterion for methylmercury for the protection of human health (USEPA, 2001). The basis for the criterion is that the major exposure pathway to humans is the consumption of mercury contaminated fish. Mercury bioaccumulates through food web trophic levels. Methylmercury is the species of mercury commonly found in higher trophic level fish. The human health criterion for methylmercury is a fish tissue residue criterion (USEPA, 2001). To translate the fish tissue criterion into a water column criterion, USEPA suggests that a bioaccumulation factor (BAF) may be developed on a national or regional scale, or on a site-specific basis. A BAF is a ratio of the expected fish tissue mercury concentration to the measured ambient water column mercury concentration. As part of the BAF approach, USEPA's suggests use of partition coefficients to convert water column methylmercury concentrations to total mercury concentrations. In effect, the use of BAFs presumes a linear linkage between a given water column total mercury concentration and the resulting fish tissue mercury concentration.

Factors cited in the methylmercury criterion document (USEPA, 2001) that may influence mercury bioaccumulation include: ambient pH, dissolved organic matter, temperature, and length of local food web. Because USEPA acknowledges that local ambient factors can influence bioaccumulation, the USEPA suggests developing site-specific BAFs.

An investigation of the available water column and fish tissue data collected in the Sacramento River Watershed (SRW) is presented below to evaluate the applicability of the BAF approach. Specifically, the data are analyzed to determine the appropriateness of a BAF for the environmental conditions within the SRW. Standard regression techniques (Neter, *et al.*, 1990) are used to test possible relationships between water quality data and fish tissue mercury concentrations. Single variate, multivariate, and single and multivariate transform regressions were developed to test relationships between water column constituent concentrations and fish tissue mercury concentrations. In single variate regressions, one independent variable (e.g. water column total mercury) is regressed with one dependent variable (e.g. largemouth bass tissue mercury concentration). Multivariate regressions relate two or more independent variables (e.g. pH and water column total mercury) to one dependent variable. In transform regressions, the variables (independent or dependent) are transformed as necessary (e.g. with the natural log) before performing the regression.

The location of the SRW within California is presented in Figure 1. The Sacramento River is the largest river in California, with an annual average stream flow volume of 22 million acre-feet (27 $km^3$/yr). The river is also the longest in the State, extending over 327 miles (526 km). Major tributaries to the Sacramento River include the Feather River, the American River, and the Pit River. Dams have been constructed over the past century on the Sacramento River downstream of the confluence with the Pit River (Shasta Dam) and on each of the other major tributaries (Oroville Dam on the Feather River and Folsom Dam on the American River). In total, there are over one thousand lakes and reservoirs throughout the watershed. River diversions are also common for transferring water to users and for flood control in the Central Valley.

The Sacramento River and several tributaries support beneficial uses potentially impacted by mercury. These include aquatic life and wildlife habitat; sport, subsistence, and commercial fishing; and rare and endangered species habitat. Predominant land uses in the SRW today are forests and rangeland, comprising 59% and 17% of the land area, respectively. This figure illustrates the large area (much of it forested and at higher elevations) owned by federal and state agencies (37% and 2%, respectively), where land management practices could be addressed by specific remediation activities. Agricultural uses (predominantly rice in poorly drained clayey soils, along with orchards, field crops, and vineyards) comprise approximately 17% of the land area and are located primarily in the floor of the Sacramento Valley. There are about 2.5 million people living in the watershed, with over half of the urbanized population located at the downstream end in Yolo, Placer, and Sacramento Counties.

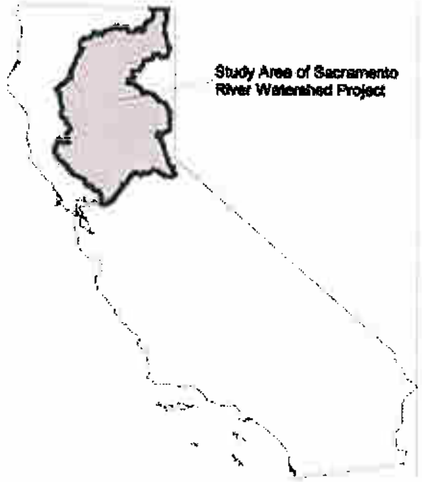

Figure 1: Sacramento River Watershed location within California.

Each fish tissue sampling location with available data is plotted within the SRW in Figure 2. Of the monitoring locations in the SRW, sufficient water quality and fish tissue data to perform a detailed site-specific investigation are only available for the "River Mile 44" site on the Sacramento River. Due to gaps in the data sets from the other monitoring locations, a pooled watershed-scale analysis is performed using long-term statistical representations of the water quality data to complete the analysis.

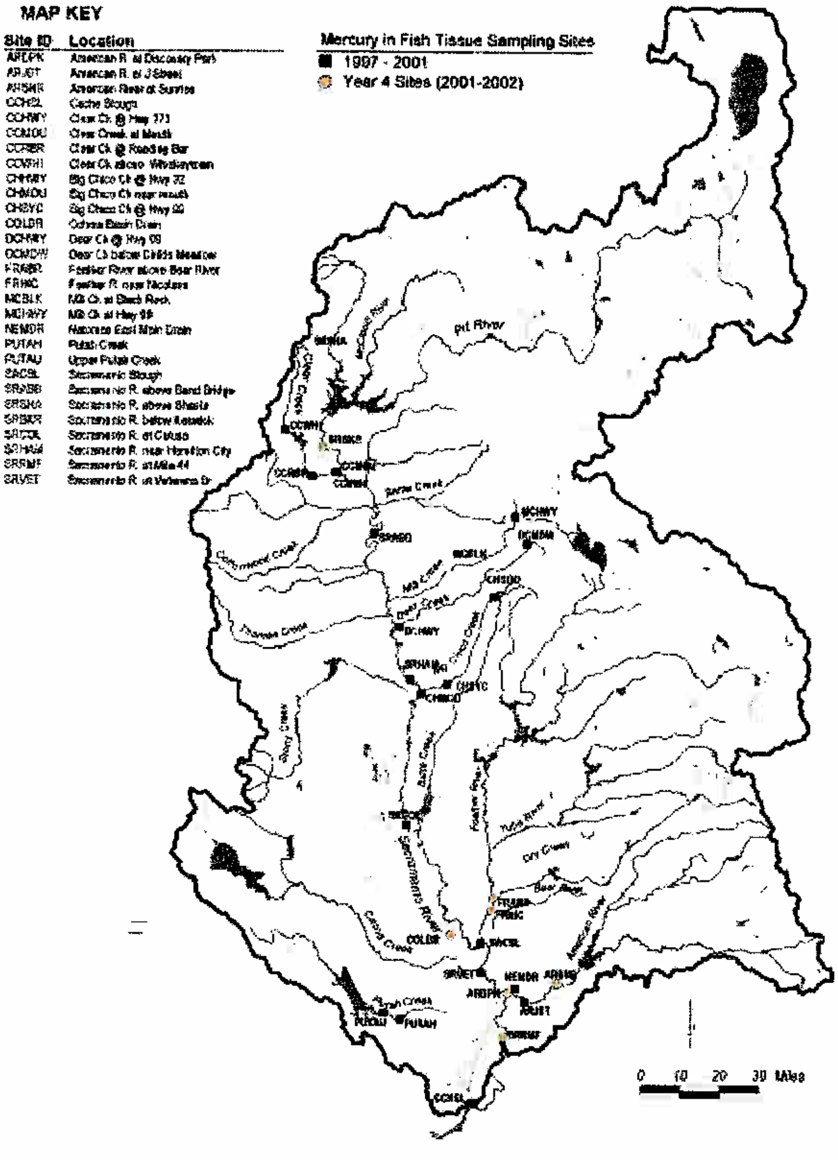

Figure 2: Sacramento River Watershed detailing fish tissue collection sites.

## METHOD OF ANALYSIS

The data analysis progressed through the following steps:

1. Analyze the fish tissue mercury concentration data to indicate any immediately obvious trends.
2. Investigate site-specific data availability for water column constituent concentrations, and species availability for monitoring sites within the SRW.
3. Analyze site-specific fish tissue mercury concentrations as a function of fish species and length, and normalize tissue concentrations as appropriate (e.g., to fish length).
4. Investigate site-specific relationships between water column constituents (e.g. total mercury, methylmercury, pH, DOC, others) and fish tissue mercury.
5. Investigate tissue concentration-length relationship for largemouth bass on a watershed-wide scale, and normalize tissue mercury concentrations to fish length.
6. Investigate watershed-wide regressions between water column constituents (e.g. total mercury, methylmercury, pH, DOC, others) and fish tissue mercury.

Each step in the analysis is detailed below. Note that the measure of significance of a relationship is calculated as a "p-value". A very low p-value (i.e., $p<0.05$) corresponds to a high level of confidence that the observed relationship between the two variables is not due to chance alone; i.e., that the variables are in fact significantly related to one another. Alternately, a higher p-value ($p>0.05$) indicates that there is no statistically significant relationship between the variables.

### General Aspects of the Datasets

Water constituent data were obtained in part from the Sacramento River Coordinated Monitoring Program (LWA, 2002). Additional water column constituent data and fish tissue mercury levels were obtained from the Sacramento River Watershed Program (SRWP, 2003).

Fish tissue mercury data are available in the SRW for largemouth bass, white catfish, trout, panfish, and striped bass. The trout dataset is comprised of one brown trout with the remainder representing rainbow trout. Bluegill, redear sunfish, and crappie represent the panfish. A summary of available fish species with tissue mercury concentration data is presented in Table 1. Log-normal probability distributions of the tissue concentration data for each species class are presented in Figure 3. Note that the mean mercury tissue concentration for white catfish, largemouth bass, and stripped bass are above the USEPA criterion level of 0.3 mg/kg.

Table 1: Summary of fish species available for analysis in the Sacramento River Watershed.

| Category | Species | TL [a] | n | % > 0.3 mg/kg [b] |
|---|---|---|---|---|
| Largemouth Bass | Largemouth Bass | 4 | 105 | 92.4 |
| White Catfish | White Catfish | 4 | 73 | 67.1 |
| Trout | Rainbow Trout | 3 | 20 | 0.0 [c] |
|  | Brown Trout | 3 | 1 |  |
| Panfish | Bluegill | 3 | 8 | 14.3 [d] |
|  | Redear Sunfish | 3 | 4 |  |
|  | Crappie | 4 | 2 |  |
| Striped Bass | Striped Bass | 4 | 8 | 100 |

Notes
[a] Trophic Level.
[b] Percent of fish with measured tissue mercury concentrations above 0.3 mg/kg (the USEPA national water quality criterion).
[c] Pooled trout data.
[d] Pooled panfish data.

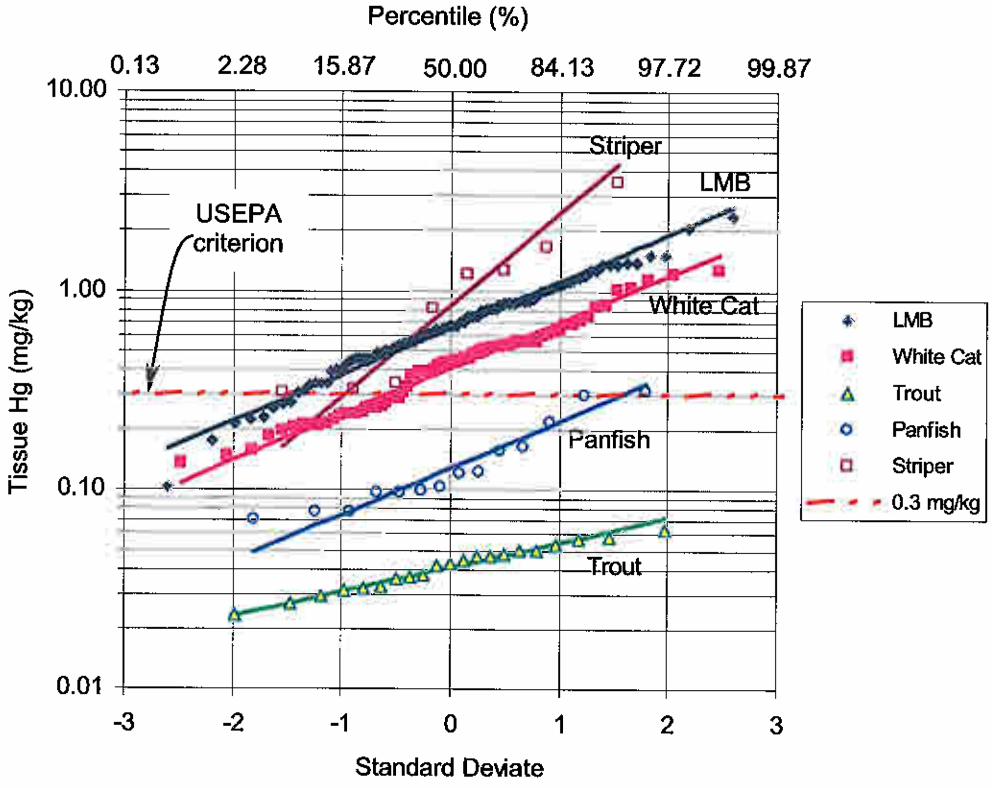

Figure 3: Log-normal probability distributions of fish tissue mercury concentrations for five species of fish. Fish samples collected from 1997-2001 at sites across the Sacramento River watershed.

To determine if a relationship exists between the length of fish and fish tissue mercury concentrations, the tissue mercury concentration for each measurement for each species listed in Table 1 is plotted in Figure 4 as a function of its measured length. Regression summaries for fish tissue mercury concentrations as a function of fish length are presented in Table 2. Only largemouth bass and white catfish regressions produced statistically significant relationships at a 95% confidence level. The striped bass relationship is significant at a 90% confidence level.

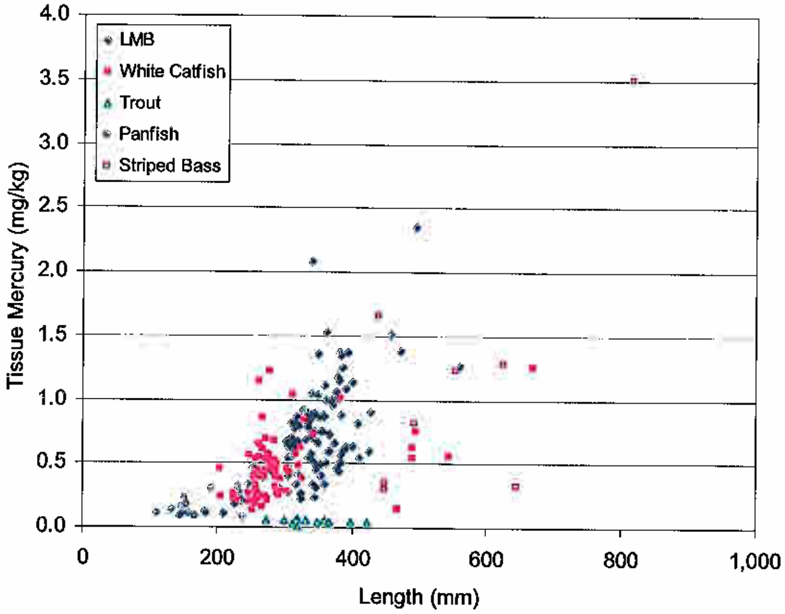

Figure 4: Fish tissue mercury plotted as a function of length for five fish species.

Table 2: Linear regression summary of fish tissue mercury concentrations vs. fish length for each species category in the Sacramento River Watershed.

| Category | n | $r^2$ | p [a] | b [b] | m [c] |
|---|---|---|---|---|---|
| Largemouth Bass | 105 | 0.33 | < 0.0001 | -0.74 | 0.0042 |
| White Catfish | 73 | 0.18 | 0.0002 | 0.062 | 0.0014 |
| Trout | 12 | 0.19 | 0.15 | NS | NS |
| Panfish | 14 | 0.13 | 0.20 | NS | NS |
| Striped Bass | 8 | 0.49 | 0.053 | -2.0 [d] | 0.0057 [d] |

Notes

[a] Regression statistically significant at a level of (1.0 – p)x100% (e.g. $p < 0.05$ means the regression is significant at a 95% confidence level).

[b] Intercept of regression. NS = not statistically significant at 95% confidence level.

[c] Slope of regression. NS = not statistically significant at 95% confidence level.

[d] Not statistically significant at 95% confidence level, but likely to be significant with additional data.

### Site-Specific Water Column and Fish Tissue Data

The available total mercury and filtered total mercury for two monitoring locations "River Mile 44" on the Sacramento River, and Feather River near Nicolaus are presented in Figure 5. Superimposed on Figure 5 are available largemouth bass tissue mercury concentrations for both locations. Because fish integrate exposures over a long time period, the relationship between tissue mercury and water column mercury was evaluated for different averaging periods (ranging from instantaneous to 3 years) for the River Mile 44 data. In the SRW the River Mile 44 site is the only data set complete enough to perform the site-specific regressions. The water column data for Nicolaus, displayed in Figure 5, is typical of other sites within the SRW, with fish tissue samples collected during gaps in water column sampling. These gaps in the data sets for other monitoring locations preclude site-specific investigations using the three-year averaging periods, which were found to have the best correlations (as explained below).

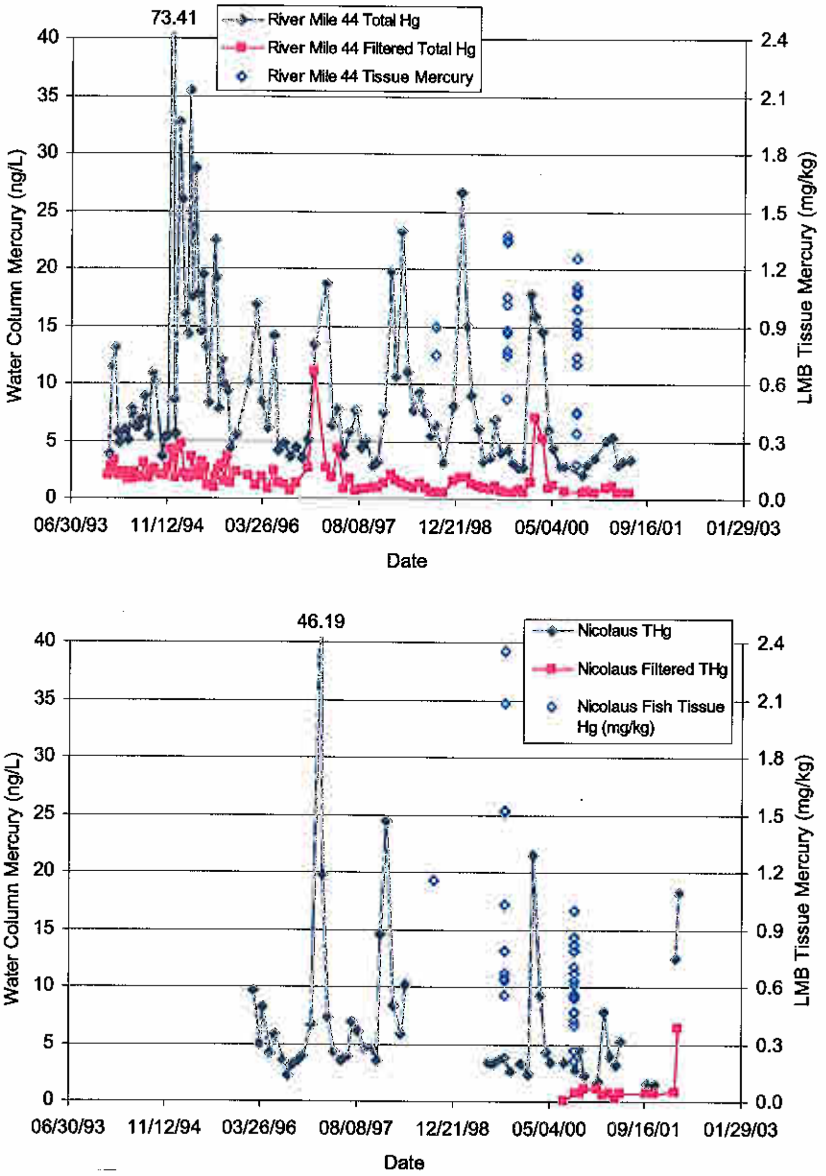

Figure 5: Total mercury, filtered total mercury, and largemouth bass data from River Mile 44 on the lower Sacramento River and Feather River near Nicolaus.

The available fish tissue data available for the River Mile 44 site is summarized in Table 3. Available fish tissue data from the Nicolaus site on the Feather River is summarized in Table 4.

Table 3: Summary of fish species available for analysis from the Sacramento River Mile 44 site.

| Category | Species | TL [a] | n | % > 0.3 mg/kg [b] |
|---|---|---|---|---|
| Largemouth Bass | Largemouth Bass | 4 | 30 | 100 |
| White Catfish | White Catfish | 4 | 33 | 50.0 |
| Trout | Rainbow Trout | 3 | 0 | NA [c] |
| | Brown Trout | 3 | 0 | |
| Panfish | Bluegill | 3 | 1 | 0 [d] |
| | Redear Sunfish | 3 | 0 | |
| | Crappie | 4 | 0 | |
| Striped Bass | Striped Bass | 4 | 1 | 100 |

Notes
[a] Trophic Level.
[b] Percent of fish with measured tissue mercury concentrations above 0.3 mg/kg (the USEPA national water quality criterion).
[c] Pooled trout data.
[d] Pooled panfish data.

Table 4: Summary of fish species available for analysis from the Nicolaus site.

| Category | Species | TL [a] | n | % > 0.3 mg/kg [b] |
|---|---|---|---|---|
| Largemouth Bass | Largemouth Bass | 4 | 30 | 93.1 |
| White Catfish | White Catfish | 4 | 10 | 100 |
| Trout | Rainbow Trout | 3 | 0 | NA [c] |
| | Brown Trout | 3 | 0 | |
| Panfish | Bluegill | 3 | 1 | 0 [d] |
| | Redear Sunfish | 3 | 1 | |
| | Crappie | 4 | 0 | |
| Striped Bass | Striped Bass | 4 | 5 | 100 |

Notes
[a] Trophic Level.
[b] Percent of fish with measured tissue mercury concentrations above 0.3 mg/kg (the USEPA national water quality criterion).
[c] Pooled trout data.
[d] Pooled panfish data.

### Site-Specific Fish Tissue Mercury Normalization to Average Fish Length

Fish tissue mercury concentrations plotted against fish length for both the River Mile 44 and Nicolaus monitoring locations are presented in Figure 6. Results from linear regressions performed for each site are included in Figure 6. Presumably, bioaccumulation occurs as the fish grow in length. Different rates of bioaccumulation would be reflected in different slopes of the regression lines in Figure 6.

The regressions of Figure 6 are used to normalize the tissue mercury concentrations to a specific length largemouth bass. The normalization removes the length dependence of fish tissue mercury concentrations on the fish length and allows evaluation of relationships with water quality data (e.g. mercury species and fractions, pH, DOC, etc.) unbiased by differences in fish length.

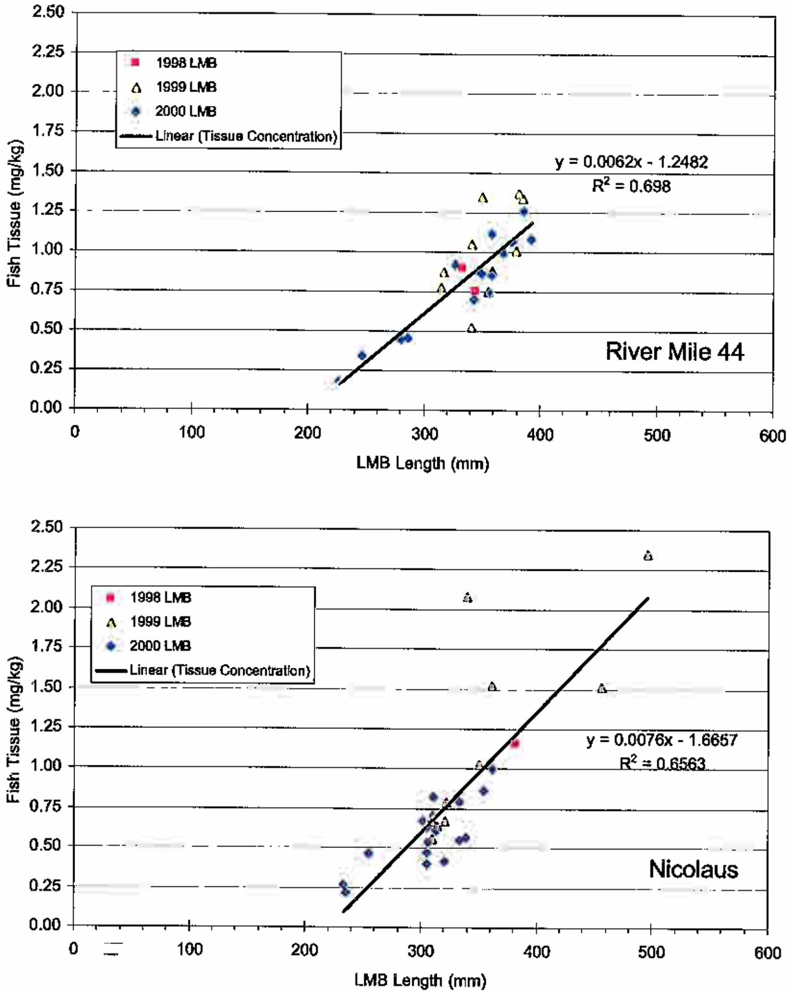

Figure 6: Largemouth Bass tissue mercury concentrations as a function of fish length for River Mile 44 and Nicolaus sites.

### Site-Specific Regressions of Water Column Chemistry to Normalized Fish Tissue

Available water column methylmercury data and largemouth bass tissue mercury concentrations are presented in Figure 7. The time frames for water column and fish tissue data do not overlap at River Mile 44, so correlations between water column methylmercury and mercury levels in fish cannot be assessed as part of the site-specific investigations.

To test the sensitivity of length of time averaging of water column constituents to the strength of the regression to fish tissue concentrations, varying averaging periods for water data were used in the regressions with fish mercury concentrations. Fish samples are collected in late summer/early fall of each year. Time averaging of water chemistry extends from September of the year the fish is collected back in time for the desired averaging period. The results for regressions with instantaneous, 1-year average, 2-year average, and 3-year average for total water column mercury concentrations are presented in Figure 8, for both raw and normalized fish tissue concentrations for the Mile 44 data set. Longer averaging periods reduced the variation in average water column values. Regressions performed with the raw tissue data tended to result in higher $r^2$ values, but normalizing by fish length properly removes the influence of fish length from the relationships. Since length biases do exist in the data, length normalization of the data is an important step in the analysis. None of the regressions in Figure 8 represents a statistically significant relationship at even a 90% confidence level (i.e., p-values are all greater than 0.10).

The very small $r^2$ values indicate that essentially none of the observed variability of the measured fish tissue mercury levels is related to the water column total mercury concentrations. The low significance levels of the regressions indicate that the calculated relationships (e.g. increasing fish tissue concentration with increasing water column concentration) are not fully supported, and in fact, there is a significant probability that no relationship exists (i.e. the slope is equal to zero). Also, it should be noted that the best-fit lines are essentially flat, indicating little change in fish tissue levels with change in water column concentrations. None of the regressions passed through the origin, indicating that a linear, 1:1 BAF relationship is not supported by the data collected in the SRW.

The median water column total mercury for the investigated time periods regressed with the fish tissue mercury concentrations are displayed in Figure 9. Results in Figure 9 are similar to results presented in Figure 8. None of the regressions in Figure 9 approaches a statistically significant relationship at even a 90% confidence level. A non-significant regression between water column total mercury and normalized fish tissue mercury indicates that the water column concentration of total mercury is not a significant factor in determining the mercury concentration in fish tissue.

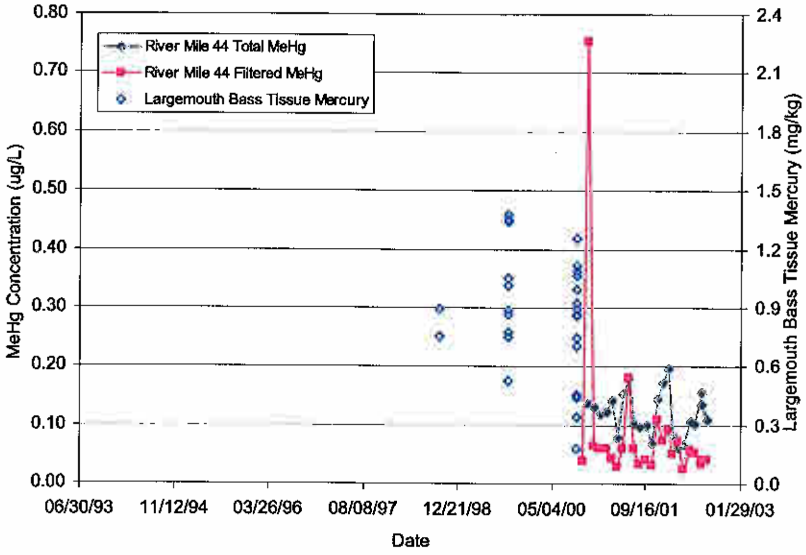

Figure 7: Time series plot of available methylmercury water column data and fish tissue mercury data for Mile 44 monitoring location.

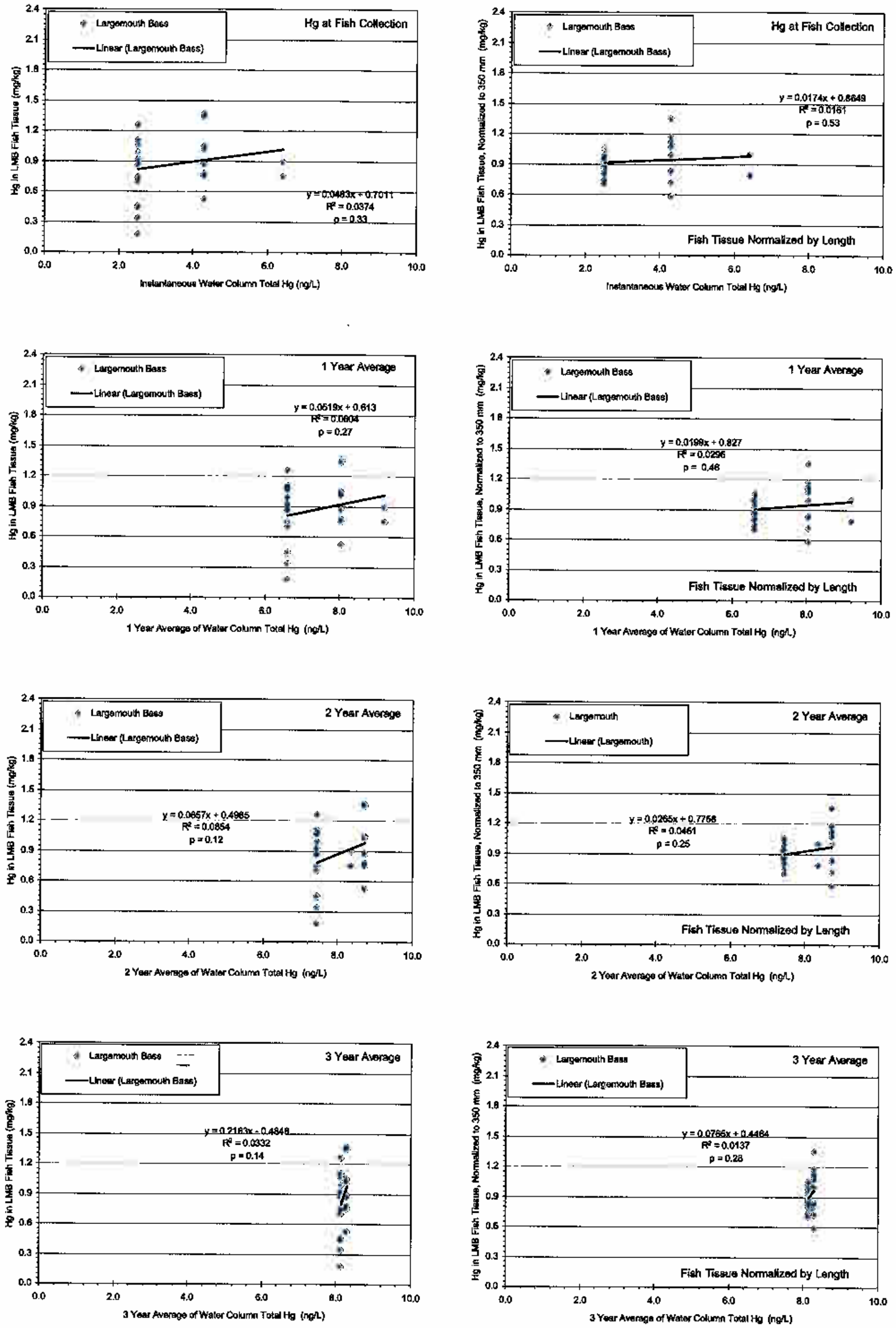

Figure 8: Un-normalized (left column of graphs) and length-normalized (right column of graphs) largemouth bass tissue concentration regressed against varying total mercury <u>averaging</u> periods (increasing from top to bottom) for the River Mile 44 site.

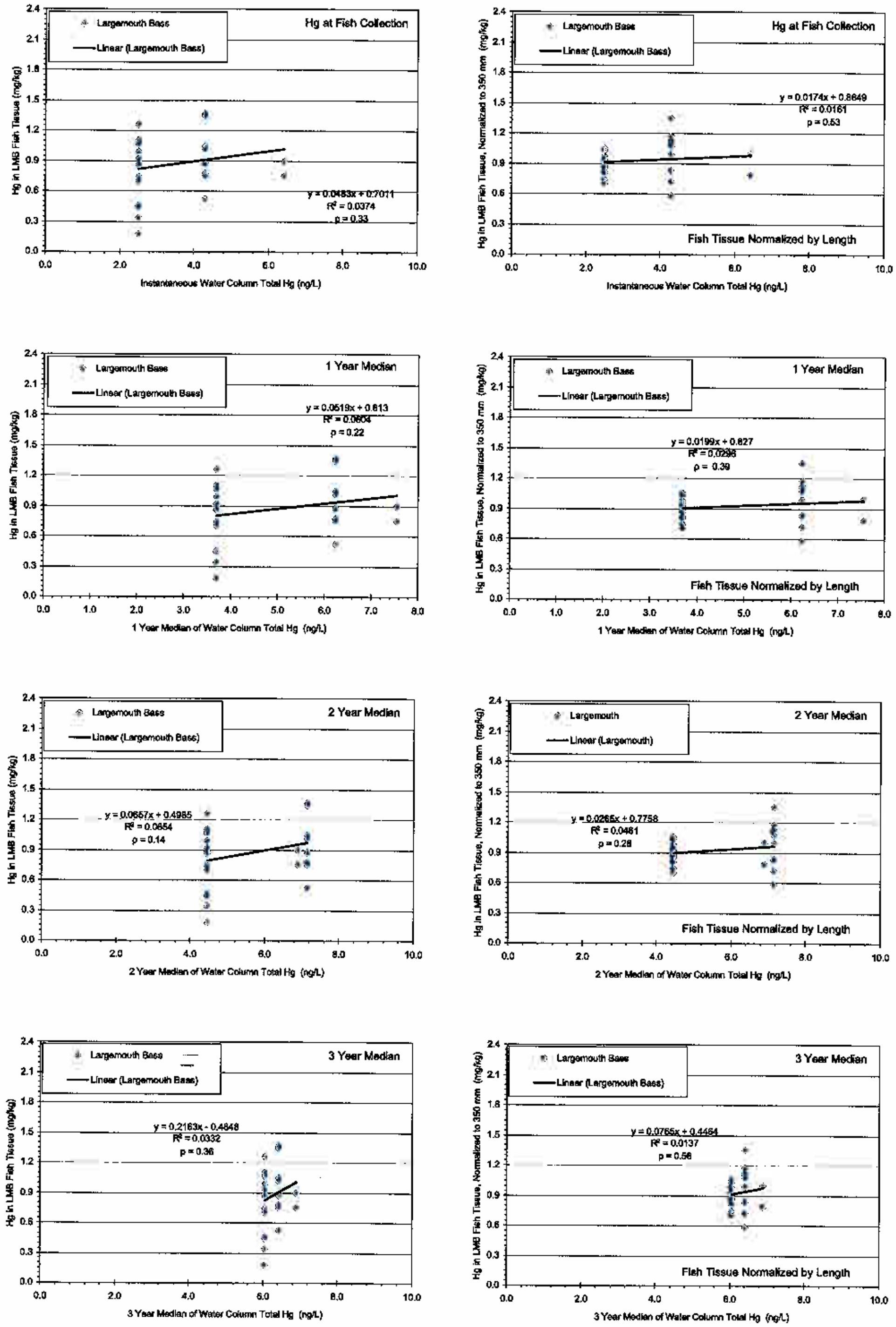

Figure 9: Un-normalized (left column of graphs) and length-normalized (right column of graphs) largemouth bass tissue concentration regressed against varying periods of data collection used to determine <u>median</u> total mercury concentrations for the River Mile 44 site.

### Watershed-wide Normalization of Fish Tissue Mercury Concentrations

Tissue mercury concentrations plotted against fish length for all largemouth bass sampled in the SRW are presented in Figure 10. The correlation is lower than either of the site-specific plots in Figure 6, as expected, owing to the effect of site-specific factors (e.g. food web). The data presented in Figure 10 represent conditions from 11 monitoring sites, and differences in conditions at each site would act to spread the data.

Largemouth bass tissue mercury concentrations were normalized by length using the regression equation listed on Figure 10. Normalization by length allows comparisons of site-specific water quality factors unbiased by differences in size of fish from each site.

Normalized largemouth bass tissue mercury concentrations are plotted against monitoring location in Figure 11.

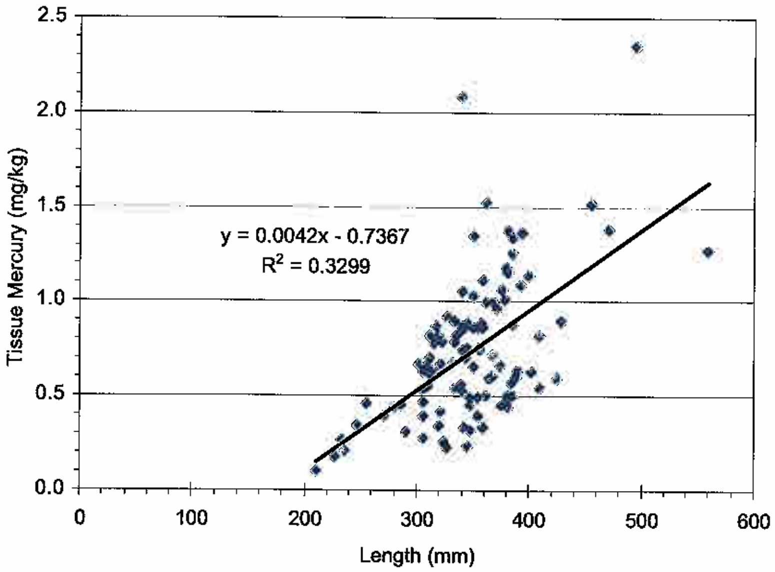

Figure 10: Largemouth bass tissue mercury concentration regressed against fish length (p-value < 0.0001).

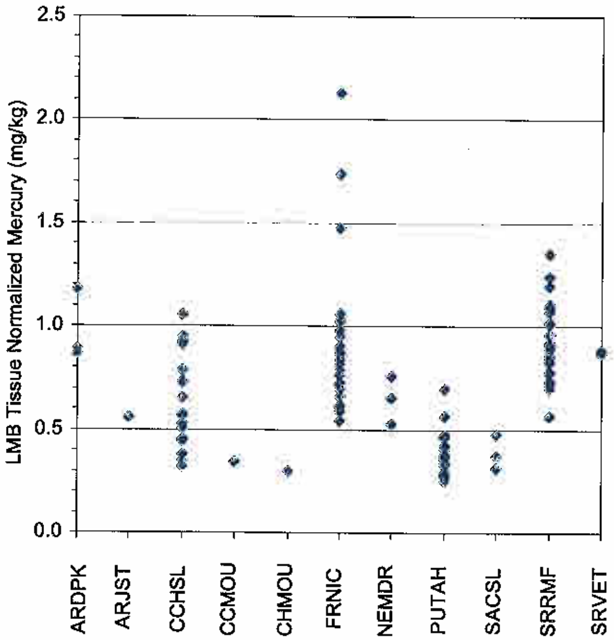

Figure 11: Largemouth bass tissue mercury concentrations, normalized to 350-mm length bass using the regression equation in Figure 10.

### Watershed-wide Regression Models Between Fish Tissue Mercury and Water Chemistry

Normalized largemouth bass tissue mercury concentrations regressed against the median water column total mercury concentrations are presented in Figure 12. Confidence intervals for the regression and predictions are included with the regression results displayed in Figure 12. The confidence interval for the regression represents the expected variability of the regression line if the entire monitoring effort was repeated. An important note is that the slope of the regression is negative; giving the counter-intuitive result that fish tissue concentrations decreasing with increasing water column mercury concentrations. The prediction confidence interval represents the expected variability in any new measurement. The predictive power of the regression is weak, as the confidence intervals essentially extend from 0.1 to 1.7 mg/kg for all values of water column total mercury. The residuals from the regression are presented in Figure 13.

The residual plot is difficult to interpret, as the long-term median representation of the water column mercury tends to clump the data. The residuals in Figure 13 may follow a pattern, indicating a transformation of the data is appropriate. Therefore, both the tissue mercury and water column total mercury concentrations were transformed with the natural log operator and the data re-regressed. The results from the transformed regression are plotted in Figure 14. The log-log model also predicts decreasing fish tissue concentrations of mercury with increasing water column total mercury. The residuals do not look more centralized (see Figure 15) than the residuals from the simple linear regression. Furthermore, the log-log model result is as improbable as the result from the simple linear model. These results provide weight to the conclusion that the tissue mercury concentrations are dependent on factors other than water column mercury concentrations.

Largemouth bass tissue concentrations of mercury regressed against filtered total water column mercury, total water column methylmercury, filtered water column methylmercury, water column pH, and water column filtered organic carbon are presented in Figure 16 through Figure 20. None of the regressions are remarkable, except the methylmercury regression results in negative slope. The negative slopes indicate an improbable result (the tissue concentrations decrease with increasing methylmercury concentrations) indicating that site-specific conditions other than water column methylmercury concentrations exert a greater influence on tissue concentrations than any long-term median water column measurement.

From a purely statistical standpoint, one of the best (low p-value and largest $r^2$ value) single variate models is the natural log of tissue mercury regressed against the natural log of the water column total methylmercury. The regression and confidence intervals are plotted with the data on Figure 21, and the residuals are plotted in Figure 22. As is evidenced by Figure 21, the model result is nonsensical, predicting infinite tissue concentrations as the water column methylmercury concentrations are reduced to zero, and decreasing fish tissue concentrations with increasing methylmercury concentrations. Because the statistically best regression model is an improbable result, site specific factors other than water column methylmercury exhibit a greater influence on fish tissue mercury.

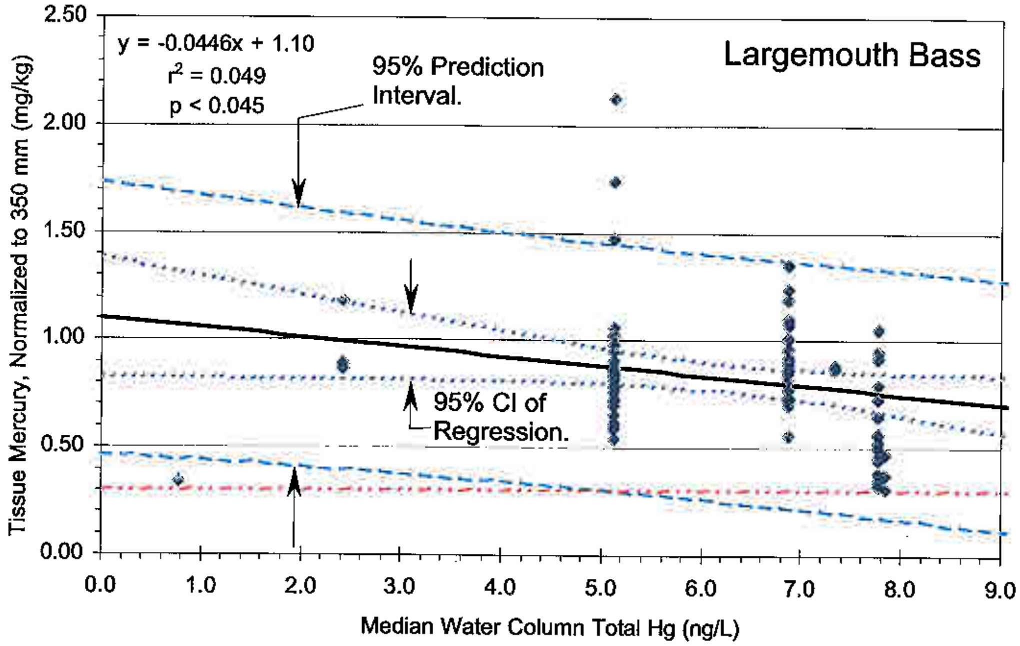

Figure 12: Confidence intervals from regression of tissue mercury concentrations in largemouth bass against water column total mercury. Linear regression and correlation coefficients presented on plot. Regression confidence interval (CI) depicts the expected variability of regression if all data were recollected and reanalyzed. Prediction interval is the expected range for any one new observation.

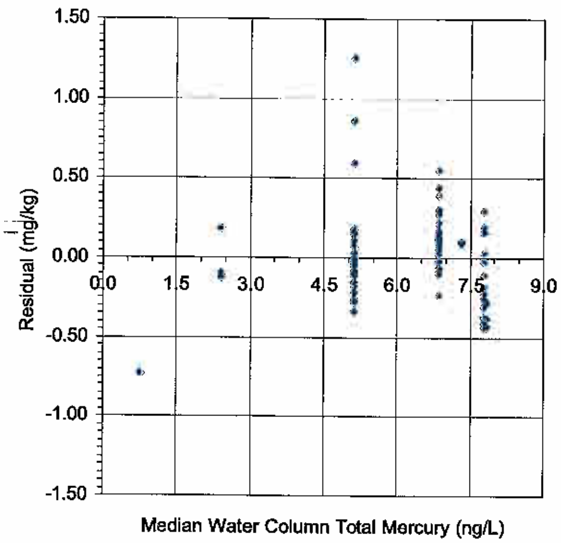

Figure 13: Residual plot for linear regression of largemouth bass tissue mercury against water column total mercury.

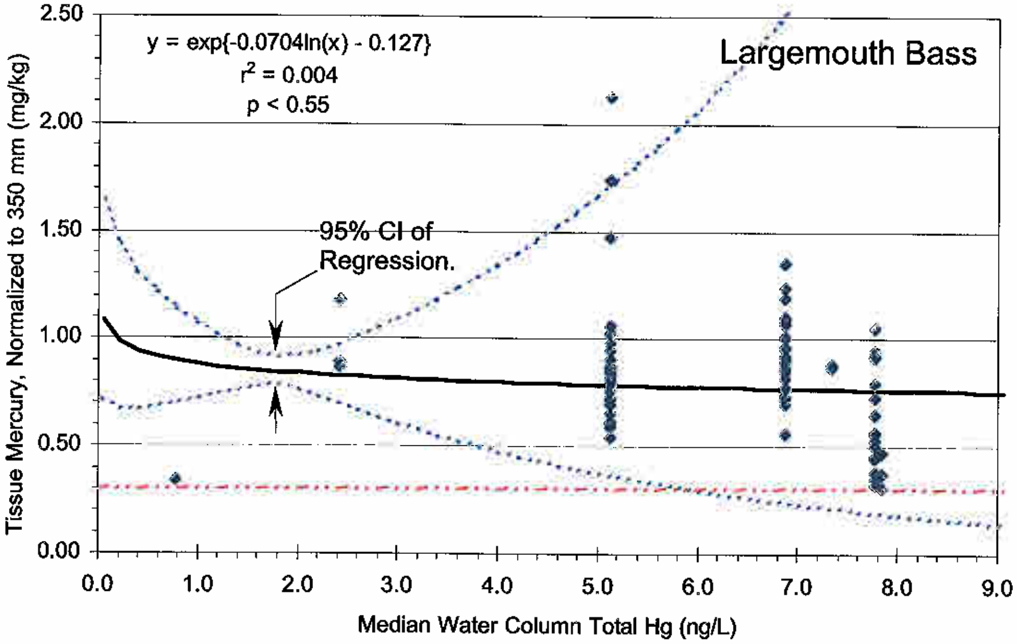

Figure 14: Regression of natural log largemouth bass tissue mercury against natural log of water column total mercury. Regression confidence interval (CI) depicts the expected variability of regression if all data were recollected and reanalyzed.

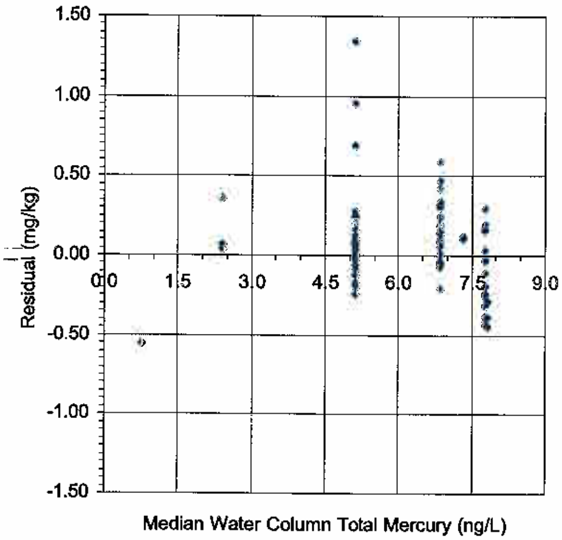

Figure 15: Residual plot of natural log-tissue mercury regression against natural log-water column total mercury.

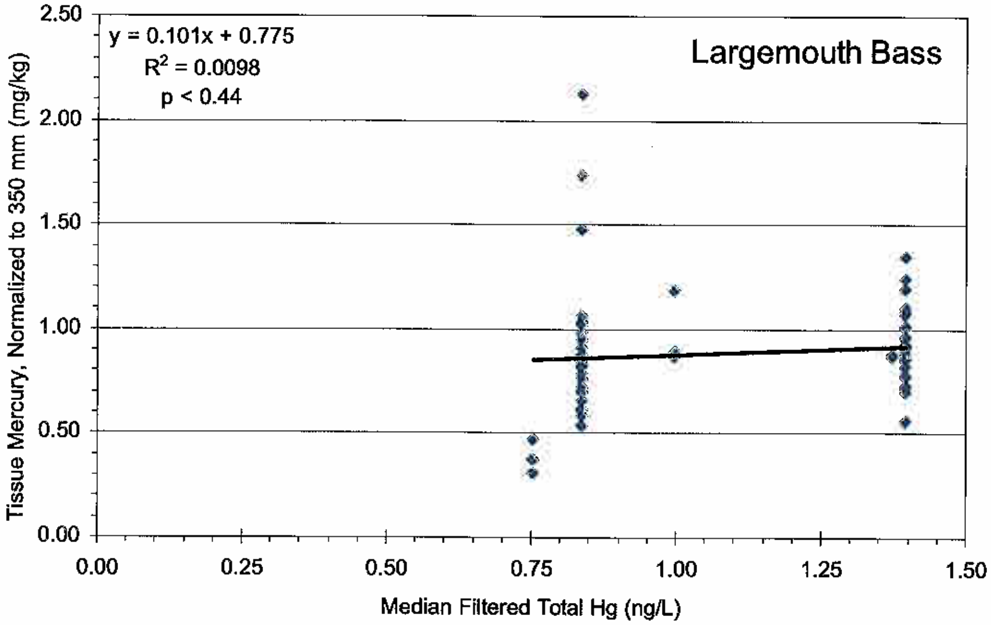

Figure 16: Conditions as in Figure 12, except plotted against filtered total water column mercury (ng/L).

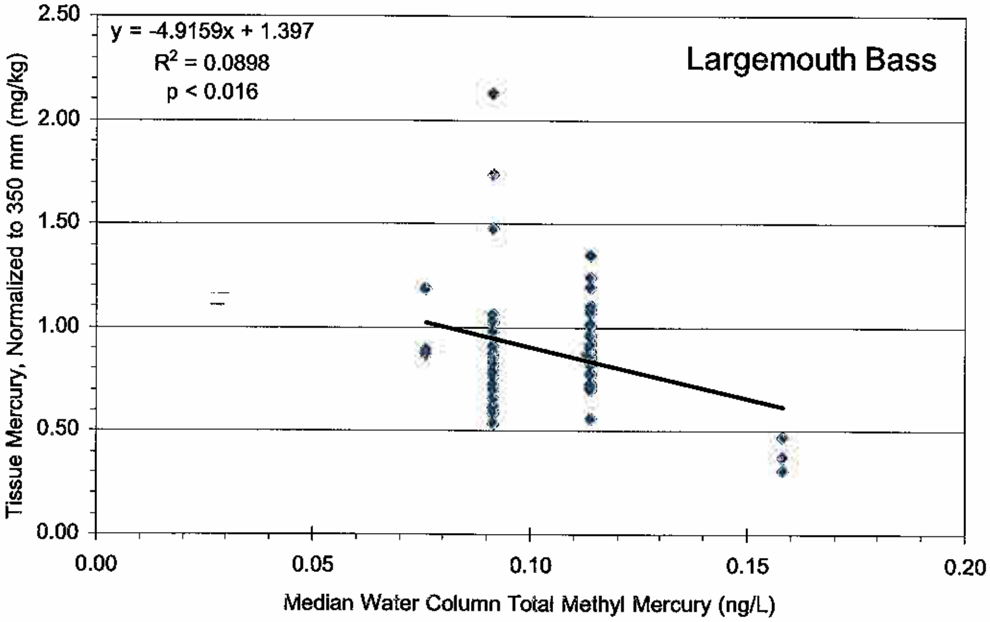

Figure 17: Conditions as in Figure 12, except plotted against total water column methylmercury (ng/L).

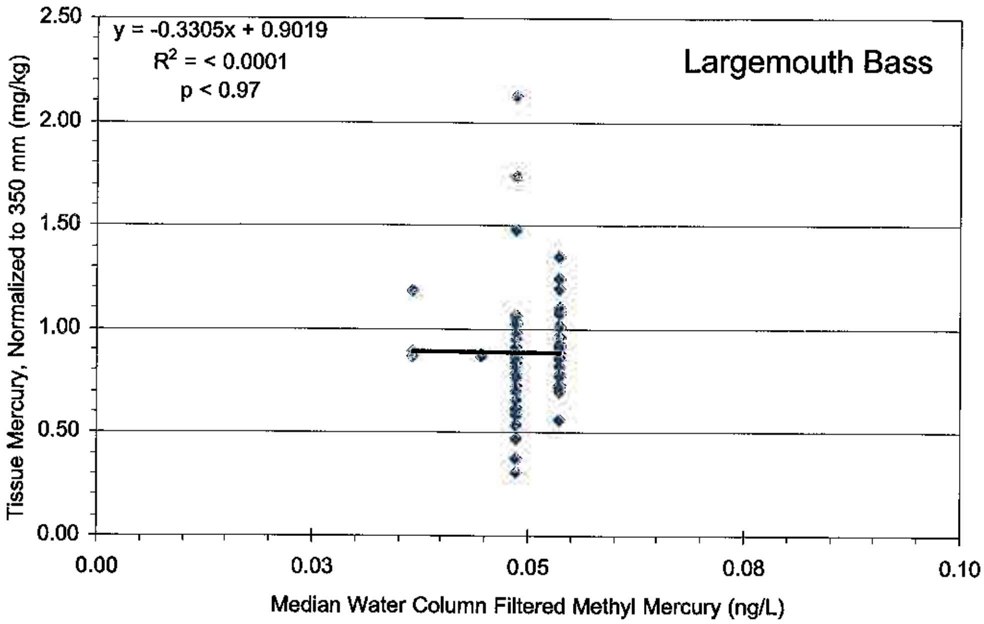

Figure 18: Conditions as in Figure 12, except plotted against filtered total water column methylmercury (ng/L).

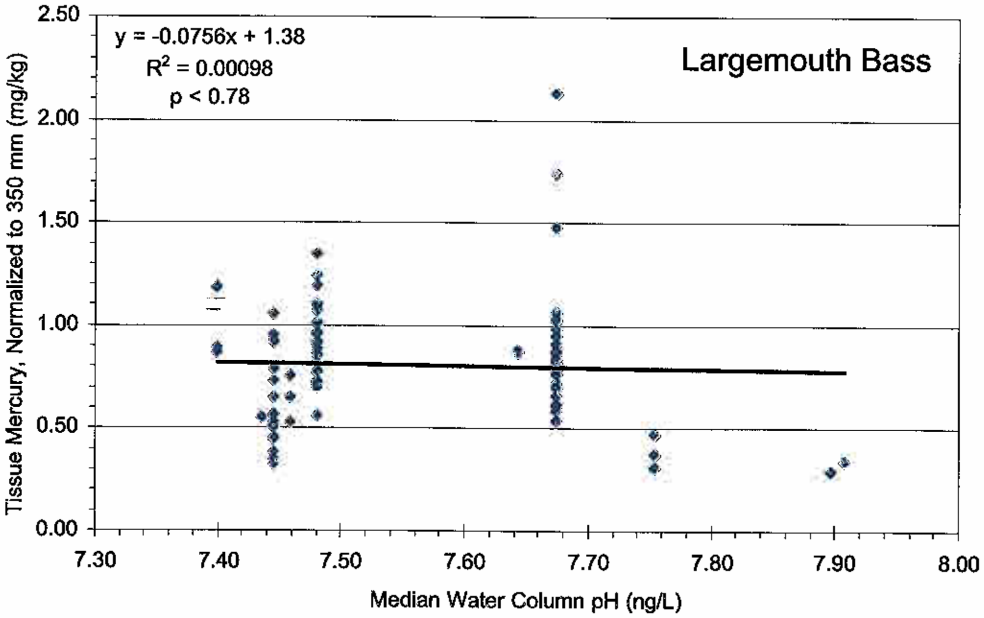

Figure 19: Conditions as in Figure 12, except plotted against water column pH.

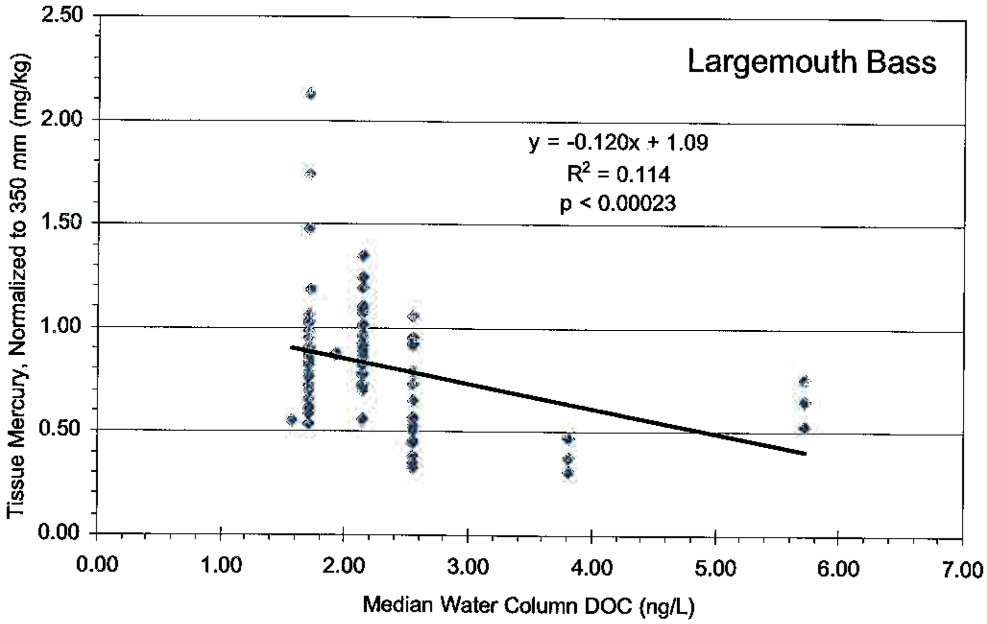

Figure 20: Conditions as in Figure 12, except plotted against filtered water column organic carbon (DOC) (ng/L).

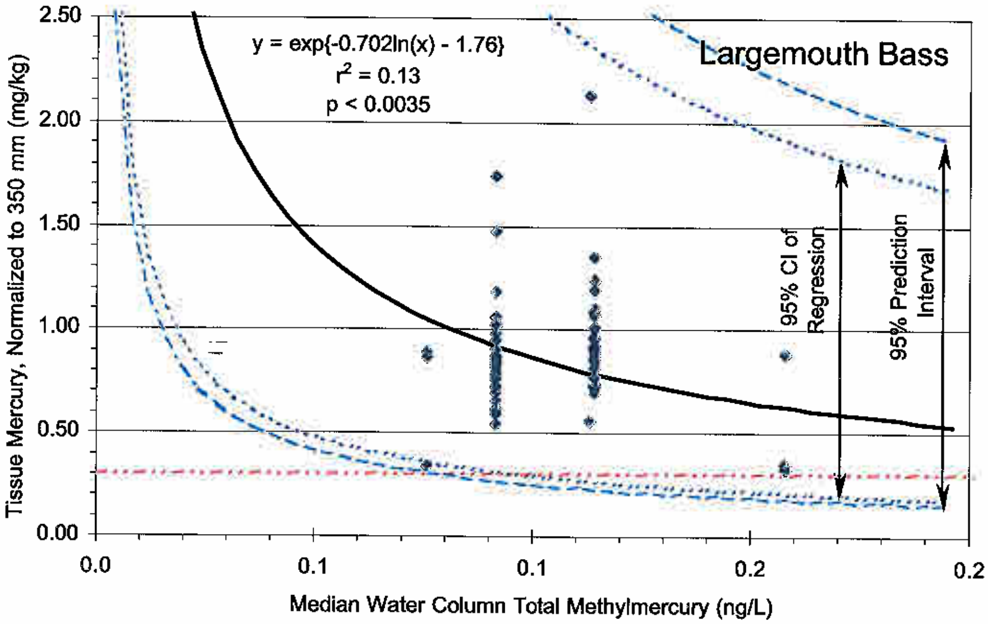

Figure 21: Transform regression model applied to largemouth bass tissue mercury concentrations as a function of water column methylmercury concentrations. Regression confidence interval (CI) depicts the expected variability of regression if all data were recollected and reanalyzed. Prediction Interval is expected range of any one new measurement.

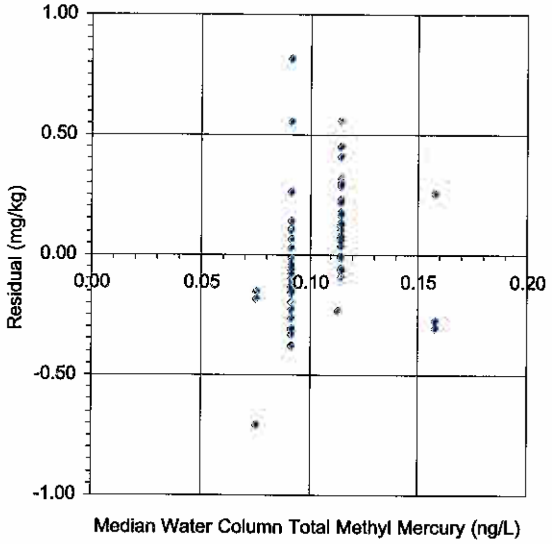

Figure 22: Residual plot for regression of natural log LMB vs. natural log water column methylmercury.

### White Catfish in the Sacramento River Watershed

The general procedure outlined for largemouth bass was followed for white catfish data collected in the SRW. Results from the white catfish data are similar to the largemouth bass. Normalized tissue mercury concentrations for each monitoring location are presented in Figure 23. Regressions of catfish tissue mercury concentrations and various water chemistry constituents are presented in Figure 24. As is evidenced in Figure 24, none of the investigated parameters were strongly correlated with white catfish mercury levels. The largest $r^2$ value was calculated to be less than 0.03, indicating less than 3% of the measured variability is accounted for by any of the water column constituents.

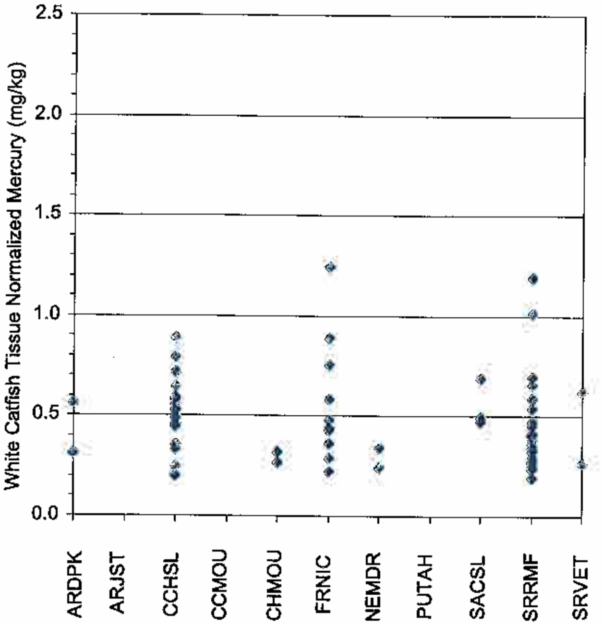

Figure 23: White catfish data available for sites in the Sacramento River Watershed. Sites ARJST, CCMOU, and PUTAH do not have any available white catfish data.

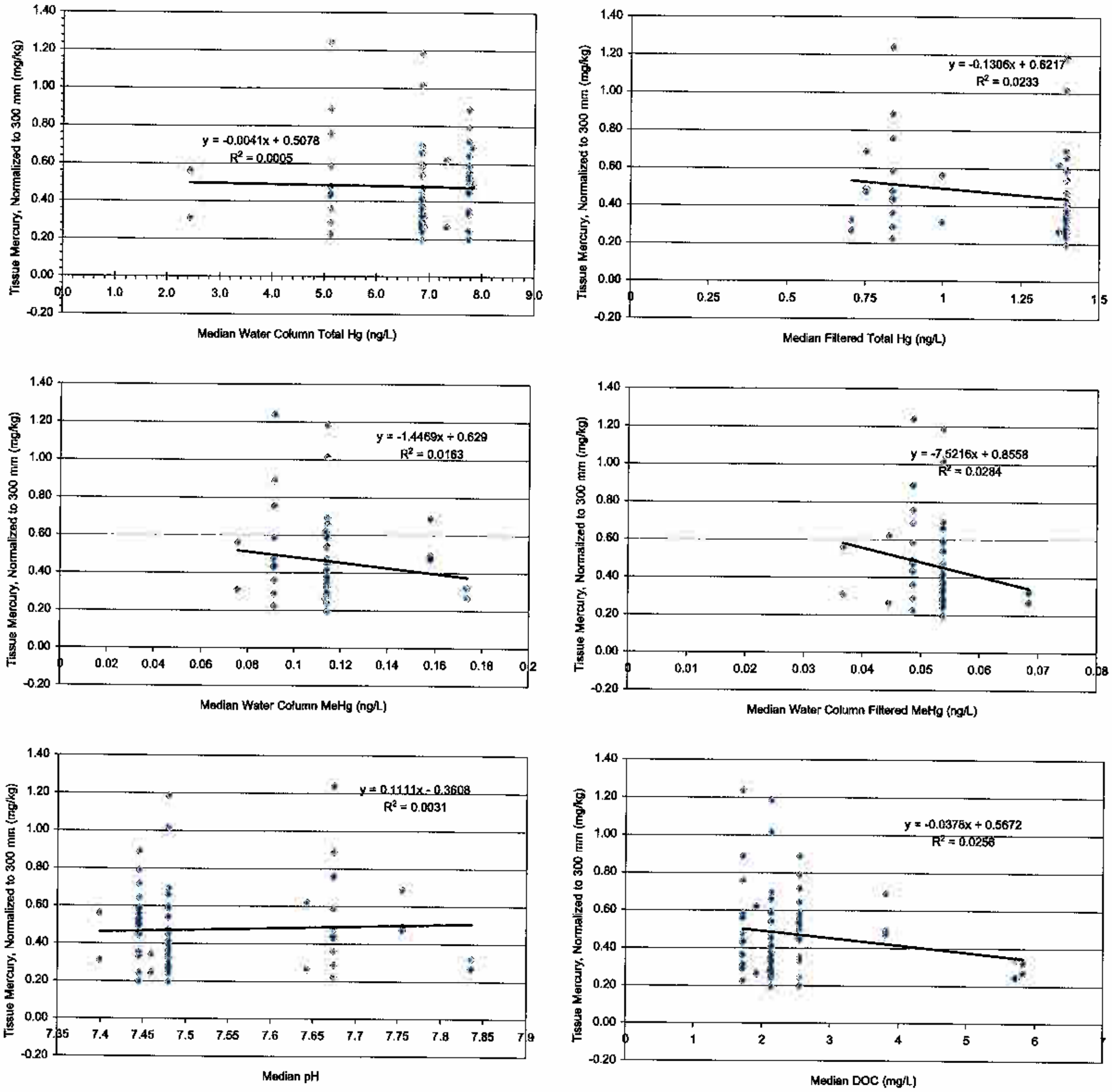

Figure 24: White catfish plotted as a function of various environmental factors in the Sacramento River Watershed.

### Site-Specific Regressions of Water Column Total Mercury to Methylmercury

Measurements of total methylmercury are compared to total mercury for the Mile 44 monitoring location in Figure 25. The regression results are included in Figure 25. Results from filtered methylmercury and filtered mercury measurements are presented in Figure 26. The total measurements appear to be slightly correlated, whereas the filtered measurements are poorly correlated.

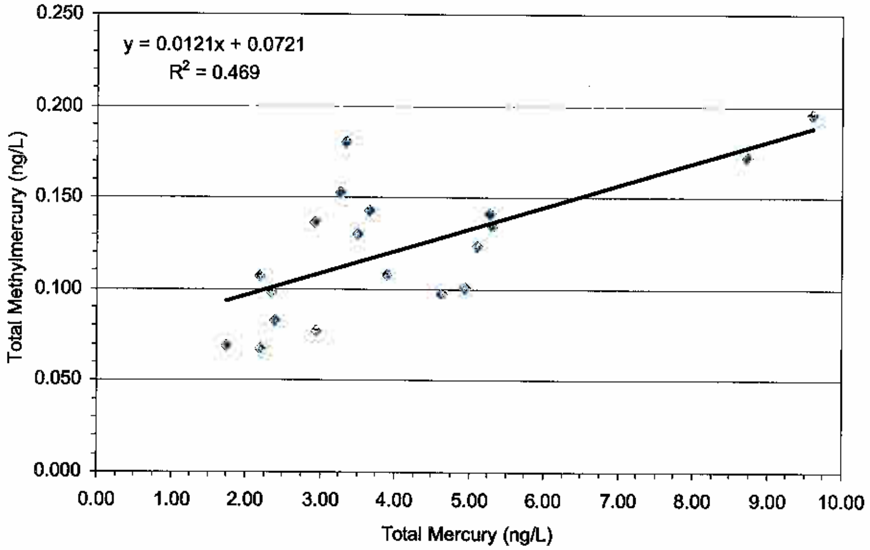

Figure 25: Total methylmercury plotted as a function of total mercury for concurrent samples collected at the Mile 44 monitoring location.

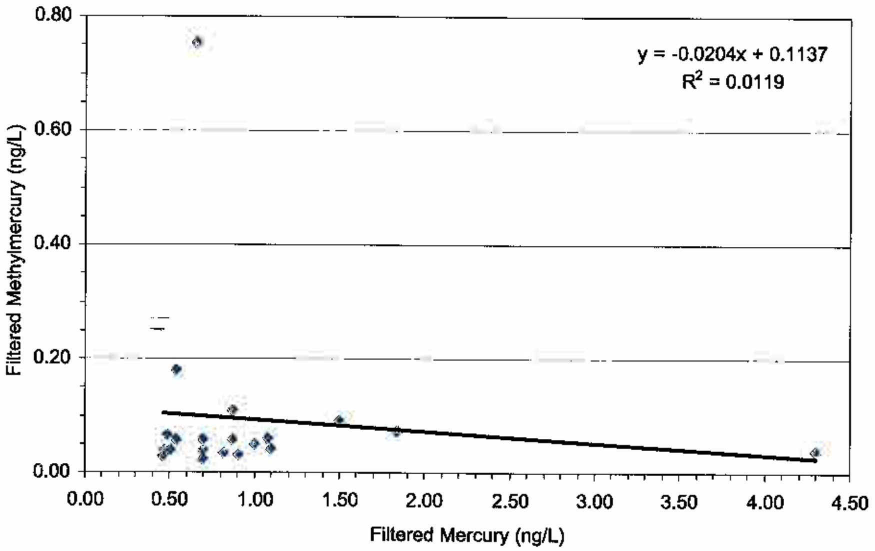

Figure 26: Filtered methylmercury plotted as a function of filtered total mercury for concurrent samples collected at the Mile 44 monitoring location.

## RESULTS

The results from the fish tissue against water chemistry and total mercury against methylmercury regressions are outlined below.

### Single Variate Regressions

Simple linear regression relationships were developed between largemouth bass tissue mercury concentrations (LMB) and the following water column constituents: total mercury (THg), filtered total mercury (THgf), total methylmercury (MeHg), filtered methylmercury (MeHgf), pH, and filtered organic carbon (DOC). The results from performing each regression are listed in Table 5. Each row in Table 5 corresponds to a separate regression model. Terms included in individual models are represented as entries in the columns of the table. With the exception of THgf, MeHgf, and pH, each constituent investigated resulted in a statistically significant regression ($p < 0.05$). However, THg and LMB are poorly correlated ($r^2 < 0.05$) and differences in THg between locations accounts for less than 5% of the variability in LMB. Total methylmercury concentrations correlated better with LMB, but the regressions resulted in inverse relationships predicting the improbable result that fish tissue mercury concentrations decrease with increasing methylmercury water column concentrations. The predicted inverse relationship is opposite of the current understanding of mercury food chain dynamics and is an indicator that other site-specific factors are more important to fish bioaccumulation of mercury than water column methylmercury concentrations. Fish tissue mercury data and the regression functions for each of the models listed in Table 5 are plotted in Figure 12 and in Figure 16 through Figure 20. Model parameters for DOC tended to be statistically significant, and the only practical model in Table 5, is the simple, single regression of LMB and DOC.

Regressions of methylmercury to total mercury (as total recoverable and dissolved fractions) at River Mile 44 indicated some correlative relationship ($r^2$=0.47) for total recoverable concentrations, but no relationship for dissolved fractions ($r^2$=0.01).

### Multivariate Regressions

The multivariate regression models are used to determine the combined influence of multiple constituents on the LMB. The multivariate models investigated for the SRW are listed in Table 5. Each multivariate model except, LMB as a function of MeHg and DOC, is statistically significant and result in higher $r^2$ values than any of the single variate models, but only DOC parameters are statistically significant. Because there are parameters in the model that are not significant, the higher $r^2$ values are simply a result of more parameters within the model, allowing a better fit to the data than the single parameter models. Insignificant parameters within the multivariate models do not imply a relationship between the water column constituents and LMB. Two variables commonly sited as contributing significantly to mercury bioaccumulation are pH and DOC. Under the conditions in the SRW, pH did not increase the utility of the regression model in comparison to the models without pH. None of the multivariate regressions listed in Table 5 are suitable for predicting linkages between water column constituents and LMB, because all include at least one insignificant parameter.

### Transform Regressions

In an effort to produce better regressions, transformations may be applied to the variables. Standard regression methods guide the selection of the appropriate transformations (Netter, *et* *al*., 1990), but artistic intuition plays an important role in performing regression transformations. Both single and multivariate transformed regressions were performed for the SRW data. Results of the regressions are listed in Table 6. Of the investigated models, only two models produced a result that was statistically significant and corresponded to accepted understanding of mercury bioaccumulation. The natural log of the LMB regressed against DOC produces a model with an $r^2$ of 0.15. Regressing the natural log of the LMB against the natural log of MeHg (ln(MeHg)), DOC, and the interaction term (cross term) of ln(MeHg) and DOC, results in the best model, with an $r^2$ of 0.35 indicating that the model accounts for approximately one third of the measured LMB in the SRW. The cross term accounts for possible interactions between ln(MeHg) and DOC affecting observed fish tissue mercury levels, such as an amplification of methylmercury sequestering efficiency of DOC as the concentration of DOC increases. When the interaction term is removed from the regression model, the $r^2$ drops to 0.30, and the ln(MeHg) term becomes statistically insignificant. Because the ln(MeHg) term is not significant, the increase in $r^2$ over the model with only DOC is simply due to including more parameters in the model. Furthermore, if ln(MeHg) is then removed from the model, the $r^2$ drops to 0.15. If only ln(MeHg) is regressed against ln(LMB), the resulting regression has an $r^2$ of less than 0.001.

Table 5: Summary table of single variate and multivariate regression models used to estimate largemouth bass tissue mercury concentration.

| | | | Model Parameters [a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| n | $r^2$ | p [b] | b | THg | THgf | MeHg | MeHgf | pH | DOC | pHxDOC | DOCxMeHg | Comment |
| *Single Variate Regressions* | | | | | | | | | | | | |
| 83 | 0.049 | 0.045 | 1.10 | -0.045 | --- | --- | --- | --- | --- | --- | --- | Improbable |
| 64 | 0.01 | 0.44 | 0.77 | --- | NS [c] | --- | --- | --- | --- | --- | --- | NS |
| 64 | 0.09 | 0.016 | 1.4 | --- | --- | -4.9 | --- | --- | --- | --- | --- | Improbable |
| 64 | 0 | 0.97 | 0.9 | --- | --- | --- | NS | --- | --- | --- | --- | NS |
| 88 | 0.001 | 0.77 | 1.4 | --- | --- | --- | --- | NS | --- | --- | --- | NS |
| 86 | 0.11 | 0.0014 | 1.1 | --- | --- | --- | --- | --- | -0.12 | --- | --- | --- |
| *Multivariate Regressions* | | | | | | | | | | | | |
| 86 | 0.11 | 0.006 | 1.0 | --- | --- | --- | --- | NS | -0.12 | --- | --- | PNS [d] |
| 86 | 0.15 | 0.004 | NS | --- | --- | --- | --- | NS | NS | NS | NS | PNS |
| 82 | 0.21 | 0.0004 | NS | NS | --- | --- | --- | NS | -0.28 | --- | --- | PNS |
| 82 | 0.20 | 0.0001 | 1.4 | NS | --- | --- | --- | --- | -0.29 | --- | --- | PNS |
| 64 | 0.13 | 0.012 | 0.76 | --- | --- | --- | --- | --- | NS | --- | NS | PNS |
| 64 | 0.004 | 0.87 | 0.92 | --- | --- | NS | --- | --- | NS | --- | --- | NS |

Notes:
[a] b = intercept, THg = Water column total mercury concentration, THgf = Filtered THg, MeHg = water column total methylmercury, MeHgf = filtered MgHg, DOC = Dissolved organic carbon, and pH = - log[$H^+$].
[b] Model is statistically significant at a confidence level of 95% if the p value < 0.05.
[c] Regressor is not statistically significant at a confidence level of 95%
[d] Parameter not statistically significant at a confidence level of 95%, meaning at least one parameter of the regression may be positive or negative at a 95% confidence level.

Table 6: Summary table of regression models with transformations used to estimate largemouth bass tissue mercury concentration.

| | | | Model Parameters [a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| n | $r^2$ | p [b] | LMB | b | THg | lnTHg | MeHg | lnMeHg | DOC | DOCxMeHg | DOCxlnMeHg | Comment |
| 64 | 0.35 | 0.0001 | ln() | 4.85 | --- | --- | --- | 2.61 | -3.61 | --- | -1.82 | Best model |
| 64 | 0.30 | 0.0001 | ln() | 2.77 | --- | --- | --- | NS [c] | -0.58 | --- | --- | PNS [d] |
| 64 | 0.27 | 0.0001 | ln() | -0.26 | --- | --- | --- | --- | NS | NS | --- | PNS |
| 86 | 0.15 | 0.0002 | ln() | 0.11 | --- | --- | --- | --- | -0.165 | --- | --- | --- |
| 61 | 0.01 | 0.75 | ln() | -0.33 | NS | --- | --- | --- | NS | --- | --- | NS |
| 64 | 0.13 | 0.003 | ln() | -1.76 | --- | --- | --- | -0.70 | --- | --- | --- | Improbable |
| 83 | 0.05 | 0.04 | ln() | 0.09 | --- | -0.056 | --- | --- | --- | --- | --- | Improbable |
| 83 | 0.004 | 0.55 | ln() | -0.13 | --- | --- | NS | --- | --- | --- | --- | NS |
| 61 | 0.001 | 0.79 | --- | -0.74 | --- | --- | --- | NS | --- | --- | --- | NS |

Notes:

[a] LMB = Largemouth Bass, b = intercept, THg = Water column total mercury concentration, lnTHg = log transformed THg, MeHg = water column total methylmercury, lnMeHg = log transformed MeHg, DOC = Dissolved organic carbon.

[b] Model is statistically significant at a confidence level of 95% if the p value < 0.05.

[c] Regressor is not statistically significant at a confidence level of 95%

[d] Parameter not statistically significant at a confidence level of 95%, meaning at least one parameter of the regression may be positive or negative at a 95% confidence level.

## SUMMARY

The evaluation of fish tissue mercury concentrations being correlated with fish length revealed that in the SRW a statistically significant relationship exists for largemouth bass and white catfish. The relationship was approximately twice as strong for site-specific tissue to length regressions developed for data collected at Sacramento River at Mile 44 and Feather River at Nicolaus, over relationships developed with data collected over the entire watershed. Tissue mercury to length relationships were used to "normalize" tissue mercury concentrations to a 350 mm length for further analyses. Normalizing avoids sample and year-specific biases in fish length.

For the River Mile 44 site, the normalized data were regressed vs. average and median water column total Hg concentrations for varying average periods (1-3 years). Site-specific regressions did not have any significant relationships between water column total mercury and fish tissue mercury, indicating that water column mercury is not a reliable or valid predictor of fish tissue mercury concentrations. It follows that BAFs generated with these data are not scientifically valid. River Mile 44 site was the only location for which this analysis was possible, due to "patchy" fish tissue monitoring data for other locations.

For all locations with fish tissue data, the normalized tissue mercury concentrations were regressed against long-term median water column data for available mercury species (including, filtered and unfiltered, total and methylmercury), and DOC and pH. Regressions were performed in different combinations of multiple regression. There were no cases of a significant positive relationship ($p<0.05$) between any mercury species in the water column, and tissue Hg. There were some "nonsensical" regressions indicating a significant inverse relationship between methylmercury in water and tissue mercury.

The examples for largemouth bass are similar to results obtained for white catfish. A detailed investigation of the white catfish relationships was not pursued.

The example regressions of total mercury against methylmercury indicated some linear relationship between total mercury and methylmercury (unfiltered samples), but no linear relationship between filtered samples.

The overall conclusions from these analyses are that based, on evaluation SRWP data, (1) site-specific factors other than water column mercury concentrations are responsible for observed differences in Hg concentrations in fish tissue, and (2) BAFs are not a valid means of deriving water quality targets from fish tissue Hg concentrations, either on a site-specific or a region-wide basis. The BAF methodology fails on both significance, and correlation levels for the fish tissue collected in the SRW. The BAF fails in most cases on the significance level because the slopes calculated in the analysis cannot be purported to be different from zero indicating no meaningful or predictive relationship, contrary to the USEPA's assertion of a direct 1:1 relationship. Even in cases where the relationship is significant, the correlation of the data and model are poor, indicating the water column mercury concentrations (regardless of speciation or fraction) account for an exceedingly small part of the variability in the measured fish tissue mercury concentrations. Site-specific factors contribute to the measured fish tissue concentrations significantly more than the water column mercury concentrations.

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