Mercury Bioaccumulation and Trophic Transfer in the Cache Creek Watershed of California, in Relation to Diverse Aqueous Mercury Exposure Conditions

By

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Abstract

Water and biota were sampled throughout the Cache Creek watershed during a 20 month period between January 2000 and August 2001. A range of mercury (Hg) exposure conditions were investigated in relation to several mining and natural Hg point sources in the watershed. The study was conducted to provide foundational information and baseline monitoring data for future point source remediation efforts and TMDL regulation. Seasonal aqueous sampling was conducted in conjunction with Hg loading studies. Mercury was characterized in adult game fishes and native fishes throughout the watershed. Bioaccumulation of methylmercury (MeHg) in several taxa of aquatic insect and small fish bioindicators was compared to diverse aqueous Hg exposure conditions and to corresponding fillet muscle Hg in the larger fishes.

The Turkey Run/Abbott complex of Hg mines and the Sulfur Creek complex of Hg mines and geothermal springs were confirmed to be dominant point sources of elevated total Hg (THg), MeHg, and MeHg bioaccumulation in the watershed. In the main stem of Cache Creek, fish Hg increased by over 100% downstream of inflows from the primary remedial targets. Fish Hg reached concentrations to over 6.00 ppm in portions of the watershed. Aqueous Hg parameters varied spatially by over three orders of magnitude between control sites and tributaries near point sources. Seasonal order of magnitude shifts were seen, greater for raw THg. Partly due to the large range of concentrations, general co-correlations were found between the different aqueous Hg parameters. On a same-site basis, strongest correlations were found between raw and filtered fractions of both THg and MeHg and between TSS and THg. While aqueous MeHg was broadly associated with general spatial patterns in aqueous THg (re loading), variable processes of methylation were indicated to play an important role in some MeHg concentrations.

On a whole watershed basis, including all individual paired seasonal samplings, aqueous raw and filtered THg and MeHg all showed substantial apparent correlations with aquatic insect and small fish MeHg bioaccumulation. However, the system-wide apparent correlations were found to be driven largely by clusters of high Hg vs low Hg site data. On an individual site basis, most of the apparent correlations broke down, with recent, seasonally averaged aqueous raw MeHg concentration remaining as by far the best predictor of aquatic insect and small fish MeHg. However, the form of the relationship with raw aqueous MeHg, as well as aqueous:biotic bioaccumulation factors (BAFs), varied between main stem and tributary sites. Study results strongly support the development of site-specific relationships for any predictive applications. Aqueous, invertebrate, and small fish MeHg were found to be seasonally dynamic, with different patterns at different sets of sites. This complicated linkages to large fish MeHg, which required the temporal pooling, by site, of aqueous and lower trophic data. Among similar sites, pooled data provided general linkages directly between unfiltered aqueous MeHg and large fish muscle Hg. Wider-ranging linkages were exhibited between MeHg in bioindicator organisms and large fish muscle. Results of this study indicate that the most useful environmental samples for regulatory and remediation monitoring for Hg include unfiltered aqueous MeHg and short-lived, relatively easily obtainable, low trophic level biota, in addition to larger fish of human health concern.

Introduction

In California, Hg loading from global atmospheric deposition is supplemented by bulk Hg contamination associated with the legacy of the historic California Gold Rush. Mercury was extensively mined and processed in the California Coast Ranges, which contain naturally enriched zones of cinnabar and other Hg minerals. Much of the resulting refined elemental Hg was subsequently utilized on the eastern side of the state in the Sierra Nevada mountains in gold mining, for amalgamation. Following the Gold Rush, the Coast Range Hg mines were generally abandoned, while in the Sierra Nevada much of the tonnage of elemental Hg used in gold mining was lost into local watersheds. Associated bulk Hg contamination and ongoing downstream transport from both sides of the state present California with a unique set of water quality issues.

The Cache Creek watershed has been identified as an important source of ongoing bulk Hg loading to the San Francisco Bay-Delta (Foe and Croyle 1998, Domagalski 2001). This is in spite of the watershed contributing a relatively minor portion of the overall water volume to the system. Loadings from Cache Creek flow into the Yolo Bypass, through the Yolo Bypass Wildlife Area, and into the North Delta Wetlands region of the Bay-Delta. Ongoing research has found elevated levels of aqueous Hg and biotic MeHg accumulation in that portion of the Delta (Slotton et al. 2000). It has also been established that major point sources of Hg are present in the Cache Creek watershed. The upper watershed contains some of California's most extensive historic Hg mining regions, now abandoned, together with natural geothermal springs that have been documented to contain highly elevated Hg (Rytuba 2000). Initial scoping studies indicated dramatic point source signatures for both aqueous loading (Foe and Croyle 1998, Rytuba 2000) and bioaccumulation (Slotton et al. 1997). The watershed has been identified for regulatory and remedial action with regard to Hg.

The U.S. EPA seeks to link aqueous Hg speciation and concentrations to MeHg movement into the aquatic food web, in relation to recent modifications of the water quality criterion for Hg (US EPA 2001). That effort additionally seeks to link bioaccumulation in lower trophic level organisms to ultimate bioaccumulation in higher trophic level organisms consumed by people and at-risk wildlife. A recent nationwide pilot study of fish Hg relative to potential watershed factors (Brumbaugh et al. 2001) found aqueous methylmercury to be the strongest correlate, even with aqueous sampling constrained to single dates. Regionally specific studies which include a temporal (seasonal) aqueous Hg component have been strongly encouraged.

In the study reported here, we characterized aqueous and biotic Hg throughout the Cache Creek watershed, both spatially and temporally, in relation to potential point source remedial targets. Monitoring techniques and baseline information were developed to help direct and assess the effectiveness of future Hg remediation projects and regulatory efforts. Most importantly, the diverse range of aqueous Hg exposure conditions in the various tributaries and main-stem Cache Creek locations, both spatially and temporally, were utilized to investigate the potential regional and localized relationships between aqueous Hg, uptake of MeHg by low trophic level indicator organisms, and bioaccumulation of MeHg in higher trophic level fish.

Specifically, this study had the following primary objectives and hypotheses:

Objectives

(1) Throughout the Cache Creek watershed, at sites spanning the range of existing aqueous Hg exposure conditions, define potential relationships (if present) between aqueous Hg concentrations/speciation and Hg bioaccumulation in lower trophic level biota.

(2) Define relationships (if present) between Hg concentrations/speciation in relatively easily obtainable, site-specific, low trophic level bioindicator organisms (e.g. benthic aquatic invertebrates and small fishes) and corresponding concentrations in large fish.

(3) Characterize aqueous Hg that is representative of predominant (non storm event) Hg exposure levels to aquatic biota, both spatially and seasonally throughout the watershed. Additionally, provide seasonal aqueous THg and MeHg data to USGS from primary tributaries and Hg source regions, across the range of predominant flow conditions, to supplement Hg loading calculations.

(4) Characterize watershed biotic Hg, both spatially and seasonally. Additionally, provide this data to USF&WS to address wildlife concerns (predation on small whole fish) and to OEHHA in relation to human health concerns (large fish muscle).

(5) Establish baseline, seasonal aqueous and biotic Hg data for representative portions of the watershed and downstream from potential remedial sites to (a) contribute to an estimate of the concentration reduction needed to significantly reduce fish Hg bioaccumulation and (b) so that potential future changes in Hg concentrations and bioaccumulation may be readily assessed once remediation is undertaken.

Hypotheses

- Locally, there are predictable relationships between (1) aqueous Hg chemistry and lower trophic level MeHg bioaccumulation and (2) Hg concentrations in lower trophic level bioindicator organisms and large fish.

- Relative biotic Hg accumulation in this region is linked to key natural and, particularly, mining-related point sources.

- Hg bioaccumulation by shorter-lived, lower trophic level organisms tracks short-term seasonal changes in aqueous Hg conditions.

Methods

Study Area and Selection of Sampling Sites

The Cache Creek watershed (Figure 1) is located on the eastern flank of the California Coast Ranges, approximately 125 km (80 mi) north/northeast of San Francisco. Precipitation and significant runoff are confined largely to the winter months of December-March. Controlled irrigation releases from a large lake (Clear Lake) and reservoir (Indian Valley Reservoir) determine downstream flows throughout the rest of the year. During this period, downstream irrigation usage removes most of the water volume, resulting in minimal flows at the outlet. Large-scale movement of Hg to downstream regions occurs primarily during winter high flow events, with Hg mainly associated with particulates at those times (Domagalski 2001).

Significant abandoned Hg mine sites are present at Clear Lake, along upper Davis Creek, and at two sites which are more hydrologically linked to ongoing downstream transport. The primary remedial targets that have been identified in this regard are (1) the Abbott/Turkey Run complex of Hg mines which drain to Harley Gulch and (2) the Sulfur Creek complex of Hg mines and geothermal springs which drain to Bear Creek (Foe and Croyle 1998, Slotton et al. 1997, Rytuba 2000). Flows from these sites are typically highly enriched in sulfate in addition to inorganic Hg, with seasonally elevated temperatures (relative to main stem Cache Creek) and ample sources of organic material just downstream. These conditions have been shown to provide an optimal environment for Hg methylation in this region (Rytuba 2000). Sulfate additions have been shown to generally enhance Hg methylation in fresh water systems (Gilmour et al. 1992). Bioaccumulation of MeHg has been found to vary directly with temperature in several studies (Maury-Brachet et al. 1990, Odin et al. 1994).

Sampling sites for this study were chosen in conjunction with a linked aqueous loading study (Domagalski et al., companion report), necessitating collections from major tributaries and along the main stem of Cache Creek at intervals to the outlet. Additional site criteria included: coverage of the range of aqueous Hg exposure conditions, presence of a majority of the trophic levels of interest, linkage to additional collaborating project components, and logistical considerations. The primary index sites and secondary sampling sites used in this project are displayed in Figure 1 and described briefly in Table 1.

Field and Laboratory Techniques

Water was collected from stream centroid locations directly into trace metal clean glass bottles, utilizing clean sampling technique. Aqueous Hg samples were double bagged at minimum, chilled immediately, and shipped overnight to Battelle Marine Laboratories for 0.45 µm filtration of sub-samples and preservation of both raw and filtered aliquots within 24 hours of collection. This approach to filtration was used partly for logistical considerations and partly due to difficulties encountered at project onset obtaining approval to routinely prepare field filtration equipment. Raw and filtered aqueous fractions were each analyzed for total and methyl Hg with standard protocols as described in the overall Quality Assurance Project Plan (Puckett and van Buuren 2000). Total suspended solids (TSS) samples were taken in parallel with Hg samples and analyzed within 96 hours by UC Davis using standard filter-based technique. Additional samples were collected in parallel for USGS analysis of cations, anions, and a wide variety of additional water quality parameters (see USGS report). Water was collected from the primary 5 index sites on 12-15 sampling dates between January 2000 and August 2001. Additional sites were sampled at a reduced frequency. Sampling was timed to characterize mean seasonal aqueous Hg conditions relative to biotic exposure, avoiding peak storm event flows, which were sampled by USGS. As noted in the companion USGS report, the study period did not include high flow events of magnitudes necessary to move large amounts of bed load to downstream receiving waters in the San Francisco Bay-Delta. However, water collections were able to characterize general seasonal Hg exposure conditions for in-stream biota.

Aquatic insects were collected from riffle zones using kick screens and from other stream habitats with a variety of hand nets. Taxa were separated directly in the field into cleaned glass jars with Teflon-lined lids. Samples were maintained live on ice and were carefully measured, cleaned, and repackaged into clean containers within 24 hours of capture. Aquatic insects were sampled from index sites at least quarterly, with monthly sampling at several sites in the final portion of the project.

Small and juvenile fishes were sampled with backpack electroshock unit and seines, with quarterly collections at most sites and more frequent collections at several index sites in the final portion of the project. Samples were maintained on ice and sorted, measured, and cleaned within 24 hours of collection. For all invertebrate and small fish sampling, efforts were made to obtain consistent samples both seasonally and spatially among the sites. Samples of several different taxa of benthic invertebrates and small fishes were generally taken, from among those types which were most universally prevalent and important components of local food webs.

Small fish and aquatic insect samples were dried (obtaining percentage moisture conversions), powdered, and analyzed consistently on a dry weight basis, with dry weight results subsequently converted to wet/fresh concentrations. Total Hg and N and C stable isotopes were analyzed at UC Davis and methyl Hg at Battelle in split, homogenous, powdered samples, using standard techniques as documented in the QAPP. In this watershed-wide initial characterization of biotic Hg across a 20 month period, multi-individual composite samples were used for the small fish and aquatic insect analyses. While all same-species composite samples were analyzed separately, invertebrate data were later combined for Hydropsychid caddisfly larvae and other predatory taxa for some comparisons. The combined predatory invertebrate measure provided more data points for analysis, exhibited very similar trends to individual taxa, and was more generally representative of the invertebrate portions of the diets of predatory fish in the system. Where invertebrate or small fish species assemblages changed between regions of the watershed, ecologically similar taxa were utilized as bioindicators, based upon literature and advise of regional experts.

Game fish and other large fish were sampled individually for fillet muscle Hg. They were collected during brief periods of minimal flow in the main stem of Cache Creek, which occurred in the late fall of 2000 at the approximate midpoint of the project. Collections were made with gill nets, seines, and backpack and boat-mounted electroshock units. Stomachs were removed and frozen directly in the field for analysis of food items. Lateral anterior scales were taken for aging, which was performed using a modified microfiche reader under the direction of Dr. Peter Moyle (UC Davis Dept of Wildlife, Conservation, and Fisheries Biology). Fresh fillet muscle samples were analyzed directly from individual fish, using dissections from the anterior dorso-lateral region. Adjacent fillet samples were dried and powdered for stable isotope analyses. Fillet muscle MeHg in the large fish samples was characterized with THg analyses, performed by UC Davis.

Quality Assurance / Quality Control

A rigorous program of QA/QC was utilized throughout the project, including oversight by Frontier Geosciences Laboratory and a minimum of 5% of all UC Davis and Battelle samples also analyzed by the third party laboratory (Frontier) for cross comparison. Standard field, preparatory, and analytical QA/QC included the collection of numerous field replicate samples and field blanks, careful preservation and assessment of actual moisture percentages of biotic samples, and extensive analytical split samples, spikes, spike replicates, calibration samples, blanks, laboratory control samples, and a range of standard reference materials with certified mercury contents. Summary and raw data tables of routine QA/QC information, as well as results of the inter-laboratory split exercise, are included at the end of the Data Appendix, as Data Appendix Tables 6-10.

The biotic THg QA/QC (Data Appendix Tables 6a and 8) was consistently well within control levels. The only exception was the relative percentage difference (RPD) for occasional field duplicates of multi-individual biotic composite samples. This was an environmental, rather than sampling, processing, or analytical source of variability. While the mean RPD for biological field duplicate THg was greater than we would prefer at 11.4%, field duplicates with RPD's beyond the 25% control level were generally samples with low Hg concentrations, for which data interpretation was identical using either value. We are, however, currently developing new approaches to reduce the variation in composite biotic field samples, including the analysis of individual organisms in multiple replicate. For this extensive, whole watershed seasonal study, however, it was necessary to utilize composite samples for the routine small organism samples.

The Battelle Laboratories MeHg analytical QA/QC for the biotic samples (Data Appendix Tables 6b and 7) exhibited greater variability than the corresponding THg results, consistent with the overall lower level of precision associated with MeHg analyses. However, laboratory split samples and a range of standard reference materials were all within control levels and, when other QA parameters were out of control, corrective action was taken and samples were re-run. As noted above for THg, field duplicates were more variable than desired, though the greatest variability occurred in low concentration samples for which either result led to the same interpretation.

Battelle analytical QA/QC for aqueous THg and MeHg (Data Appendix Tables 6c, 6d, and 9) was generally well within control limits and, when QA parameters were out of control, corrective action was taken and samples were re-run.

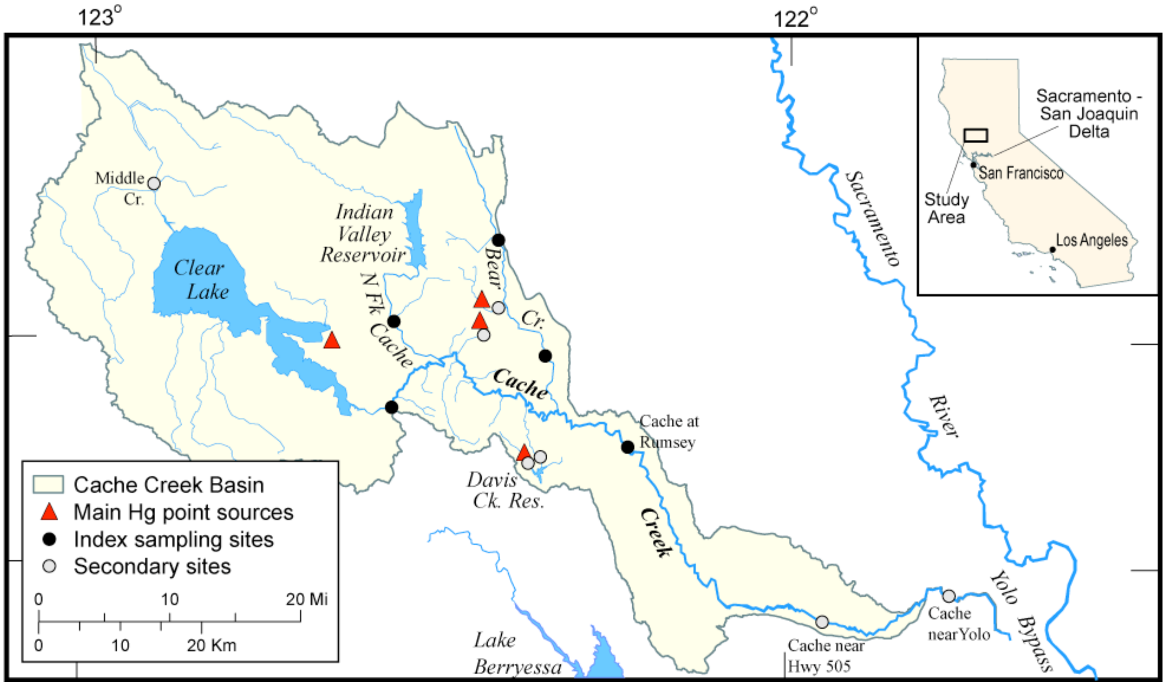
Results of the 5% inter-laboratory comparison splits can be found in Data Appendix Tables 10a-d. Mean relative percentage difference between laboratories was assessed including both positive and negative RPDs (to determine direction of apparent bias, if any) and also on an absolute basis (for the mean absolute relative differences between labs). In the inter-comparison of split, homogenous, powdered biotic samples for THg analysis, the mean of absolute RPDs between UC Davis and Frontier Geosciences was 10%. Similarly, the mean absolute RPD between Battelle Laboratories and Frontier Geosciences was 20% for MeHg in the same samples. We believe that these are fairly typical, and acceptable, levels of difference for inter-laboratory biotic Hg analyses. Three of 37 THg comparisons between UC Davis and the oversight laboratory (8%) were flagged by the oversight laboratory as being out of compliance, i.e. exhibiting relative percentage differences greater than or equal to 25% with respect to corresponding oversight laboratory results. A more concerning 7 of 25 comparisons (28%) were indicated to be out of compliance for the project biotic MeHg laboratory, Battelle. Further examination of the inter-laboratory data (Data Appendix Tables 10b and 10c) indicates that the primary project MeHg laboratory (Battelle) reported a somewhat low biotic MeHg bias (mean RPD = -14%) relative to the oversight laboratory (Frontier). Additionally, the primary project biotic THg laboratory (UC Davis) reported a slightly elevated bias for THg (mean RPD = +7%) relative to Frontier. We note that, in Frontier intra-lab analyses of both MeHg and THg in same, homogenous, powdered samples, MeHg:THg percentages substantially greater than 100% were generated in 11 of 25 biotic inter-comparison samples (120-169%, mean = 147%). MeHg:THg ratios greater than 100% are an impossibility chemically and reflect analytical variability rather than actual ratios. The high incidence of anomalous MeHg:THg ratios in Frontier intra-laboratory analyses indicates that the oversight laboratory experienced under-recoveries of THg, over-recoveries of MeHg, or both, and that this was the primary source of differences reported between project and oversight laboratories for biotic Hg.

Eight aqueous field duplicate samples were tested by both Battelle Laboratories (the primary project aqueous Hg laboratory) and Frontier Geosciences (the oversight laboratory). Results can be found in Data Appendix Table 10d. These aqueous inter-comparison samples were analyzed for raw THg and MeHg, together with 0.45 µm filtered THg and MeHg. Filtration was performed at the respective laboratories, within 24 hours of sample collection. Mean absolute relative percentage difference between the two labs was 29% for both raw and filtered THg. No consistent inter-lab bias was apparent. The corresponding mean RPD for raw MeHg was 38%, with an apparent elevated bias in Battelle samples relative to Frontier. While greater than desirable, we believe that these differences are within the normal range for inter-laboratory or even intra-laboratory analyses of duplicate environmental water samples. The filtered MeHg comparisons were more problematic, averaging 92% RPD between laboratories. While filtered MeHg typically demonstrates the greatest analytical variability of the four water fractions, due to lowest absolute concentrations and potentially variable filtration, the high RPD for filtered MeHg in this inter-comparison derives mainly from three samples taken along a transect that has historically been found to be enriched in MeHg. Very low filtered MeHg in the oversight laboratory analyses of these three samples, including two results of apparently zero filtered MeHg, contrast with moderate to high levels of the same parameter as analyzed by Battelle from this region throughout the project. It is not clear if the inter-comparison results indicate systematic over-recoveries by Battelle or relative under-recoveries by Frontier. However, it is notable that the most problematic samples came from the most problematic sites, analytically, that were utilized throughout the project. The Sulfur Creek and downstream Bear Creek samples were found to require site-specific protocols to avoid unusual analytical interferences. Battelle had more experience dealing with these waters, which may have influenced the outcome of these particular inter-comparisons.

Several qualifications must be made in viewing the data that follow: (1) The aqueous MeHg data contained cases of greater apparent filtered than total fractions, an impossibility similar to MeHg:THg ratios >1.00. These cases were linked to proximity with the level of detection, with the additional possibility of the filtration process lysing cells. Anomalous filtered:raw MeHg ratios substantially greater than 1.00 were mainly seen in very low concentration MeHg samples. In these cases, we used the convention of reducing the reported filtered value to the corresponding raw MeHg concentration, which, if accurate, was the greatest it could actually be. (2) Because it was important to include low-end aqueous Hg data in our relationships with biota, another convention was to utilize reported values even when they were at or slightly below the calculated analytical detection level, rather than omitting them. Both of these conventions effected only samples in the < 0.03 ng/L MeHg range and did not in any way alter interpretations. (3) A degree of added analytical variability was introduced into aqueous Hg data from Sulfur Creek and downstream mid Bear Creek as a result of the necessity for 50-fold and 10-fold dilutions, respectively. This was brought about by additional water quality constituents from Sulfur Creek which, undiluted, destroyed Hg analytical columns and created additional detection interferences. (4) Calculations of aqueous particulate MeHg and THg were complicated by the propagation of errors in samples that were near the levels of detection for TSS and filtered and/or raw Hg, all of which were used in the calculation of aqueous particulate Hg. For comparison with biotic MeHg, particulate Hg data were omitted for aqueous samples with TSS less than 1.00 mg/L and for samples with apparent filtered THg or MeHg greater than or equal to corresponding raw concentrations.

For clarity, many of the data presented in this report have been reduced to various mean values, together with statistical confidence intervals. The reader is also referred to the accompanying Data Appendix, which includes extensive information for all of the individual analytical samples and associated QA/QC.

Figure 1.
Map of Cache Creek watershed, UC Davis study sites, and primary mercury point sources.
(Modified from a USGS base map)



Close-up view of central study region.

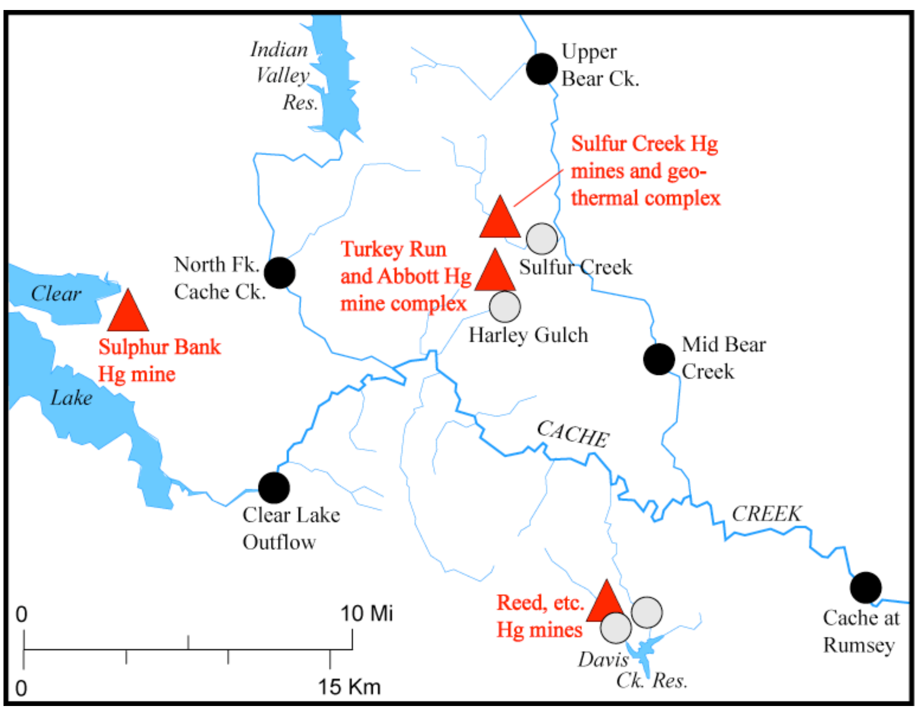


Table 1a. Cache Creek main stem sampling sites

Site Name: **North Fork Cache Creek**
Lat/Long: N: 38° 59.23’ W: 122° 32.17’
General Site Description: North Fork Cache Creek just downstream of Hwy 20 bridge crossing. Cobble to gravel bottom, relatively clear water. Smaller fork of Cache, emanating from Indian Valley Reservoir.
Description of Mine Impact: None known. Relative control site in the watershed.

Site Name: **Cache Creek below Clear Lake**
Lat/Long: N: 38° 55.48’ W: 122° 33.88’
General Site Description: Cache Creek (South Fork) just downstream of the Clear Lake Dam, at top of high gradient canyon portion of Cache. Rocky bottom, mesotrophic to eutrophic lake water. Primary fork of Cache Creek, emanating from Clear Lake.
Description of Mine Impact: Major mercury mine site at Oaks Arm of Clear Lake, but relatively little transport of mercury out of the lake during this study. Relative control site on Cache main stem.

Site Name: **Cache Creek at Rumsey**
Lat/Long: N: 38° 53.41’ W: 122° 14.33’
General Site Description: Cache Creek just upstream of Rumsey Bridge. Near outlet of canyon, higher gradient stretch and near top part of Capay Valley and low gradient stretch. Rocky bottom, transitioning to lower grain sizes downstream.
Description of Mine Impact: Below all major mercury mine inputs, though with much dilution.

Site Name: **Cache Creek below Highway 505**
Lat/Long: N: 38° 41.47’ W: 121° 55.40’
General Site Description: Cache Creek app. 1 km downstream of Hwy 505, well downstream of Capay Valley, in Sacramento Valley. Bottom primarily gravel to small cobble with some rocky riffles.
Description of Mine Impact: Same as Rumsey, with additional distance and potential dilution.

Site Name: **Cache Creek below Yolo**
Lat/Long: N: 38° 43.66’ W: 121° 46.78’
General Site Description: Cache Creek just upstream of Hwy 113. Near Settling Basin and outlet to Yolo Bypass. Bottom almost entirely sand to gravel, few riffles.
Description of Mine Impact: Same as above two sites, with additional distance and potential dilution.

Table 1b. Cache Creek tributary sampling sites

Site Name: **Middle Creek**
Lat/Long: N: 39° 10.96 W: 122° 54.64'
General Site Description: Middle Creek at gaging station. Tributary to Clear Lake. Clear water, bottom cobble to gravel.
Description of Mine Impact: None known. Chosen as a control site.

Site Name: **Davis Creek above Davis Creek Reservoir**
Lat/Long: N: 38° 51.82' W: 122° 22.14'
General Site Description: Davis Creek at upstream crossing of County Road 40, above Davis Creek Reservoir. Small tributary creek with primarily rocky bottom.
Description of Mine Impact: Major point source direct impact: historic Reed mercury mine just upstream, including calcine piles into creek.

Site Name: **Davis Creek below Davis Creek Reservoir**
Lat/Long: N: 38° 51.10' W: 122° 21.26'
General Site Description: Davis Creek just downstream of spillway from Davis Creek Reservoir. Small tributary creek with rocky bottom mixed with pools with fine sediments.
Description of Mine Impact: Region long studied by UC Davis. Water spilling to downstream Davis Creek may seasonally contain relatively high MeHg.

Site Name: **Harley Gulch**
Lat/Long: N: 39° 0.58' W: 122° 25.99'
General Site Description: app. 500 m downstream of Turkey Run and Abbott mercury mine complex. Small seasonally dry creek, bottom mostly gravels to rocky.
Description of Mine Impact: Major point source mine impact: Flows typically diluted app. 2:1 from relatively clean small creek from east.

Site Name: **Upper Bear Creek**
Lat/Long: N: 39° 5.83' W: 122° 24.71'
General Site Description: Bear Creek at Bear Valley Road bridge crossing. Small, valley creek.
Description of Mine Impact: Site chosen as upstream control for Bear Creek watershed mine-impacted sites. Believed to be upstream of all known mine loading.

Site Name: **Sulfur Creek**
Lat/Long: N: 39° 2.21' W: 122° 24.56'
General Site Description: Sulfur Creek near confluence with Bear Creek. Small, canyon creek with extremely poor water quality even disregarding mercury. Geothermal sulfur etc.
Description of Mine Impact: Major point source direct impact: directly downstream of all Sulfur Creek watershed historic mercury mines and geothermal springs.

Site Name: **Middle Bear Creek below Sulfur Creek**
Lat/Long: N: 38° 58.88' W: 122° 20.94'
General Site Description: Bear Creek approximately 10 km downstream of Sulfur Creek.
Description of Mine Impact: Downstream of Sulfur Creek loading, diluted app. 10-fold.

Results and Discussion

Large Fish

Approximately 200 large game fish and native fish were individually sampled throughout the watershed for fillet muscle Hg during a period of reduced water releases from Clear Lake and Indian Valley Reservoir and between storm events. This window of sampling opportunity occurred approximately mid-project in late November through mid December 2000. The large fish collections focused on top predator (piscivorous) species likely to accumulate greatest concentrations of MeHg. In the tributary streams, fish communities consisted almost exclusively of the native assemblage of California roach (*Hesperoleucas symmetricus*), Sacramento sucker (*Catastomus occidentalis*), and predatory Sacramento pikeminnow (*Ptychocheilus grandis*). Suckers (lower trophic level bottom browsers) were present throughout the watershed and were sampled as a consistent large fish index species. Pikeminnows were sampled from the tributary sites as the only available piscivorous species. Within the main stem of Cache Creek, largemouth bass (*Micropterus salmoides*) constituted the dominant top predatory fish species in the upper portion of the creek near the Clear Lake outflow. Smallmouth bass (*Micropterus dolomieui*) were dominant downstream of the North Fork of Cache Creek. Smallmouth bass and pikeminnows coexisted at two of the sampling sites; very similar length:Hg relationships were found between the two species across a useful normalization size window, indicating that the native pikeminnows of the tributary reaches could provide measures relatively analogous to the bass data.

Stomach content analyses found aquatic macro-invertebrates and small fish to be the dominant food items of the pikeminnows and both bass species, though many stomachs of the predatory fish samples were empty in these late-season collections (Table 2). The literature indicates that macro-invertebrates may be important components of the diets of these species, in addition to fish (Moyle 1976, 2002). Thus, the invertebrate and small fish data collected in the project, in addition to providing bioindicator measures of localized MeHg exposure, may also be linked by diet to Hg accumulations in the predatory fishes. The three predatory fish species contained similar food items. No significant inter-site differences in diet were seen among the predatory fish or the detritivorous Sacramento suckers, indicating that dietary differences could not explain observed spatial differences in fish Hg bioaccumulation. Interpretation of stable isotope analyses is still underway to investigate the possibility that localized food webs of varying complexity may have played a role. However, interpretation of the stable isotope data has been confounded by the presence of a very large, chemical-based ^{15}N signature emanating from Sulfur Creek. Additionally, interpretation has been hampered by the lack of consistent herbivorous biota samples for use as relative trophic baselines. Stable isotope data are compiled in the Data Appendix, in Appendix Table 4. Determination of fish ages (Figure 2) indicated some differences in apparent growth rates from similar fish of different sites. However, the primary observed spatial differences in large fish Hg (between main stem Cache Creek reaches located upstream and downstream of the identified Hg point sources, and between Bear Creek and Cache Creek), cannot be explained by differences in growth rate. Apparent differences in growth rate *were* found to possibly play a role in anomalously elevated fish Hg at the upper Bear Creek site, discussed later in this section.

Size vs fillet muscle Hg is plotted by site for bass, pikeminnows, suckers, and additional large fish in Figures 3(a)-3(d). The majority of the data sets were best fit by exponential curves. Relatively strong curve fits were seen for most same-site/same-species data sets, indicating relatively consistent, localized populations. However, between sites, some divergent size:Hg trends were apparent. Mid Bear Creek (below the Sulfur Creek Hg mines and geothermal complex) was dramatically higher in large fish Hg than all other sampling locations containing large fish. Fish were not present directly in Sulfur Creek, apparently due to impaired water quality, or in Harley Gulch beneath the Abbott-Turkey Run mine complex due to physical barriers to upstream migration. Fish Hg concentrations of concern were clearly present in the Cache Creek watershed. At mid Bear Creek, fillet muscle Hg concentrations in predaceous pikeminnows greatly surpassed all standard consumption guidelines, with fresh/wet weight levels to over 6000 ng/g (6.00 ppm) and most individuals in the 2000-4000 ng g^{-1} (2.00-4.00 ppm) range. Even lower trophic level Sacramento suckers from this site exhibited fillet muscle Hg well above the US FDA 1000 ng/g (1.00 ppm) action guideline in 83% of samples. It is notable that this species is a frequent target of bald eagles which winter in the region (D.G. Slotton personal observation). One of the most important findings of the study was that, along the main stem of Cache Creek, piscivorous fish fillet muscle Hg increased by 2-3 fold between the Clear Lake outflow and sites at and downstream of Rumsey, indicating a substantial increase in MeHg bioaccumulation and presumed exposure between those points. The identified remedial targets discharge into Cache Creek within this transition zone. Lower trophic level Sacramento suckers exhibited a similar increase across this stretch. At the Cache Creek sites downstream of the primary remedial targets, concentrations above the EPA 300 ng/g (0.30 ppm) guideline were seen in all but young-of-year bass, with concentrations measured to over 1500 ng/g (1.50 ppm). In these portions of the main stem creek, fillet muscle concentrations over 300 ng/g were additionally seen in Sacramento suckers over approximately 400 mm in length.

Among the sampling sites containing large fish, Cache Creek at the Clear Lake outflow and the North Fork Cache Creek index station (representing the primary upstream source flows) demonstrated consistently lowest fish Hg levels, except for the largest suckers taken from the North Fork of Cache Creek. While the smaller suckers demonstrated low Hg consistent with the overall lower Hg environment of this control site, the larger individuals captured here were very similar in Hg to main stem Cache Creek fish. This suggests that the larger/older individuals at this site may have been transitory migrants for spawning, in contrast to the younger individuals which typically remain within their birth tributary prior to attaining sexual maturity (Moyle 1976, 2002). Spatial comparisons thus focused on suckers in pre-spawning size classes.

Both piscivorous pikeminnows and bottom browsing suckers exhibited unexpectedly elevated Hg at the upper Bear Creek site, which was presumed to be an unimpacted, clean upstream control relative to mid Bear Creek. Fish Hg at this site was greater than at all other sites in the watershed that contained large fish, other than mid Bear Creek which was an order of magnitude greater.

For direct inter-site comparison, site data for each species were normalized to the concentration corresponding to a single inter-comparable size. The sizes used for inter-site normalization were 270 mm (total length) for bass and pikeminnows, and 290 mm for Sacramento suckers. Size-normalized mercury data are presented in Figure 4. The relatively small normalization sizes (corresponding to 10.6 and 11.4 inches respectively) were dictated by size structures of the various sampled fish populations and inter-species relationships; they do not represent either mean or maximum concentrations. Size normalization simply provides relatively consistent measures with which to compare spatial variation in Hg content. The normalization size chosen for the piscivorous fish additionally falls within the range of age 3 bass and pikeminnows in the watershed (Figure 2), the age group utilized by the USGS in a recent nationwide pilot study of fish Hg vs watershed factors (Brumbaugh et al. 2001). The normalized data demonstrate the same trends previously noted. The Clear Lake outflow and North Fork Cache Creek index sites showed lowest fish Hg. Main stem Cache Creek sites downstream of the Harley Gulch and Bear Creek drainage remedial targets were elevated above these control levels by 136% in the piscivorous fish and by 95% in Sacramento suckers. Interestingly, normalized fish Hg from the main stem of Cache Creek below all of the major point source inputs showed moderate but consistent incremental rises of 9-20%, moving downstream from Rumsey to Highway 505 to the site near the Settling Basin. Normalized data from mid Bear Creek were an order of magnitude greater than the main stem Cache Creek values below Bear Creek: approximately 7-fold greater in the piscivorous fish and approximately 9-fold greater in the suckers. Relative to the Clear Lake outflow and North Fork Cache Creek control sites, fillet muscle mercury in mid Bear Creek piscivores and suckers was elevated 16 and 18 times respectively.

The normalized fish data also indicated upper Bear Creek to be anomalously elevated, relative to corresponding aqueous Hg samples which were extremely low for THg and moderate for MeHg. Piscivorous fish exhibited 56% greater Hg than comparable main stem Cache Creek fish from Rumsey and downstream and 270% more than those from the other "control" sites. Sacramento suckers were similarly elevated by 64% over the same main stem Cache Creek sites and by 220% over the Clear Lake outflow and North Fork Cache Creek controls. The most obvious explanation for the relatively elevated fish Hg at this site is that they simply swam upstream from the highly Hg-elevated reach of Bear Creek located below Sulfur Creek. However, we believe that the pikeminnows and suckers collected at upper Bear Creek were resident fish, as they exhibited internally consistent size:Hg trends that differed from those seen at mid Bear Creek. Localized aquatic insects and small fish were also relatively elevated in MeHg at this site. Stable isotope results (Data Appendix Table 4) suggest that the anomalously elevated fish Hg at Upper Bear Creek may be explained in part by the apparent presence of a longer net food web length at the site, relative to other watershed sites. The difference in the $^{15}N/^{14}N$ ratio between predatory pikeminnows (at the top of the aquatic foodweb) and *Hydropsyche* (near the bottom) was notably larger here. Slightly lower growth rates for pikeminnows and, to a greater extent, suckers from this site (Figure 2) may have influenced net fish Hg accumulation, resulting in somewhat higher concentrations in same-sized fish relative to some other sites. Additionally, we may have simply missed sampling important pulses of aqueous MeHg, either seasonally or diurnally (this site was sampled less frequently than most of the primary index sites). Photodegradation of MeHg has been shown to strongly effect Hg cycling in lakes (Sellers et al. 1996), and may also be important at this clear, unshaded section of Bear Ck, potentially obscuring more elevated aqueous MeHg conditions at periods other than those sampled (generally mid-day). Finally, this site was unique in containing a dense mat of benthic algae in extended pooling sections. It may be that Hg is methylated near anoxic zones located beneath these mats and that MeHg then enters the foodweb through a benthic rather than water column pathway. Cleckner et al. (1999) found periphyton communities to be active and important Hg methylation sites in the Florida Everglades. A similar phenomenon may be taking place in the upper Bear Creek environment, in which case, benthic-oriented lower trophic level species from this site would be expected to demonstrate elevated Hg also. Indeed, the upper Bear Creek benthic invertebrate (Figure 10) and benthic-feeding California roach small fish data (Figure 17) show a corresponding elevation in Hg bioaccumulation relative to the suite of sites not directly impacted by known mercury point sources. In Figures 23 and 24 in a later section, large fish mercury from this site can be seen to correspond to both invertebrate and roach Hg, but not to mean aqueous concentrations, relative to the majority of other sites that contained large fish. However, as discussed later in the report in relation to bioaccumulation factors (BAFs), this may be partially or largely a function of differential efficiency of bioaccumulation into the base of the foodweb at this and certain other sites. In any case, the presence of substantially elevated fish Hg at an apparent control site with no known Hg point source highlights the potential complexity of the relationships between Hg loading and corresponding MeHg bioaccumulation. The upper Bear Creek site may represent a regional case of general natural erosion and atmospheric trace Hg loading combining with an ideal environment for methylation and bioaccumulation to result in elevated fish Hg.

The large fish fillet muscle Hg data provide a general backdrop of existing Hg bioaccumulation patterns within the watershed. In the following sections, we discuss the potential within-watershed relationships between the fish Hg trends, aqueous Hg concentrations and speciation, and MeHg bioaccumulation in a variety of lower trophic level indicator organisms.

Table 2.
Summary stomach content data for project top predator fish samples.

"Number of Fish" = individuals containing the given food item, of total from a site-sampling.
"Avg # Ingested" = average number of the given item per fish containing the item.
"Avg % Tot. Vol." = average volume percentage of the given item (re all contents) per individual containing it.

Sampling conducted in late season low-feeding period due to flow constraints on sampling

			Aquatic Insects			**Fish**		
Sample Date	Location	Type	Number of Fish	Avg # Injested	Avg % Tot. Vol.	Number of Fish	Avg # Injested	Avg % Tot. Vol.
Nov-00	Cache Ck. bel. Clear Lake	Largemouth Bass	4 of 14	3	60%			
Dec-00	Cache Creek at Rumsey	Smallmouth Bass	5 of 20	1	70%			
Nov-00	Cache Ck. bel. Hwy 505	Smallmouth Bass	2 of 10	2	80%			
Dec-00	Cache Ck. below Yolo	Smallmouth Bass				1 of 2	1	90%
Nov-00	North Fork Cache Creek	Pike Minnow				7 of 10	1	71%
Nov-00	Upper Bear Creek	Pike Minnow				2 of 12	1	60%
Nov-00	Mid Bear Creek	Pike Minnow	2 of 15	1	40%			
Nov-00	Cache Creek at Rumsey	Pike Minnow	1 of 11	1	90%	2 of 11	1	70%
Nov-00	Cache Ck. bel. Hwy 505	Pike Minnow	2 of 10		45%	1 of 10	1	90%

			Crayfish			**Amphibians**		
Sample Date	Location	Type	Number of Fish	Avg # Injested	Avg % Tot. Vol.	Number of Fish	Avg # Injested	Avg % Tot. Vol.
Nov-00	Cache Ck. bel. Clear Lake	Largemouth Bass	2 of 14	1	83%			
Nov-00	Cache Creek at Rumsey	Pike Minnow				1 of 11	1	90%

Figure 2(a-c).
Age vs. length relationships for large fish from main-stem and tributary stream locations in the Cache Creek watershed. *(fish age in years determined by scale analysis)*

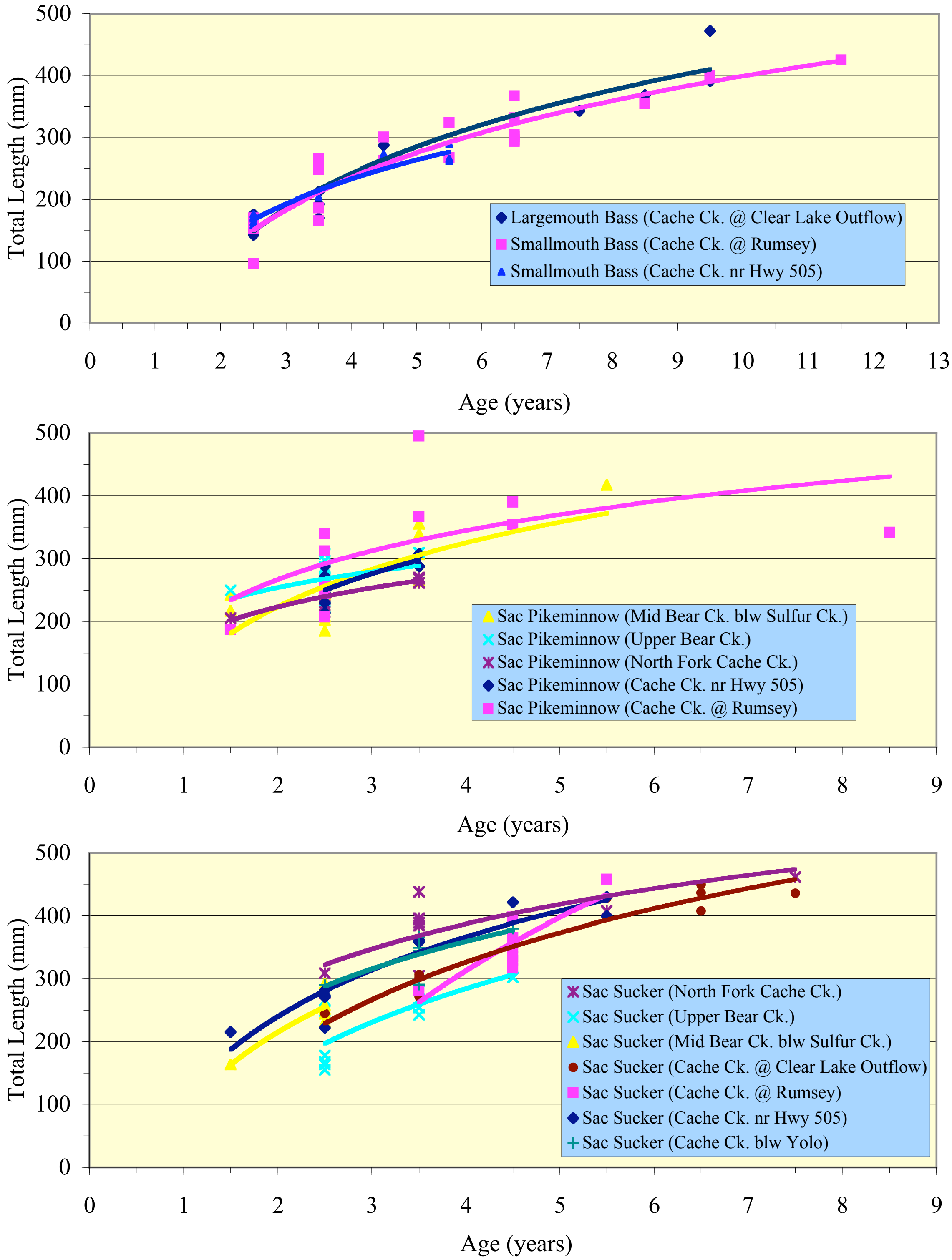
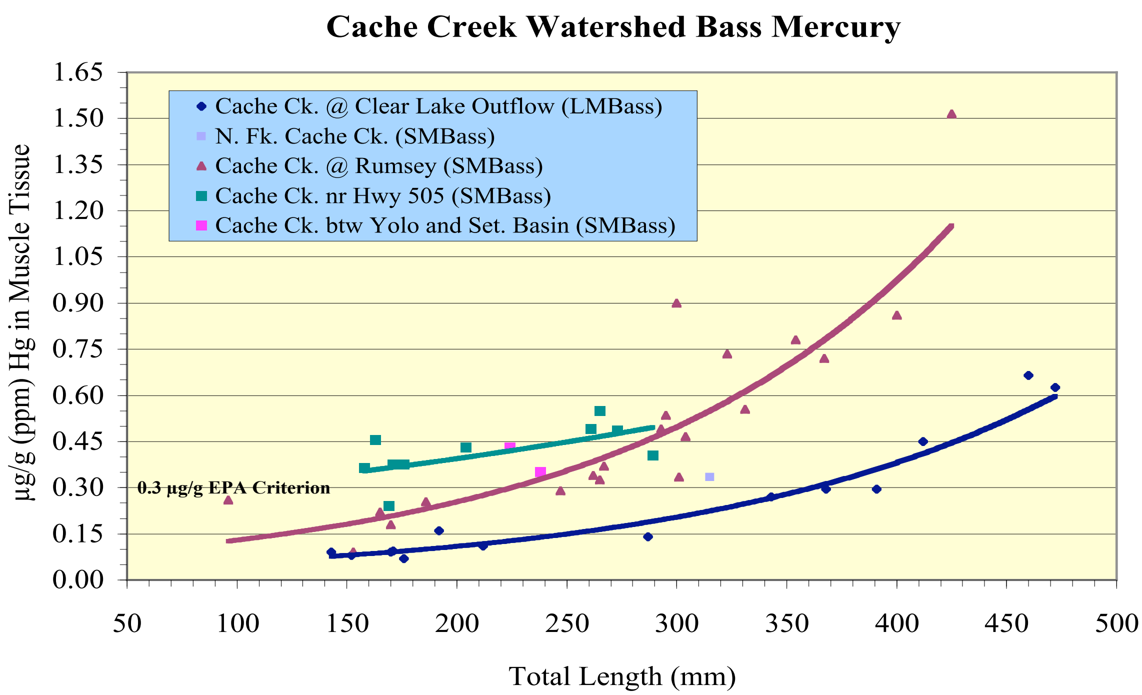


Figure 3 (a-d).
Muscle mercury in large fish from locations throughout the Cache Creek watershed.
(fish total length vs. muscle Hg, with exponential curve fits)



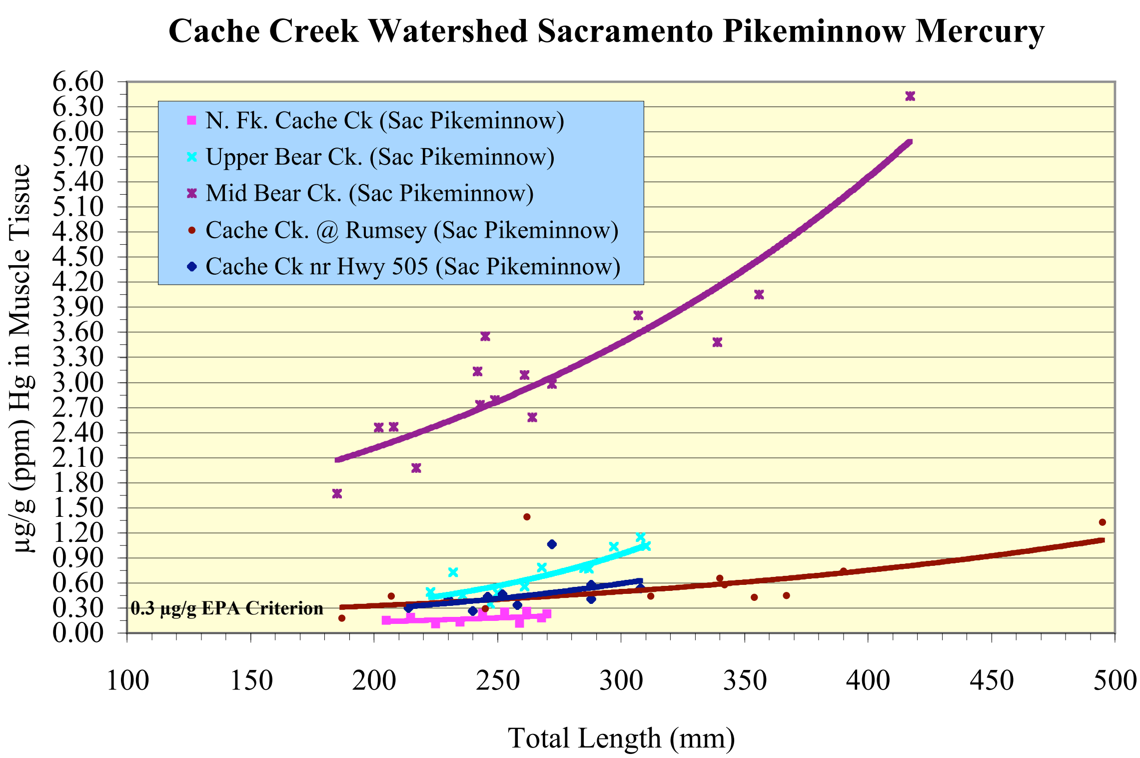
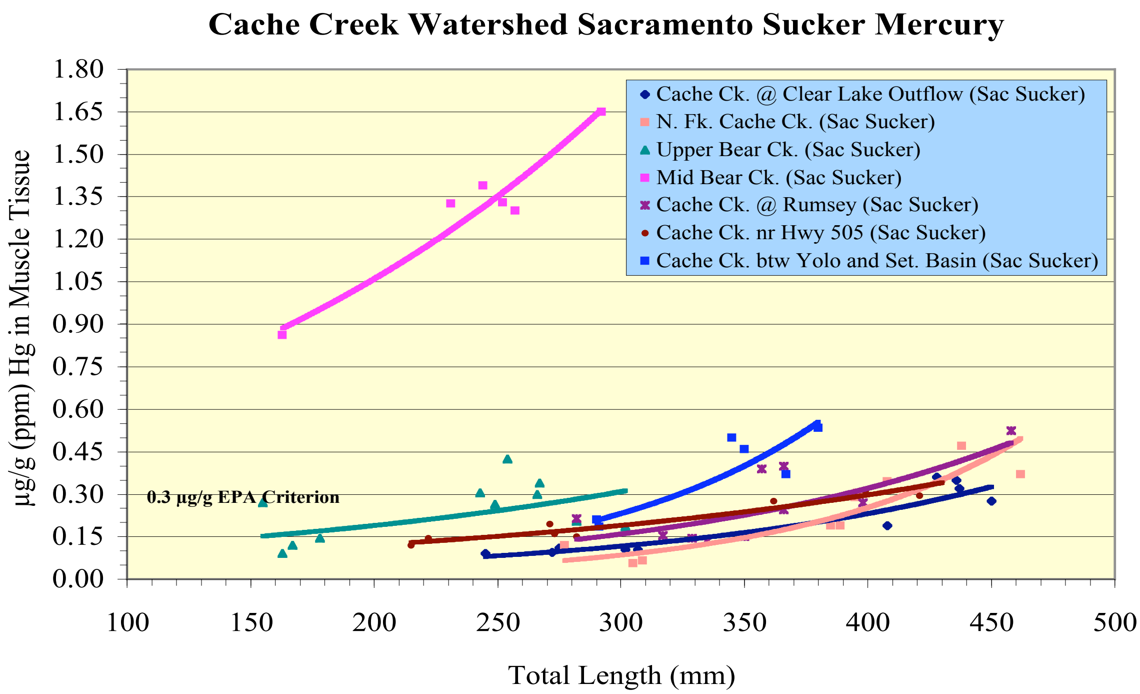


Figure 3 (a-d) continued.



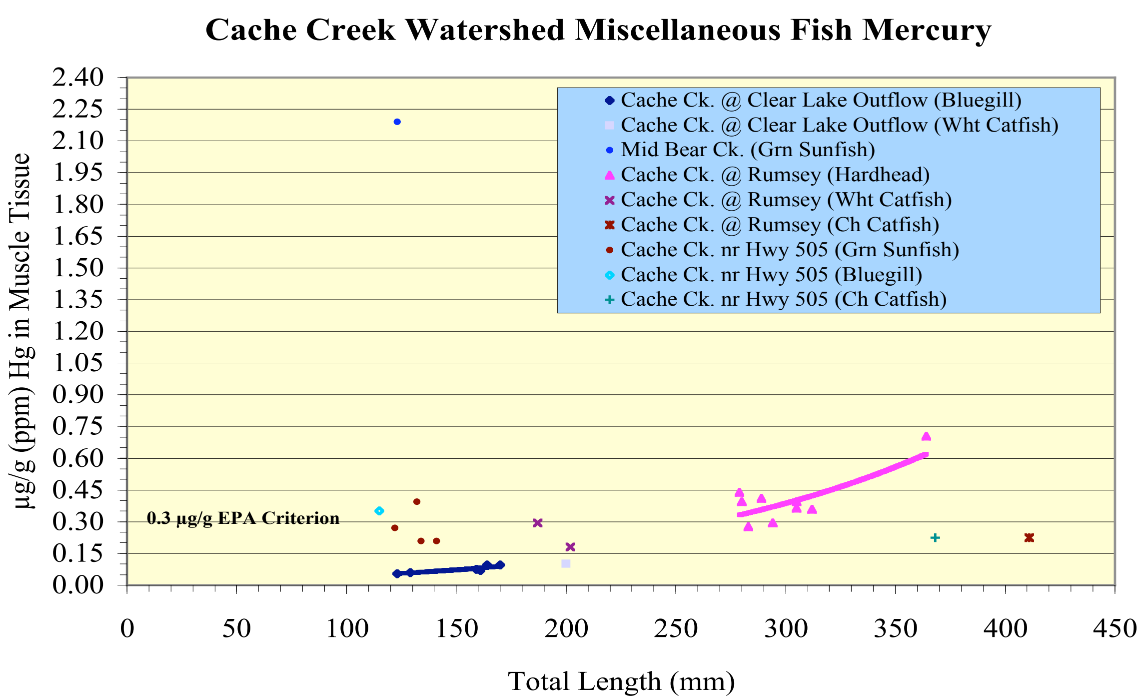
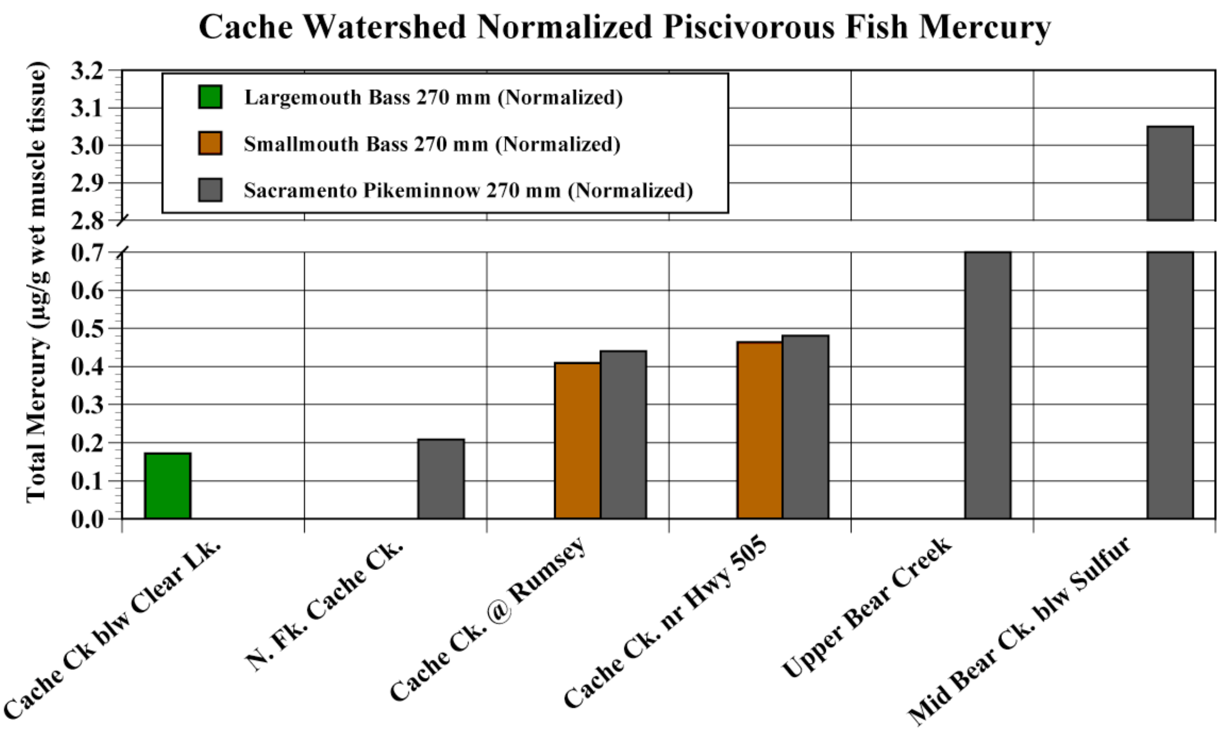
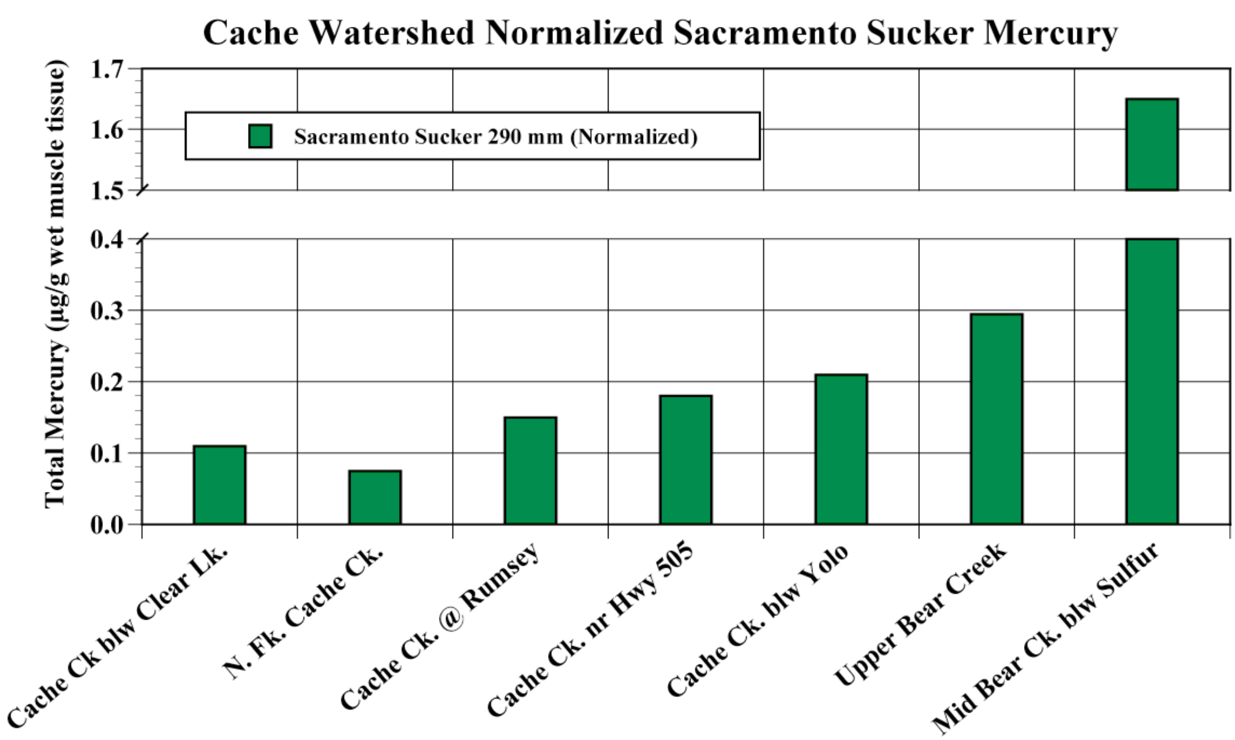


Figure 4.
Normalized muscle mercury in fish taken throughout the Cache Ck. watershed.
(Piscivorous fish = 270 mm normalization. Sacramento Sucker = 290 mm normalization.)
(Normalization = intersection of length (270 mm or 290 mm) with length:Hg regressions)





Water

It should be stressed that, for the comparison of biotic MeHg accumulation with corresponding aqueous Hg exposure, it was *concentration*—rather than loading—that was the object of the aqueous research described here. The Hg loading of this watershed to downstream receiving waters is discussed in the companion report by Domagalski et al.; loading from the point source remedial targets is covered in the companion report by Suchanek et al. Here, we focus on characteristic localized aqueous Hg *concentrations*, in relation to corresponding biotic MeHg accumulation. Efforts were made to collect water during conditions representative of the predominant flow regime of the given period, avoiding short-term events. In Figure 5, all of the project aqueous THg and MeHg concentration data are condensed for each of the sites into "box and whisker" plots displaying the ranges (10% through 90%), the median 50%, and overall median values for each site and aqueous Hg parameter. Aqueous Hg occurred over a wide range of concentrations and partitioning among the locations. By aqueous "partitioning", we refer to raw vs 0.45 µm filtered THg and MeHg. For biological context, sites are arranged in these plots in general order of increasing invertebrate MeHg found in the study. Invertebrates were the biota which were sampled most extensively and were the only studied biotic group that was present at all sampling locations.

The sites divided most notably into one group of highly elevated sites for all aqueous Hg parameters, associated with Hg point sources, and the remainder of sites which were typically dramatically lower. Among the low-moderate concentration sites removed or at distance from Hg point sources, a further division was apparent, with main stem Cache Creek elevated in aqueous Hg at sites downstream of the primary identified point sources. Relative control sites, with lowest overall concentrations of aqueous Hg, included Middle Creek, Cache Creek at the Clear lake outflow, North Fork Cache Creek, and upper Bear Creek. Upper Bear Creek was distinctive in demonstrating the very lowest THg in combination with moderate levels of MeHg.

Among sites which supported piscivorous fishes, median aqueous raw THg spanned over an order of magnitude, from concentrations of <1-3 ng/L at control sites (i.e. North Fork Cache Creek, Upper Bear Creek) to approximately 50 ng/L at mid Bear Creek. Dramatically higher THg occurred during storm flows and closer to mine sites at sampling locations such as Sulfur Ck and Harley Gulch, which did not contain fish. Corresponding median concentrations of aqueous MeHg at fish-containing sites ranged between approximately 0.08 ng/L at control sites and 0.35 ng/L at mid Bear Creek. Significantly higher concentrations were found at some of the near-mine sites where fish were not present.

Although aqueous THg concentrations from the primary point sources were in a similar range to those documented from other highly contaminated sites, aqueous MeHg concentrations appeared to be somewhat elevated. Highly contaminated sites have been noted to produce proportionally lower methylation rates than more typical environmental locations (e.g. Krabbenhoft et al. 1999). While this was the case in the current project as well, absolute concentrations of aqueous MeHg in Harley Gulch, Sulfur Creek, and Bear Creek were generally greater than concentrations reported from the Carson River in Nevada (Bonzongo et al. 1996), the New Idria Mine in California (Ganguli et al. 2000), Poplar Creek below the Oak Ridge, Tennessee nuclear weapons facility (Southworth et al. 2000), and the Idrija River in Slovenia

(Hines et al. 2000). We hypothesize that this may have been due to enhanced Hg methylating conditions within some of the Cache Creek tributaries, relative to typical highly contaminated sites, with potentially related factors including concurrent sulfate additions (Gilmour et al. 1992) relative to sulfide (Benoit et al. 1999), organic substrate (Ullrich et al. 2001), periphyton/biofilm layer (Cleckner et al. 1999), and water temperature (Ullrich et al. 2001).

In Table 3, the relationship between total suspended solids (TSS) and total or methyl Hg are summarized. Suspended solids samples were taken in parallel with all aqueous Hg samples. Because of the expense of aqueous mercury analyses, a considerable effort has been undertaken by various groups (most notably the US Geological Survey) to try to establish simple linkages between aqueous mercury and the more readily analyzed TSS or its surrogate, turbidity. Varying degrees of success have been realized, particularly in relation to storm event flows and associated loading within individual streams. In the current study, which did not focus on storm event flows, the relationship between TSS and mercury was less consistent. At most individual sites (for those sites with sufficient samplings to test statistically), strong relationships were found between TSS and THg, with correlation coefficients of 0.78-0.99. The interesting exceptions were the streams draining the primary mercury point sources, Harley Gulch and Sulfur Creek. We hypothesize that this was due to seasonal production of dense algal material at these sites, confounding the relationship between sediment-based TSS and THg. Additionally, though strong TSS:THg relationships were seen in the primary non-point source streams, this relationship was generally site-specific. When the parameters were compared across all of the sites and samples, no relationship was apparent. This was also the case for TSS vs. aqueous methylmercury. Methylmercury, however, showed weak or no relationships with corresponding suspended solids on an individual site basis as well, for most of the primary sampling sites. Only two of the sites, North Fork Cache Creek and Cache Creek at Hwy 505, demonstrated moderate to high correlations with TSS (0.66-0.77). We conclude that measurements of suspended solids and/or turbidity may offer promise as surrogates for aqueous total mercury, on a site-specific basis and with a locally calibrated TSS:THg relationship. This approach does not appear to be available for aqueous methylmercury.

General co-correlations of varying apparent strength were noted between virtually all of the different aqueous Hg fractions, when assessed across a watershed-wide scale that incorporated large gradients of concentration (Figure 6). This may be at least partly explained by the fact that the data were largely divided into two groups with divergent concentrations, generating potentially false apparent correlations. Log-log transformation was used to more normally distribute the data sets. Log-log “power” regression r^2s of between 0.45 and 0.81 were seen for all aqueous Hg inter-comparisons, with the strongest apparent correlations between raw and filtered THg (0.81) and between raw and filtered MeHg (0.67). The positive correlations between the different Hg parameters indicate that, on a broad regional scale, concentrations of each of the fractions may be generally inter-related and relative loading of THg may be somewhat predictive of other aqueous Hg fractions. However, when examined at individual sites, most of these apparent positive correlations disappeared. Figure 7, displaying the various inter-relationships of aqueous Hg parameters in samples collected throughout the study at mid Bear Creek, is typical of within-site trends. The relationship between raw THg and filtered THg remained strong ($r^2 = 0.76$), as, to a lesser extent, did that between raw and filtered MeHg ($r^2 = 0.52$). All other aqueous Hg comparisons gave no statistical relationship. It is notable, however, that, with the exception of a specific group of elevated MeHg points, an apparently strong correlation occurred at this mine-impacted tributary site between both aqueous raw and filtered THg and corresponding raw MeHg. The anomalous, high MeHg points that confounded these otherwise strong correlations were consistently associated with late spring through early summer data, indicating enhanced localized MeHg production. These results indicate that aqueous MeHg may be broadly linked to concurrent aqueous raw and filtered THg concentrations during a portion of the year, but that seasonally elevated production of MeHg may result in different and variable relationships at other times. Seasonally elevated MeHg production may occur directly in the water column under enhanced methylating conditions, as well as beneath the water column in the sediments and/or the periphyton/biofilm layers that are present at various sites. Evidence for the potential importance of a benthic methylation pathway can be found in the discussion of the anomalous upper Bear Creek large fish data (above) and in subsequent biota sections (below). Large fish Hg bioaccumulation was found to be strongly linked to corresponding bioaccumulation in benthic invertebrates and benthic-feeding small fish.

Aqueous Hg parameters were not consistent over time, demonstrating considerable variability seasonally and, to some extent, interannually. Temporal variation across an order of magnitude was seen for most aqueous Hg parameters at index sites, with total Hg in the main stem of Cache Creek below the dominant point sources varying by up to two orders of magnitude. During previous years with larger winter storm flows, aqueous THg variation of over three orders of magnitude was recorded in this reach (Foe and Croyle 1998). In Figure 8, raw and filtered aqueous THg and MeHg are plotted by time across the study period for three representative index sites: Cache Creek at the Clear Lake outflow (the primary upstream water source), mid Bear Creek (a tributary site downstream of a primary Hg point source region), and Cache Creek at Rumsey (main stem Cache Creek below the tributary inflows from the identified remedial targets). Concentrations are plotted with a log scale to allow simultaneous display of the widely disparate aqueous Hg fractions, though note that this approach compresses the apparent variability in concentrations for each individual aqueous fraction. During the 20 month course of the study, the dominant source of flow to the watershed, Clear Lake, never contributed aqueous THg at concentrations over approximately 5-10 ng/L. This indicates that the major Hg point source of the Sulphur Bank Hg mine, located directly on the shores of Clear Lake, may primarily impact the lake itself, with outflowing water to downstream Cache Creek only lightly elevated in THg under the types of conditions tracked during the study period. The seasonal patterns in filtered THg and raw and filtered MeHg at the Clear Lake outflow index site were apparently driven by within-lake processes, exhibiting elevations mid-winter through mid-summer, with MeHg peaking in the spring. This was generally consistent with extensive research conducted at Clear Lake throughout the 1990s (Suchanek et al. 1997). A different seasonal pattern was seen at the mid Bear Creek tributary site, with elevated concentrations of both THg and MeHg during early winter storm flows, presumably in conjunction with the mobilization of enriched former depositional material. The tributary sites also exhibited substantial elevations in MeHg between mid spring and mid summer, indicating enhanced localized methylation at that time, as discussed above. Within the main stem of Cache Creek at Rumsey and downstream, temporal patterns in aqueous Hg appeared to consist of a combination of the upstream trends, with reservoir release patterns influencing the effect of higher Hg tributary input (Bear Creek, Harley Gulch, Davis Creek). The proportional contribution of these tributary sources increased when main stem flows were not dominated by reservoir releases.

Maximal MeHg and THg concentrations in the main stem of Cache Creek were thus recorded during early winter storm flows prior to major reservoir releases. Throughout the irrigation season of April-September, flows and aqueous Hg trends in lower Cache Creek were determined mainly by releases from Clear Lake and Indian Valley Reservoir.

Figure 5.
Summary aqueous Hg concentration data from study sites in the Cache Creek Watershed.
(Data from Jan. 2000 – Aug. 2001)
(Boxes denote median 50% of site data. Lines at overall median concentrations)

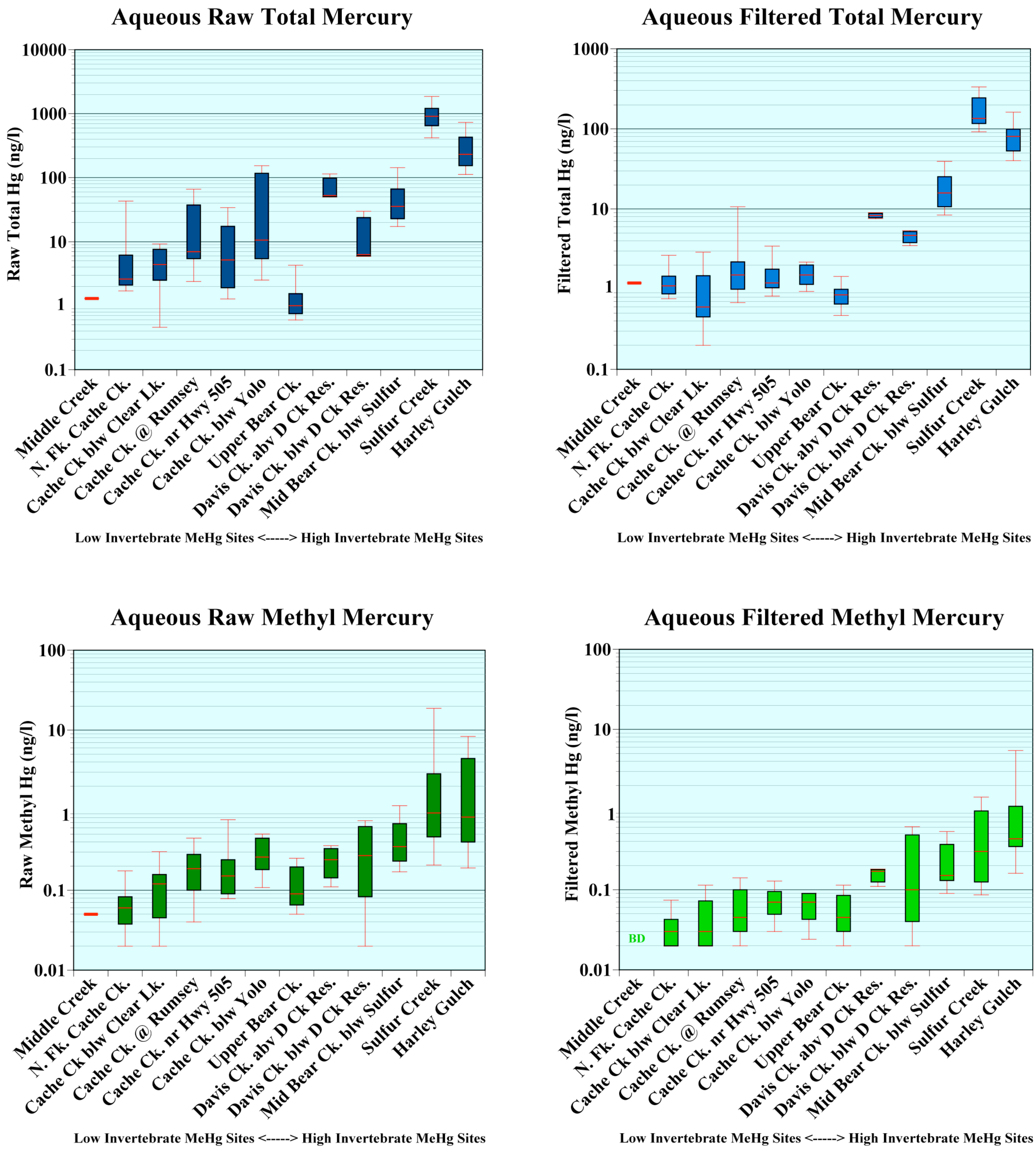


Table 3.
Relationship between Total Suspended Solids (TSS) and aqueous THg and MeHg

(Summary of linear regression correlations, 0.000 – 1.000)
(Summary of all primary site water samples taken throughout the project, Jan 2000 – Aug 2001)
(na = "not applicable"; relationships with correlation coefficients <0.400)

Location	Corr. Coefficient (r^2) TSS vs. Raw THg	Number (n) of Sampling Events	Equation of Regression
All Watershed Sites Combined	0.000	108	(na)
North Fork Cache Creek	0.996	12	THg(ng/l)=0.184(TSS(mg/l)) + 0.658
Cache Creek below Clear Lake	0.777	12	THg(ng/l)=0.183(TSS(mg/l)) + 0.951
Cache Creek at Rumsey	0.944	13	THg(ng/l)=0.334(TSS(mg/l)) - 4.532
Cache Creek below Highway 505	0.920	9	THg(ng/l)=0.226(TSS(mg/l)) + 2.516
Cache Creek below Yolo	0.911	7	THg(ng/l)=0.414(TSS(mg/l)) - 0.780
Upper Bear Creek	0.955	11	THg(ng/l)=0.125(TSS(mg/l)) + 0.776
Middle Bear Ck. bel. Sulfur Creek	0.882	13	THg(ng/l)=3.463(TSS(mg/l)) + 13.60
Sulfur Creek	0.088	13	(na)
Harley Gulch	0.367	8	(na)

Location	Corr. Coefficient (r2) TSS vs. Raw MeHg	Number (n) of Sampling Events	Equation of Regression
All Watershed Sites Combined	0.000	107	(na)
North Fork Cache Creek	0.282	10	(na)
Cache Creek below Clear Lake	0.003	10	(na)
Cache Creek at Rumsey	0.769	13	MeHg(ng/l)=0.0008(TSS(mg/l)) + 0.132
Cache Creek below Highway 505	0.661	9	MeHg(ng/l)=0.0043(TSS(mg/l)) + 0.096
Cache Creek below Yolo	0.362	7	(na)
Upper Bear Creek	0.000	11	(na)
Middle Bear Ck. bel. Sulfur Creek	0.042	12	(na)
Sulfur Creek	0.224	12	(na)
Harley Gulch	0.091	8	(na)

Figure 6.
Log/log (power) regressions comparing the relationships between aqueous Hg fractions.
(Data from all Cache Creek watershed sites, combined)

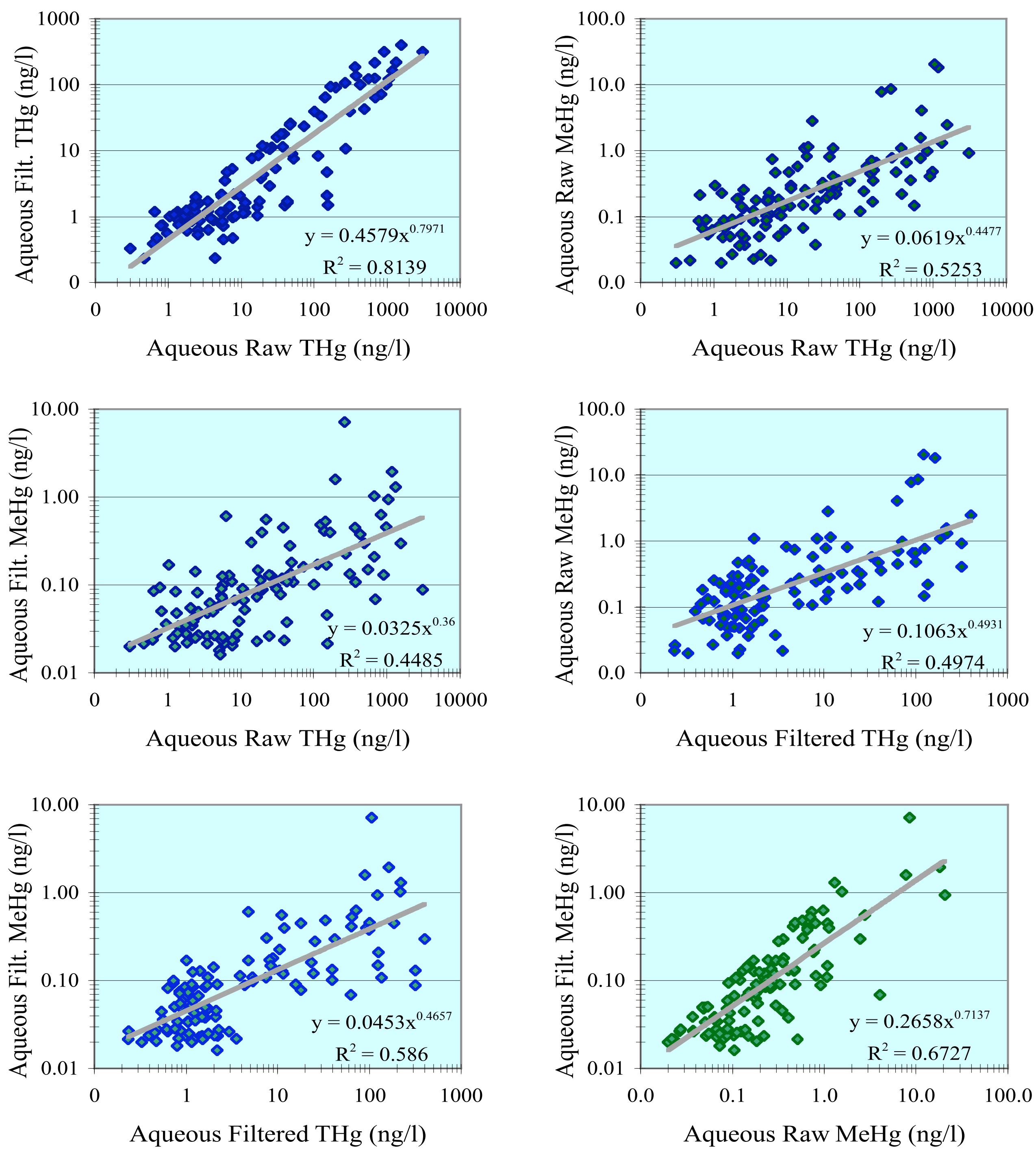


Figure 7.
Log/log (power) regressions comparing the relationships between aqueous Hg fractions at an <u>individual site: mid Bear Creek below Sulfur Creek</u>.

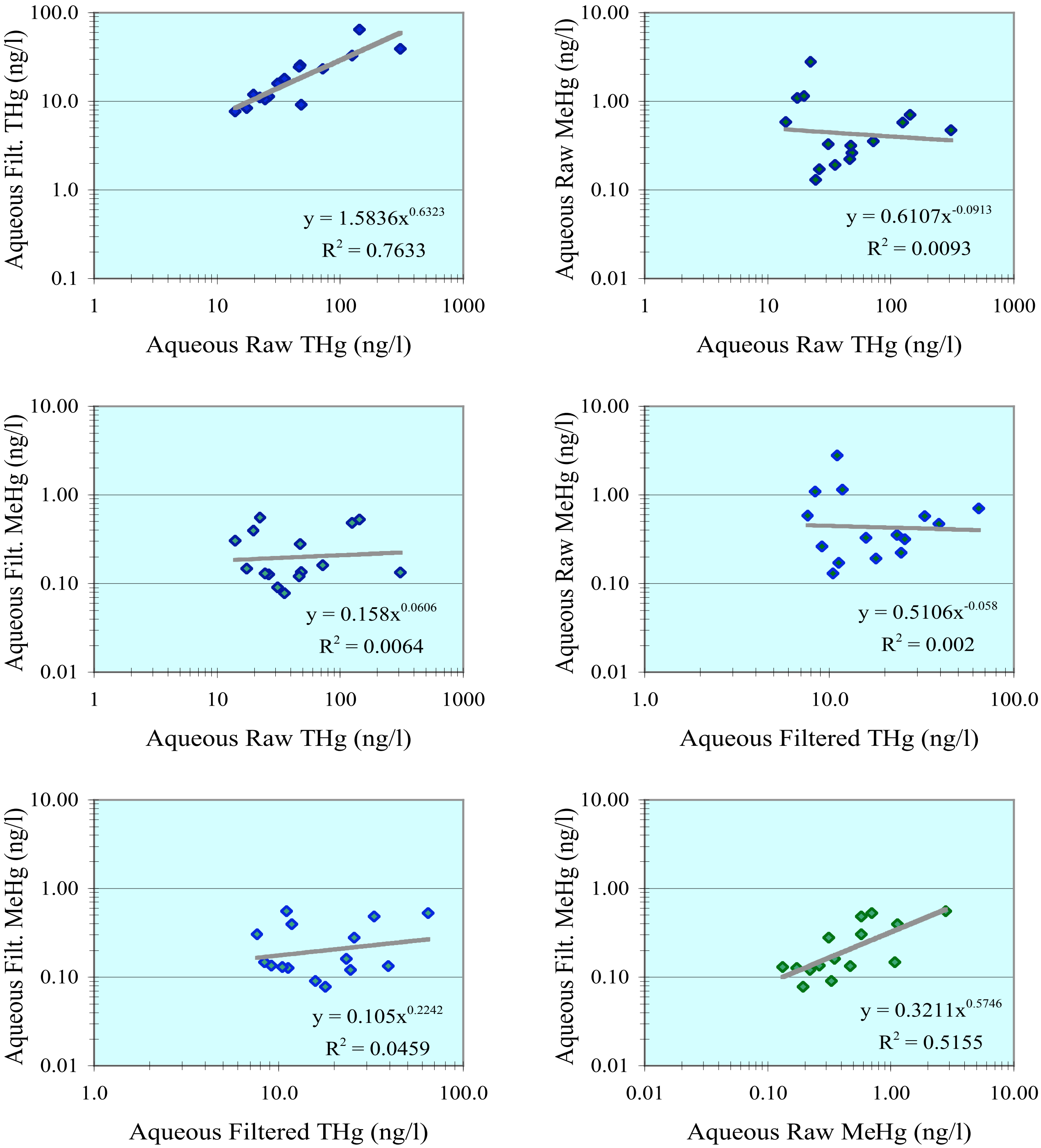
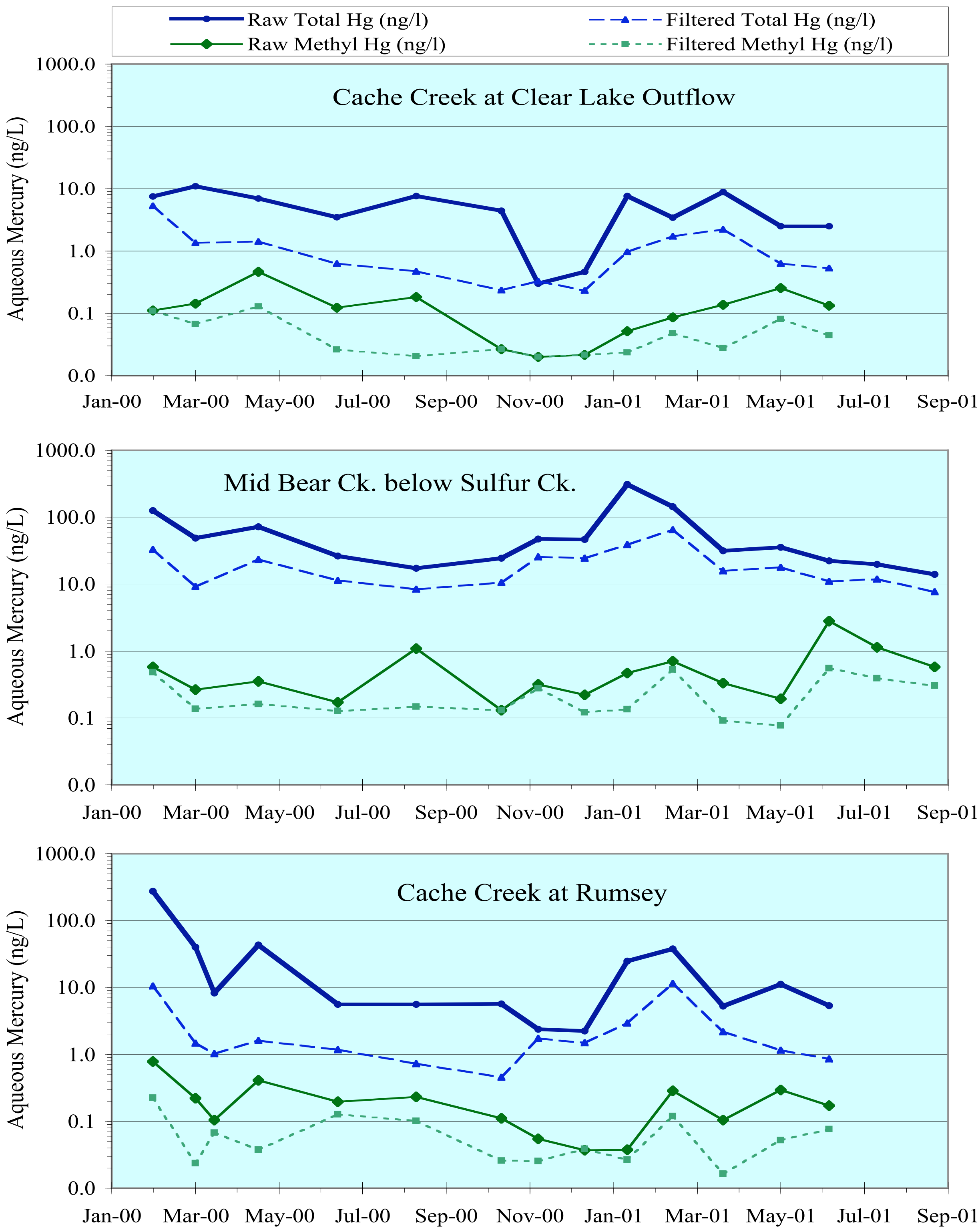


Figure 8.
Aqueous mercury over time at two main-stem and one high Hg tributary index sites.
(Note log axes for Hg concentrations)



Aquatic Insects

A suite of in-stream benthic aquatic insects was sampled throughout the project at a range of sites. These, together with small fish samples, were explored as relatively readily available, site-specific, and seasonally specific integrators of Hg exposure to biota. The different invertebrate taxa were additionally of interest as diet items for the small and large fish.

With the exception of herbivorous invertebrate species such as Baetid mayflies, which in most cases were clearly lower in both methyl and total Hg, the majority of prevalent large aquatic insects, including Hydropsychid caddisfly larvae and a variety of predatory taxa, exhibited relatively consistent MeHg and THg in individual site samplings. In Table 4, data for all primary aquatic insect samples taken at individual site samplings during a representative sampling period are compiled, demonstrating this general similarity in mercury accumulation across most predatory taxa and *Hydropsyche*. This trend was in contrast to our Sierra Nevada studies, where drift-feeding caddisfly larvae were generally lower in Hg than obligate predators such as Perlid stoneflies, which were consistently lower than top invertebrate predators such as Corydalid hellgrammites (Slotton et al. 1995). Similar trends of increasing aquatic invertebrate MeHg percentage with trophic position were observed by Mason et al. (2000) in two Maryland streams, and by Tremblay and others (1996) in a Canadian lake and two reservoirs. While individual site samplings in the current study frequently yielded similar MeHg concentrations among co-occurring taxa, on a watershed-wide basis invertebrate MeHg percentages increased as would be predicted, moving from lower trophic level species to obligate predators (Figure 9). For the Cache Creek watershed, a composite invertebrate MeHg value was found to be the best and most consistent invertebrate measure for use in inter-site, temporal, and aqueous Hg comparisons. A combined value was also felt to be the most realistic measure in relation to subsequent trophic transfer. The Cache Creek invertebrate MeHg data are thus summarized here with same-site, same-date mean values of a defined set of separately analyzed insect families. These composite values consist of the mean MeHg of Hydropsychid caddisfly larvae and co-occurring predatory species, including Perlodid and Perlid stonefly nymphs, Coenagrionid damselfly nymphs, Libellulid and Gomphid dragonfly nymphs, and Corydalid hellgrammites.

The proportion of methyl to total Hg in the various aquatic insect families is shown in Figure 9. Cache watershed aquatic insects contained relatively high proportions of MeHg (60-94%, Figure 9a) throughout the watershed, except at the most extreme, near-mine locations (16-45%, Figure 9b). Figure 9b includes only sites located along the initial tributaries from major Hg point sources: upper Davis Creek, Harley Gulch, and Sulfur Creek. Significant uptake of inorganic Hg was apparent for all taxa at these sites. For all other portions of the watershed, including mid Bear Creek, methyl percentages were very consistent at a high proportion of total invertebrate Hg in the taxa utilized for comparisons (70-94%, Figure 9a). Similar increases in biotic MeHg percentage, moving away from a strong Hg point source, were observed by Southworth et al. (1995) and Hill et al. (1996) downstream of the Oak Ridge, Tennessee nuclear weapons facility, also attributed to a decrease from extreme inorganic Hg exposure.

In Figure 10, aquatic insect MeHg data across the project period are condensed by site in box and whisker plots. Concentrations exhibited a spatial pattern that was similar to those of both the large fish and the aqueous MeHg data. Lowest median composite aquatic insect MeHg

(15-20 ng/g, wet wt) occurred at Middle Creek and the two primary source flows to the watershed, the Clear Lake outflow and North Fork Cache Creek. Concentrations were elevated by approximately 50% at Rumsey (27 ng/g) and increased somewhat more at the most downstream Cache Creek stations (30-35 ng/g). Upper Bear Creek was elevated above these sites at 45 ng/g, also consistent with the large fish data. Despite containing lower invertebrate MeHg:THg percentages, the sites located on initial tributaries downstream of primary Hg point sources exhibited absolute invertebrate MeHg at dramatically elevated levels, with median wet weight concentrations between 230 and 625 ng/g. Harley Gulch showed the very highest levels. Interestingly, mid Bear Creek, several km downstream of the Sulfur Creek source inflows and considerably diluted, had similar invertebrate MeHg as compared to Sulfur Creek itself, though Sulfur Creek insects contained much greater total Hg.

We begin to address the potential relationship between aqueous Hg exposure and bioindicator invertebrate MeHg in Figure 11. For these comparisons, we utilized the composite Hydropsychid + additional predatory taxa MeHg value for each site sampling, as described above. The choice of appropriate corresponding aqueous data was not straightforward. After considerable data trials and consideration of seasonal flow and biotic patterns, we adopted the convention of using the average of aqueous data collected within the period from 9 weeks prior through 3 weeks following each given invertebrate collection. This provided replication across a seasonal period centered 3 weeks prior to the corresponding invertebrate collection. With the sampling scheme utilized, this approach generally captured average conditions during the various distinct seasonal flow regimes. Composite invertebrate MeHg is plotted as a function of corresponding aqueous Hg concentration for raw and filtered THg and MeHg. Log-log treatment of the data was used as a statistical approach to the aqueous Hg vs invertebrate MeHg relationships, with corresponding power regressions. Log-log transformation expands the otherwise clustered distribution of lower-end points, while also giving less individual weight to high-end outliers in this diverse data set. The resulting power regression r^2 values for the aqueous Hg vs invertebrate MeHg data range from 0.49 for raw THg to 0.63 for raw MeHg, 0.68 for filtered THg, and 0.76 for filtered MeHg. These relatively strong correlations indicate that, when comparing these highly divergent Hg exposure sites throughout the watershed, a significant portion of the overall variation in the invertebrate bioindicator MeHg data may be attributable to aqueous Hg concentrations. However, we recognize the potential limitations of these apparent correlations. There is a strong likelihood of co-correlation among the aqueous Hg parameters, as noted in the water discussion above. Additionally, despite the relative damping effect of log-log transformation, the apparent correlations may be considerably influenced by the persisting effect of clusters of low concentration vs high concentration data.

Consequently, we collected additional data at several representative index sites in order to examine the potential relationships between aqueous Hg parameters and biotic MeHg on an individual site basis. In Figures 12 and 13, site-specific data from mid Bear Creek and Cache Creek at Rumsey are displayed. Log-log transformation was not necessary with same-site data, which were more normally distributed. While the watershed-wide data set demonstrated relatively strong general correlations between each of the aqueous Hg fractions and invertebrate MeHg, the data from the individual sites yielded quite different results. In particular, both raw and filtered THg showed virtually no direct relationship with corresponding invertebrate MeHg. In fact, on a same-site basis, some of the highest invertebrate MeHg corresponded to minimal raw and filtered aqueous THg. In contrast, relatively strong relationships were apparent between invertebrate MeHg and raw aqueous MeHg, with linear correlations of 0.65-0.66 at both mid Bear Creek ($p < 0.002$) and Cache Creek at Rumsey ($p < 0.005$), two very divergent sites. While relatively strong within-site relationships were seen with raw aqueous MeHg, it is important to note that the form of those relationships differed substantially between sites. Thus, while at Mid Bear Creek aqueous unfiltered MeHg exposure of 0.5 ng/L corresponded to an invertebrate MeHg concentration of 415 ng/g, an equivalent exposure at Cache Creek at Rumsey yielded invertebrate MeHg of only 43 ng/g. These results demonstrate the importance of developing site-specific relationships for regions that may vary widely in their mercury cycling processes.

The observed correspondence between raw aqueous MeHg and low trophic level biotic MeHg in the Cache Creek watershed contrasted to some extent with the preliminary results of Cleckner et al. (1998) in the Florida Everglades, who found low trophic level MeHg (particularly periphyton) to occasionally vary apparently independently of aqueous MeHg. Filtered aqueous MeHg in the Cache study demonstrated considerably lower apparent direct relationship with invertebrate MeHg, with correlations of 0.19 at Cache Creek at Rumsey and 0.29 at mid Bear Creek. This may be due in part to many concentrations occurring near the level of detection (note scale) and/or the sampling scheme which included a ≤24 hour delay prior to filtration. Alternatively, it may indicate that invertebrate MeHg was more greatly influenced by the particulate portion of the aqueous MeHg.

Aqueous particulate THg and MeHg are compared to invertebrate MeHg in Figure 14. On a whole watershed basis, particulate THg demonstrated an apparently strong relationship with invertebrate MeHg, with a power regression r^2 of 0.69. However, this can be almost entirely attributed to the bi-modal clusters of low-Hg vs high-Hg points. Within each of these clusters, no relationship was apparent. The particulate MeHg data were more normally distributed, but the resulting power regression r^2 was relatively weak at 0.27. On a site-specific basis, neither particulate Hg parameter showed any meaningful relationship with invertebrate MeHg, as demonstrated by the characteristic data shown for mid Bear Creek and Cache Creek at Rumsey. This was somewhat surprising, as we had hypothesized that particulate MeHg, in particular, would be linked to invertebrate MeHg bioaccumulation. The weak relationships found in this study between aqueous particulate MeHg and invertebrate MeHg may indicate that the propagation of analytical errors near the various levels of detection of the parameters used to calculate particulate MeHg masked a potentially stronger relationship, despite exclusion of the most questionable analytical data. In any case, raw aqueous MeHg was found to provide by far the most consistent and predictive aqueous measure of corresponding invertebrate MeHg.

General seasonal trends in invertebrate MeHg are presented for all of the sites in Figure 15. In Figures 16(a-e), the seasonal patterns for the primary index stations are examined in conjunction with aqueous raw MeHg, filtered MeHg, and small fish MeHg (to be discussed in the following section). Invertebrate MeHg was not static, instead demonstrating increases/decreases of 2-4+ fold across the year and at all of the sites. Seasonal patterns generally followed aqueous MeHg. General trends repeated in the second year of the study at most of the sites, with varying degrees of inter-annual variability. At the Clear Lake outflow, invertebrate MeHg peaked strongly in the spring, declining to minimum levels in the early winter. The pattern, as discussed above for water, appeared to be closely linked to within-lake processes in Clear Lake. We noted large *Daphnia* cladocerans in spring collections of water at this site. Exported zooplankton from lakes and reservoirs have been shown to provide an important mechanism for transfer of MeHg into downstream food webs (Schetagne et al. 2000). In contrast, mid Bear Creek demonstrated bimodal invertebrate MeHg peaks in winter and summer, with strong declines between. The winter peaks may be attributable to storm flow-related resuspension of enriched bed material and transport from upstream point source regions. The summer peaks indicate localized maxima in MeHg at those times, presumably linked to local Hg methylation. Seasonal trends at Upper Bear Creek were studied intensively for only one annual cycle and were consistent with the general Bear Creek trend, with a primary peak in the early-mid summer, a secondary peak in early winter, and declines between. North Fork Cache Creek had the lowest variability and lowest overall concentrations of the index sites, transitioning gradually between an apparent minimum in the spring and higher levels toward winter, possibly linked to cycles within Indian Valley Reservoir. Cache Creek at Rumsey exhibited a relatively muted trend that apparently incorporated upstream patterns, increasing between winter and early summer and declining into the fall. Aquatic insect MeHg concentrations at this index site were also intermediate between those at the dominant release water source flows and those from higher Hg tributaries.

As discussed above for water, the relative contribution of the high-Hg tributaries to main-stem Cache Creek was maximal during early winter runoff flows, when Clear Lake and Indian Valley Reservoir were not releasing substantial water. In Table 5, the relative early winter impact of Bear Creek inflow on main stem Cache Creek aquatic insects is apparent. Total Hg, MeHg, and nitrogen and carbon stable isotopes all demonstrated strong signatures of the Bear Creek influence in early winter. Though irrigation releases from Clear Lake and Indian Valley Reservoir dominate water quality in main stem Cache Creek throughout much of the year, the seasonal inputs from the Hg point source regions may provide lasting effects to downstream regions. Clearly, these inflows can have a significant seasonal impact.

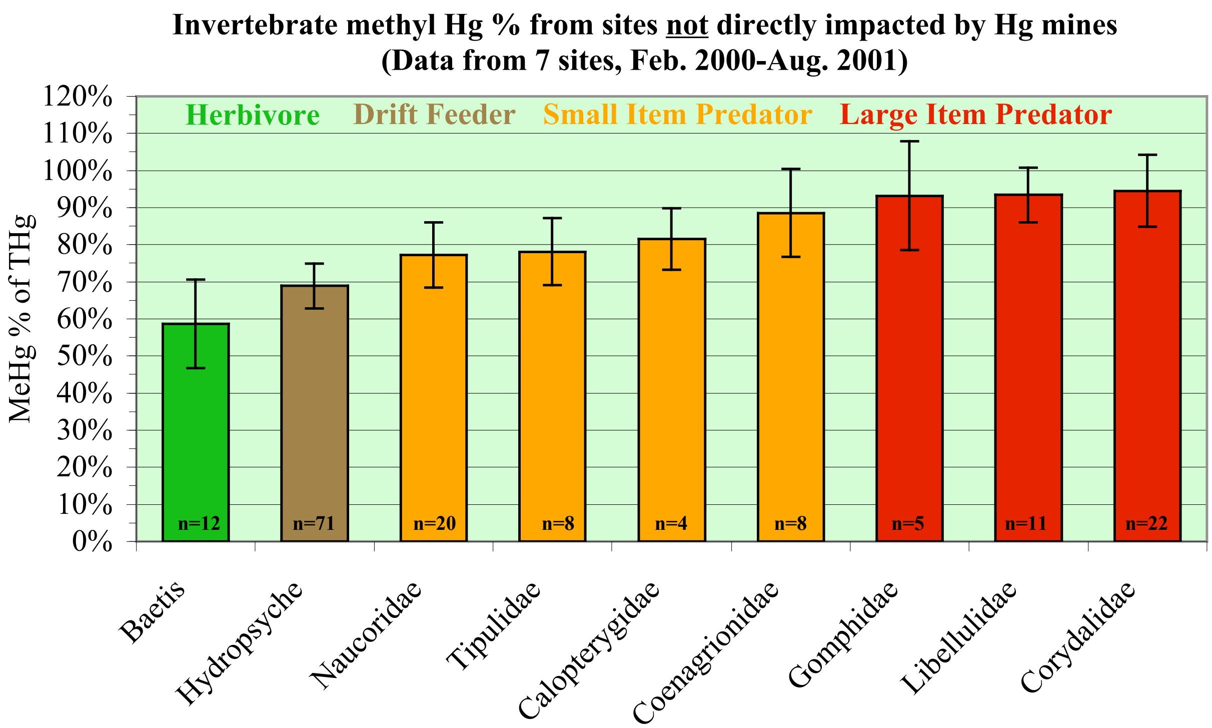
It was notable how rapidly the aquatic insect bioindicators changed their MeHg concentrations throughout the year. Increases of up to 100% per month and decreases up to 50% per month were seen at the most volatile locations. These swings in standing crop MeHg concentration may represent a combination of (a) continuous new recruitment under altered aqueous exposure conditions and (b) actual concentration changes in individual organisms. In Table 5, it can be seen that the smallest, most rapidly growing (and most rapidly replaced) organisms sampled, Baetid mayfly nymphs and Hydropsychid caddisfly larvae, exhibited more radical short-term seasonal influence from the relatively concentrated Bear Creek inflows, as compared to the large Corydalid hellgrammites, which grow more slowly and can live over a year. (This data also illustrates the large ^{15}N signature deriving from Sulfur Creek, which has confounded our interpretation of the stable isotope results).

Table 4.
Representative invertebrate data showing similar within-site/date Hg among *Hydropsyche* and predatory invertebrate taxa

(data in italics are from non-predatory taxa, not used in "predatory insects" composite means)
(samples collected from all sites during May 2000)

Sample Date	Location	Insect Family	n	Avg Size *(mm)*	MeHg *(ww ng/g)*	THg *(ww ng/g)*	% MeHg
24-May-00	Cache Ck. bel. Clear Lake	Hydropsyche	73	12	**74**	**67**	112%
8-May-00	North Fork Cache Creek	*Baetis*	*250*	*6*	***7***	***10***	*66%*
		Hydropsyche	96	11	**16**	**21**	79%
		Perlodidae	52		**16**	**18**	88%
		Tipulidae	14	30	**15**	**18**	81%
9-May-00	Cache Creek at Rumsey	Hydropsyche	163	11	**35**	**38**	91%
		Perlodidae	59	9	**37**	**36**	102%
		Corydalidae	7	50	**41**	**40**	102%
22-May-00	Cache Ck. bel. Hwy 505	*Calopterygidae*	*7*	*30*	***14***	***15***	*91%*
		Hydropsyche	91	12	**32**	**44**	72%
		Libellulidae	17	19	**29**	**24**	121%
22-May-00	Cache Ck. below Yolo	*Calopterygidae*	*14*	*26*	***16***	***21***	*74%*
		Hydropsyche	30	10	**43**	**59**	74%
		Coenagrionidae	5	15	**48**		
24-May-00	Middle Creek	*Ephemerellidae*	*45*	*12*	***6***	***8***	*77%*
		Hydropsyche	5	11	**14**		
		Naucoridae	9	9	**20**	**25**	83%
		Corydalidae	3	38	**15**	**20**	77%
8-May-00	Harley Gulch	Hydropsyche	77	12	**274**	**1513**	18%
		Coenagrionidae	28	14	**296**	**1204**	25%
		Naucoridae	45	10	**937**	**1893**	50%
		Corydalidae	9	38	**582**	**1451**	40%
17-May-00	Davis Creek abv. DCR	Perlidae	4	21	**96**	**428**	22%
		Libellulidae	3	21	**143**	**875**	16%
		Naucoridae	14	10	**131**	**193**	68%
		Corydalidae	3	38	**107**	**909**	12%
17-May-00	Davis Ck. bel. DCR	Hydropsyche	4	8	**291**		
		Naucoridae	5	7	**243**	**607**	40%
		Corydalidae	6	32.5	**238**	**329**	73%
8-May-00	Upper Bear Creek	Hydropsyche	46	9	**31**	**34**	92%
		Libellulidae	4		**30**	**31**	97%
		Naucoridae	57	11	**33**	**41**	81%
		Tipulidae	27	30	**18**	**19**	93%
8-May-00	Sulfur Creek	*Ephemerellidae*	*151*	*9*	***32***	***185***	*17%*
		Coenagrionidae	87	21	**290**	**1987**	15%
		Naucoridae	18	10	**139**	**416**	33%
9-May-00	Mid Bear Creek	*Elmidae*	*80*	*7*	***297***	***370***	*80%*
		Hydropsyche	126	12	**359**	**425**	84%
		Coenagrionidae	61	20	**138**	**168**	82%
		Libellulidae	12	20	**286**	**343**	84%
		Naucoridae	38	11	**306**	**465**	66%

Figure 9 (a-b).
Invertebrate methyl Hg % of total Hg in Cache Creek watershed samples.
(mean MeHg % ± 95% confidence intervals)



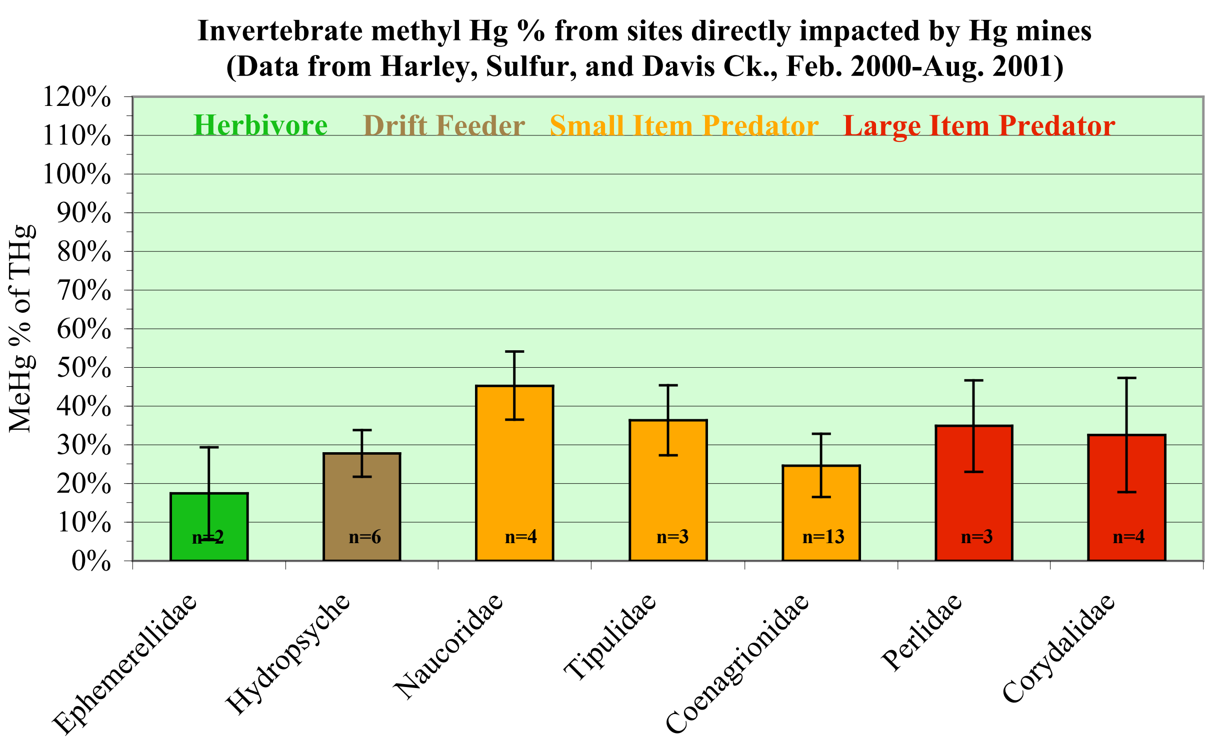


Figure 10.
Summary aquatic invertebrate Hg data from study sites in the Cache Creek Watershed.
(Data from Feb. 2000 – Jul. 2001)
(Boxes denote median 50% of site data. Lines at overall median concentrations)
(*invertebrate MeHg = mean site-sampling MeHg of Hydropsyche and predatory taxa*)

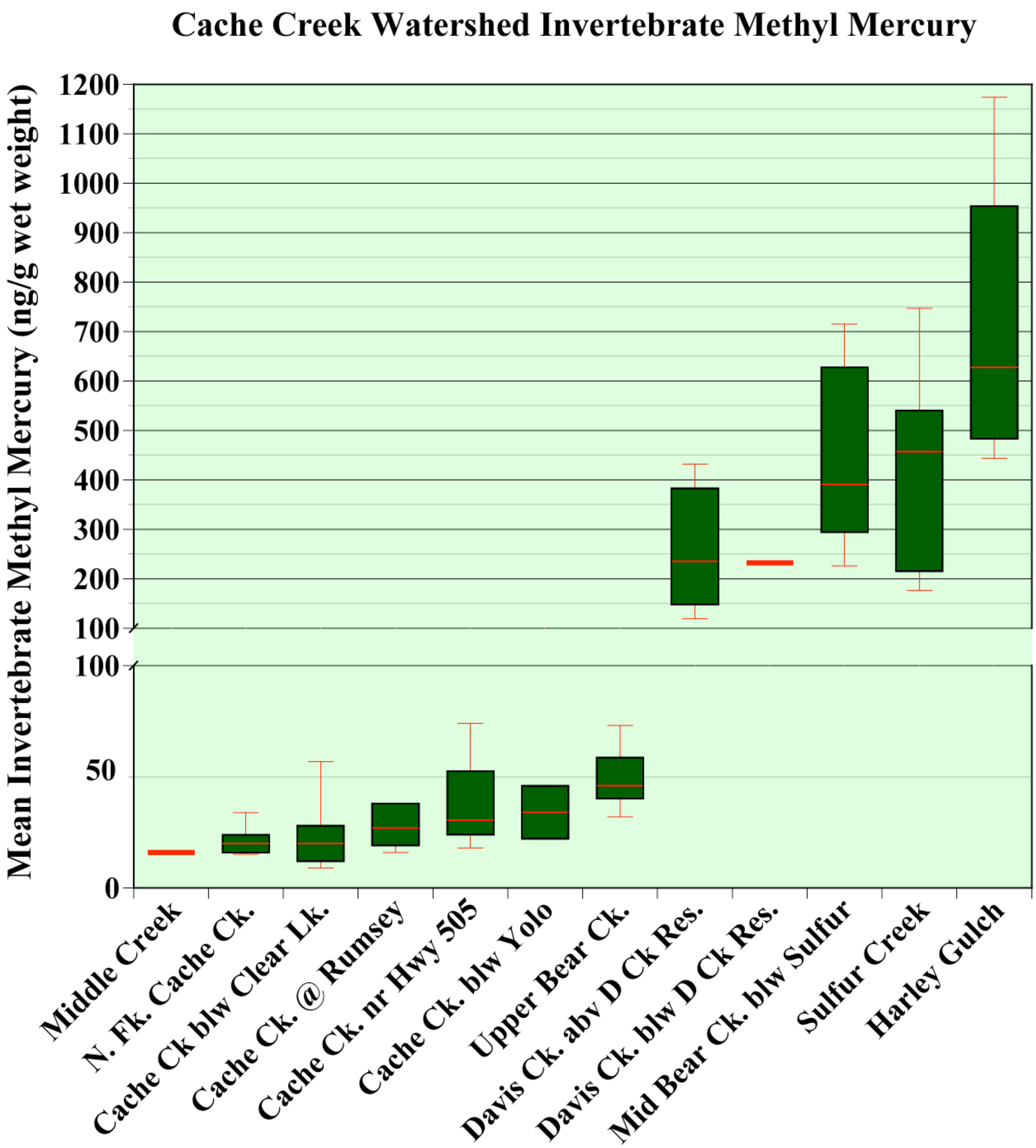


Figure 11.
Log/log (power) regressions of aqueous Hg fractions vs composite invertebrate MeHg.
Data from all Cache Creek watershed sites combined.
(composite invertebrate MeHg = mean site-sampling MeHg of Hydropsyche and predatory taxa)
(corresponding water data from the most representative samplings prior to invertebrate collection)

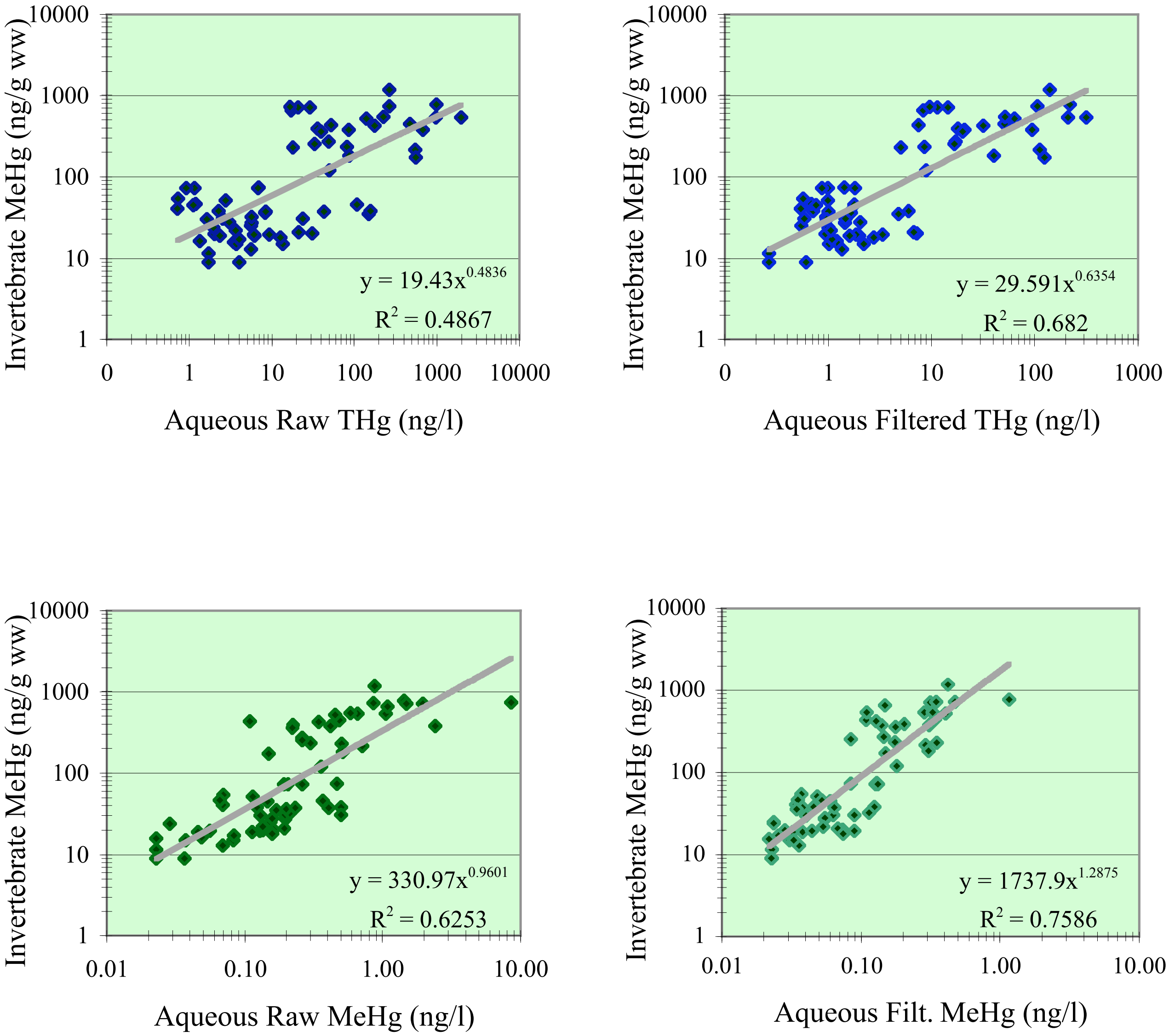


Figure 12.
Linear regressions of aqueous Hg fractions vs composite invertebrate MeHg at an individual site: Middle Bear Creek below Sulfur Creek.
(composite invertebrate MeHg = mean site-sampling MeHg of Hydropsyche and predatory taxa)
(corresponding water data from the most representative samplings prior to invertebrate collection)

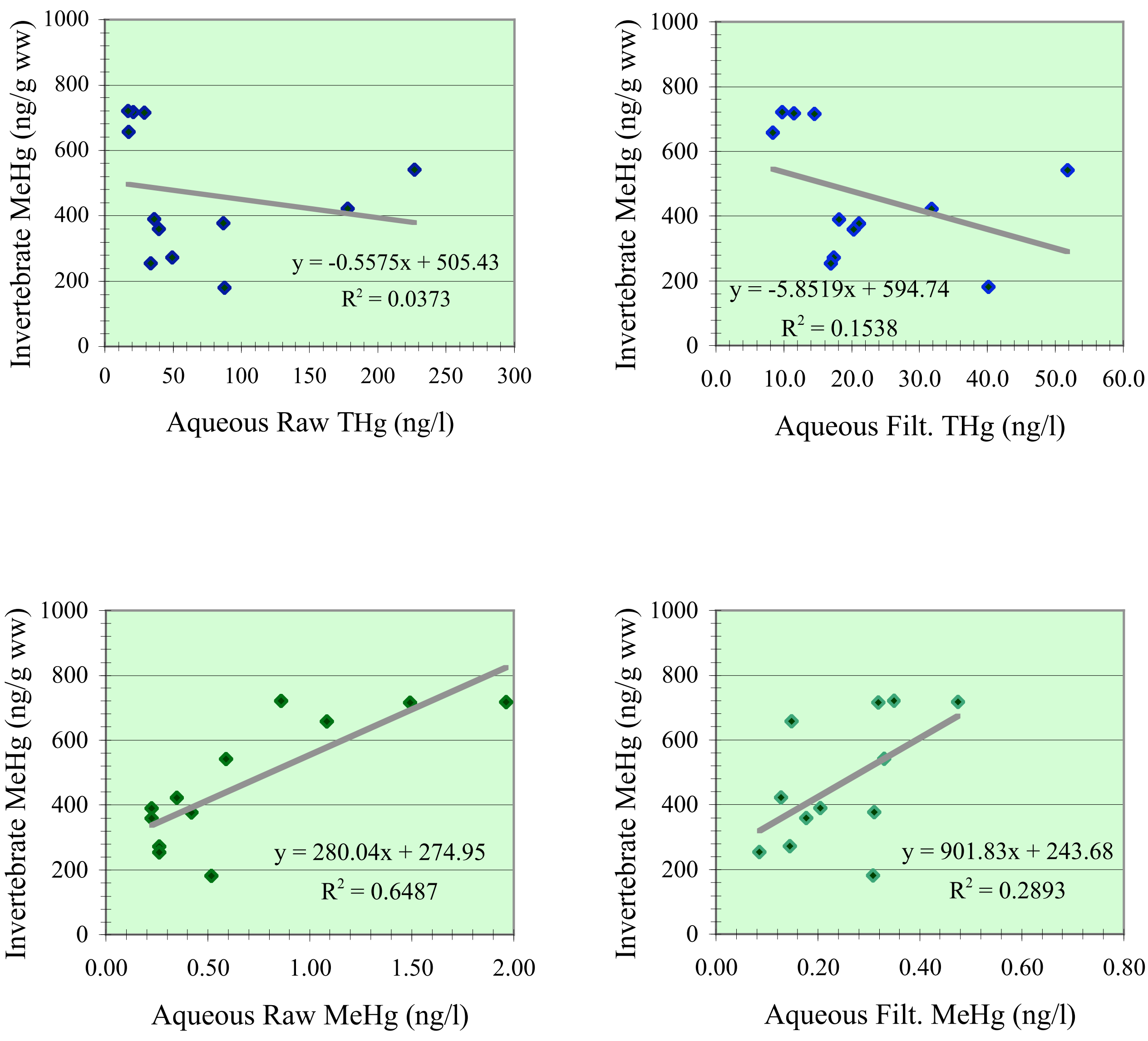


Figure 13.
Linear regressions of aqueous Hg fractions vs composite invertebrate MeHg at an individual site: Cache Creek at Rumsey.
(composite invertebrate MeHg = mean site-sampling MeHg of Hydropsyche and predatory taxa)
(corresponding water data from the most representative samplings prior to invertebrate collection)

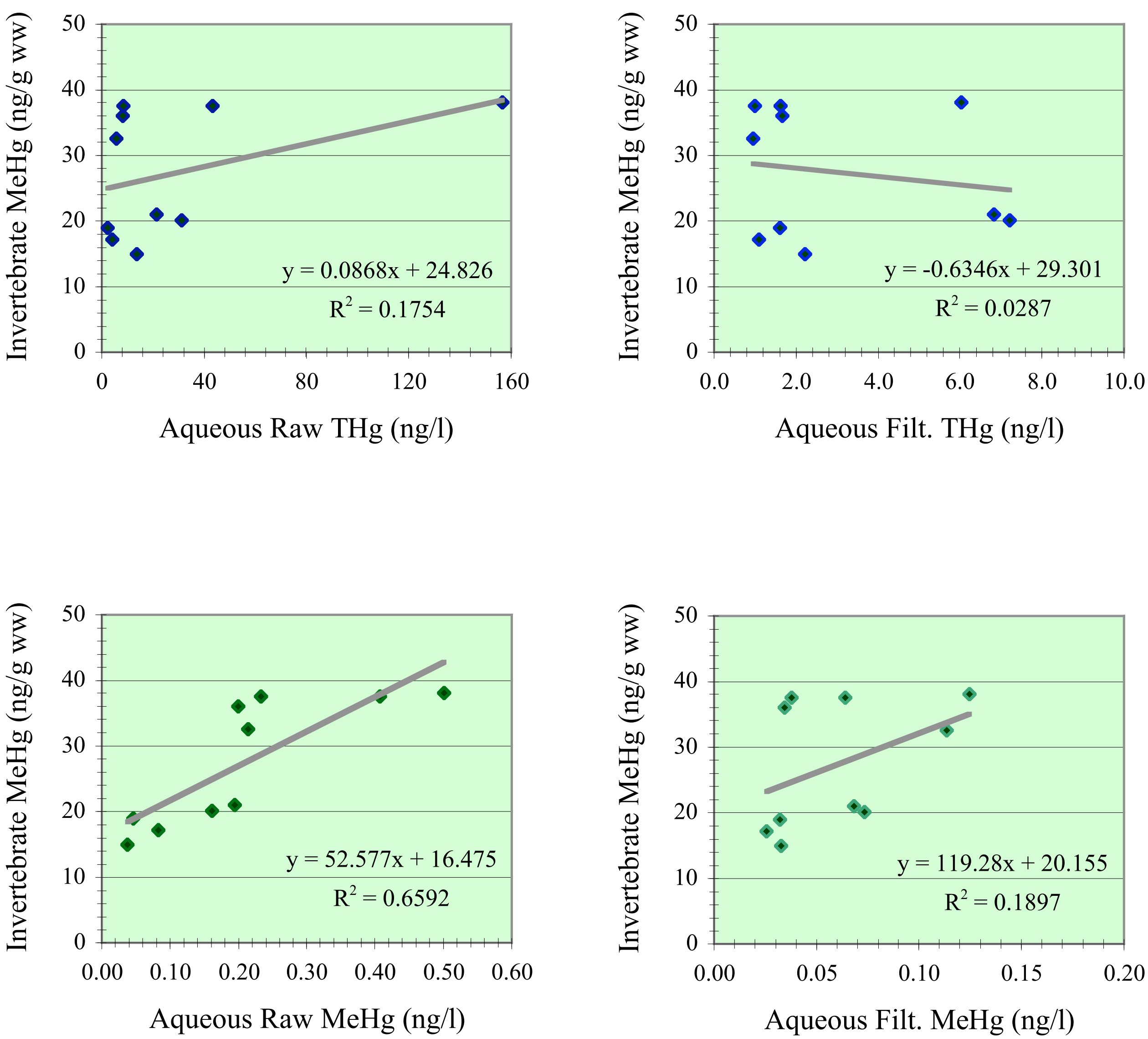


Figure 14.
Log-log and linear regressions of <u>particulate Hg fractions</u> vs composite invertebrate MeHg.
(composite invertebrate MeHg = mean site-sampling MeHg of <u>Hydropsyche</u> and predatory taxa)
(corresponding water data from the most representative samplings prior to invertebrate collection)

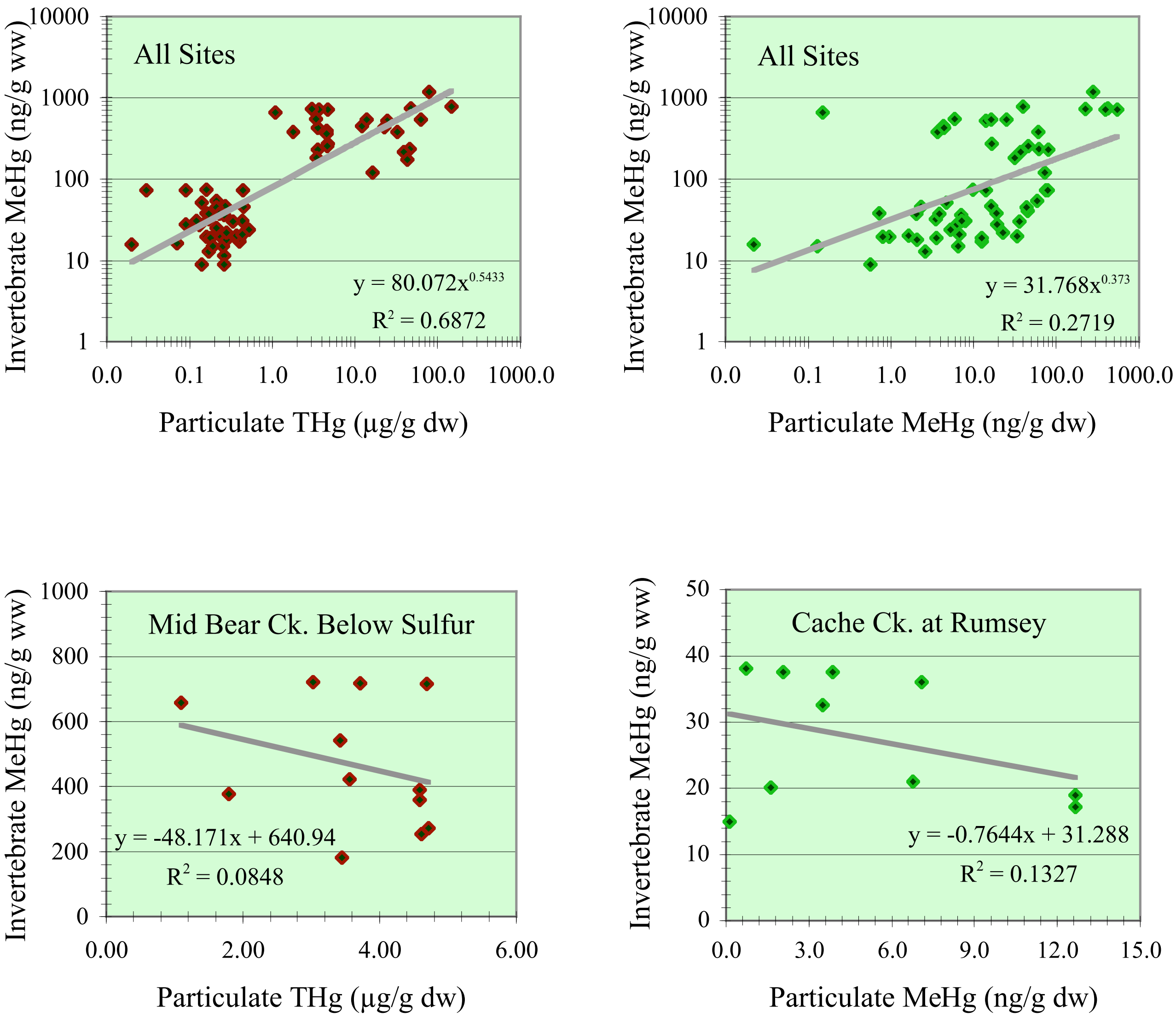


Figure 15.
Condensed mean invertebrate methylmercury concentration vs. site and date.
(Composite invertebrate MeHg = mean site-sampling MeHg of Hydropsyche and predatory taxa)
(Top chart includes all sites; bottom chart includes low invertebrate concentration sites only)
(Note difference in concentration scale between charts)

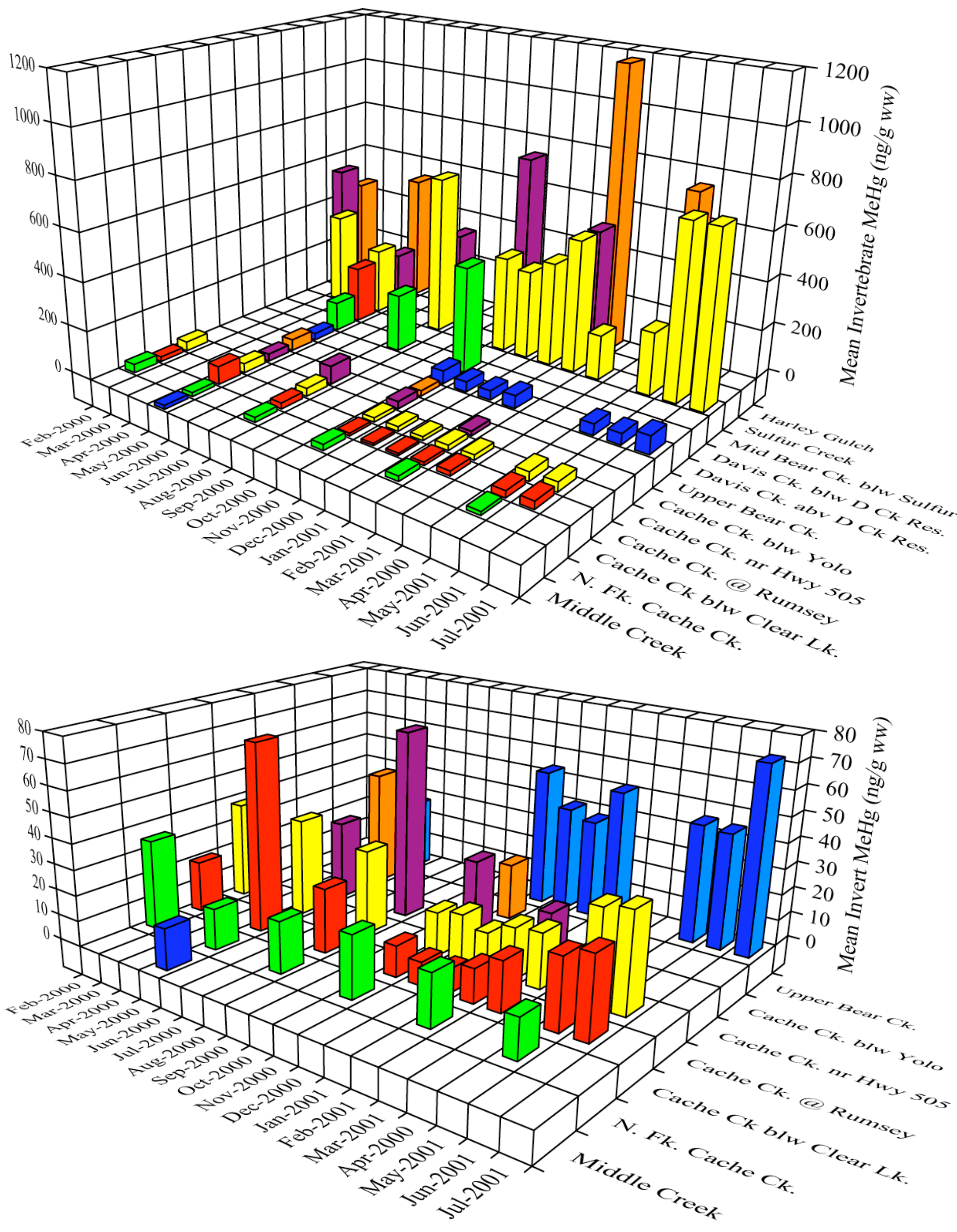
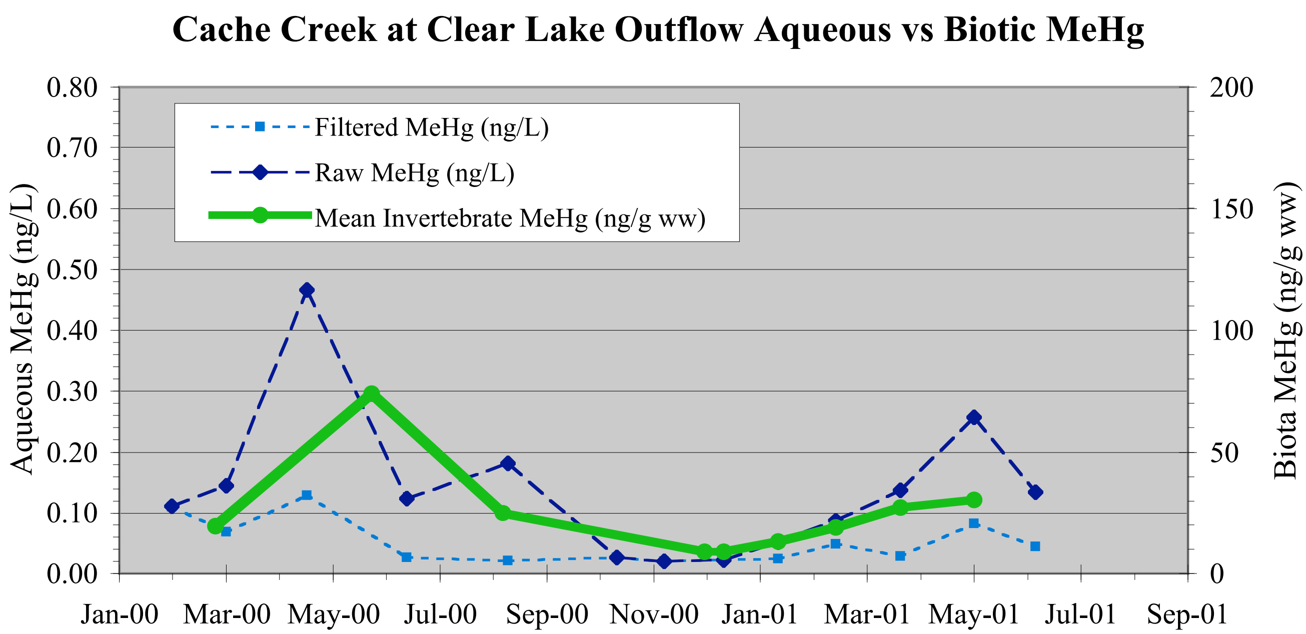


Figure 16 (a-e).
Seasonal trends in aqueous, invertebrate, and small fish methylmercury at index sites.
(invertebrate MeHg = mean site-sampling MeHg of Hydropsyche and predatory taxa composites)
(small fish MeHg = mean of three multiple individual whole body composites)



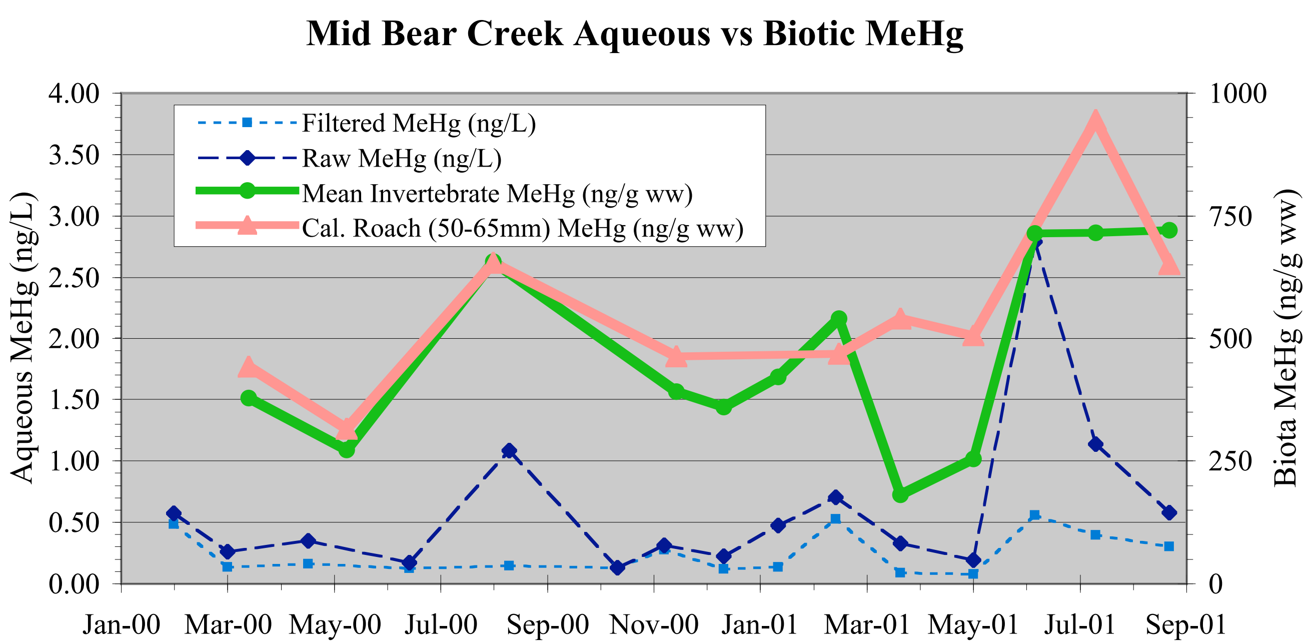
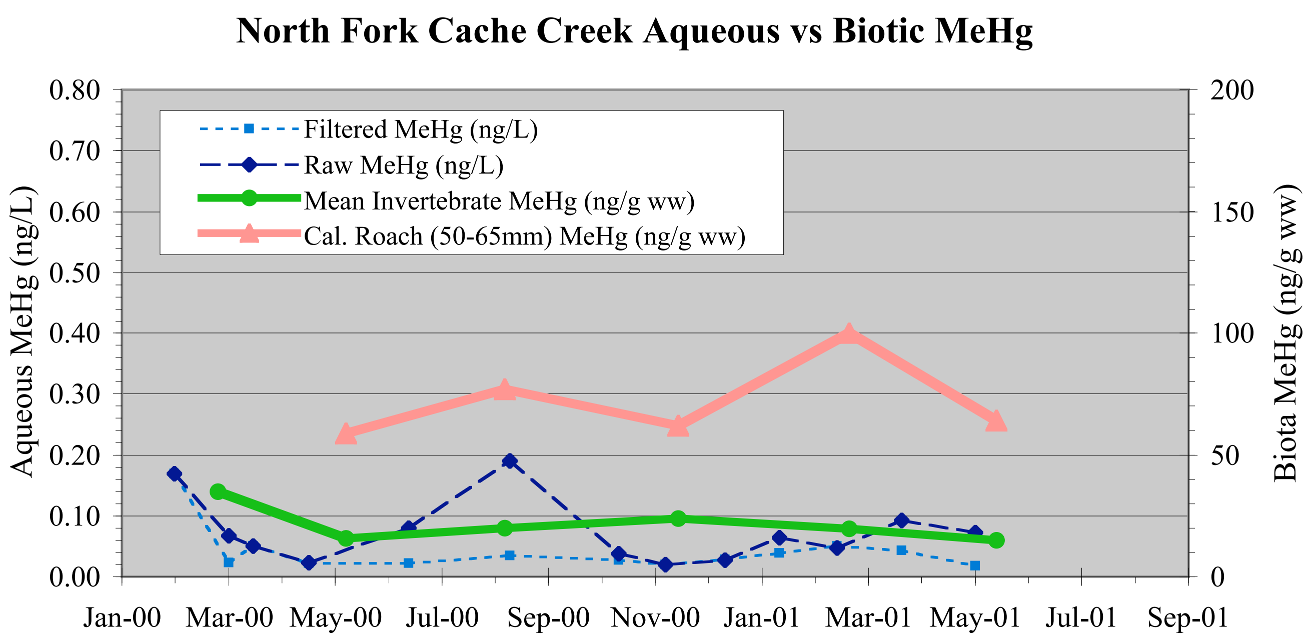


Figure 16 (a-e) continued.



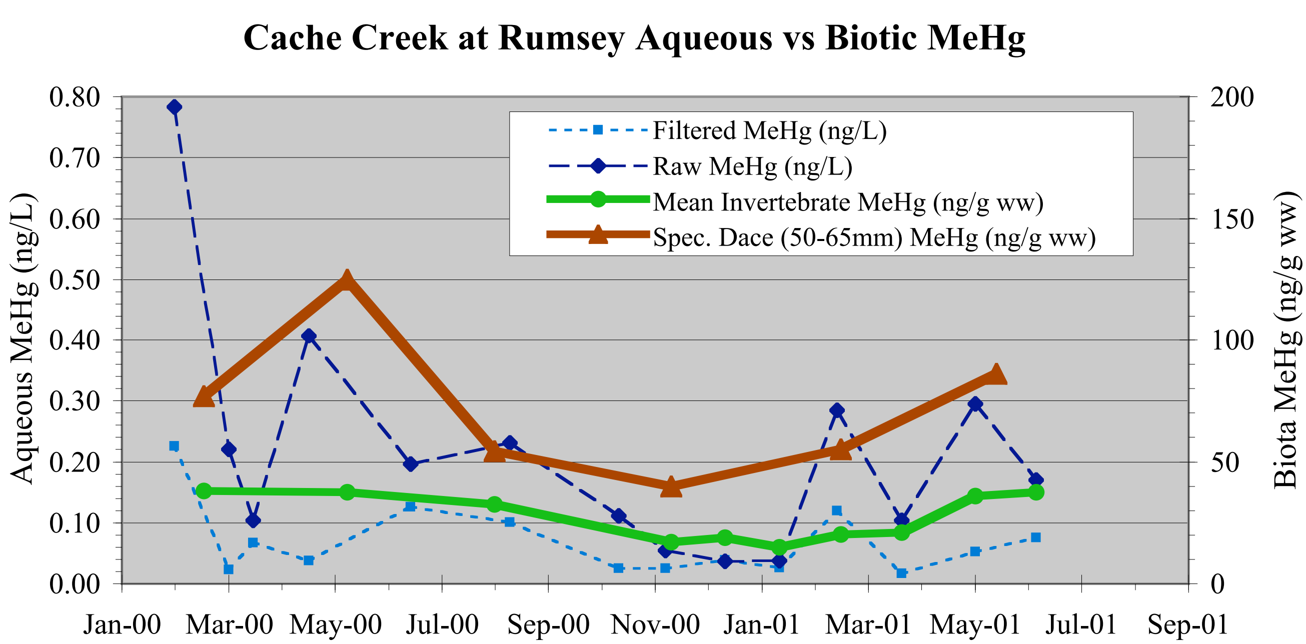


Figure 16 (a-e) continued.

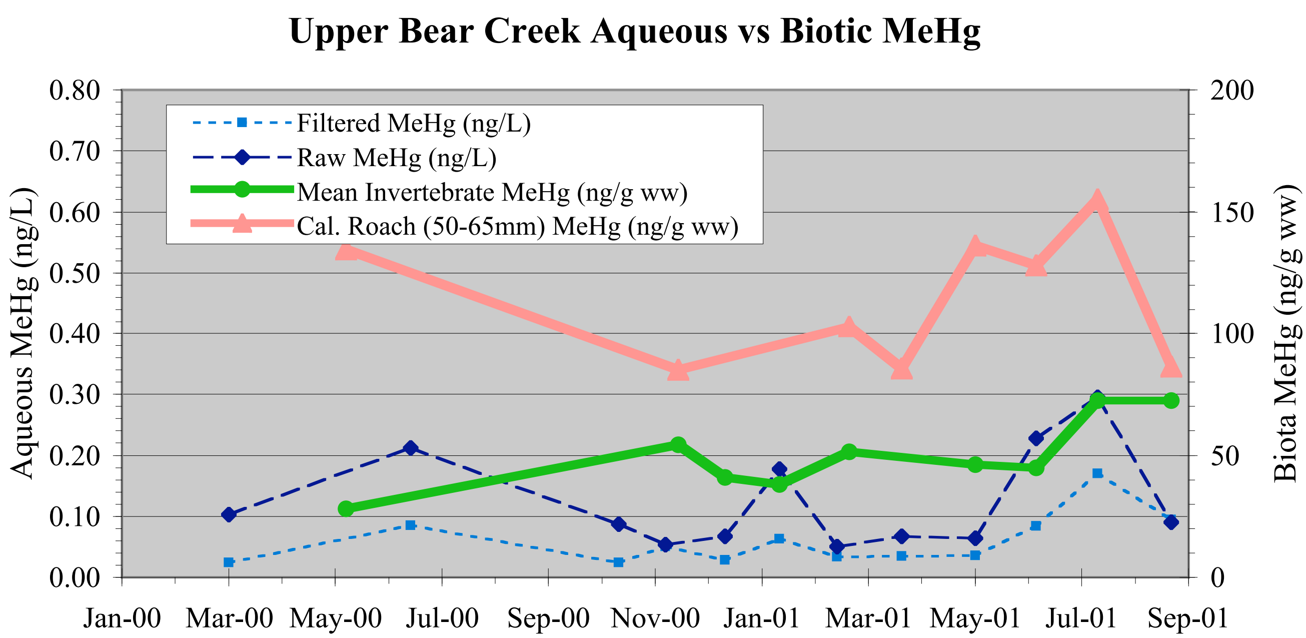


Table 5.
Early winter influence of Bear Creek inflows on main stem Cache Creek invertebrates.
(samples collected 15-Feb-2001)
(i.s.) = insufficient sample for analysis

Insect Family	n	Avg Size *(mm)*	THg *(ww ng/g)*	MeHg *(ww ng/g)*	% MeHg	$^{15}N/^{14}N$ ratio	$^{13}C/^{12}C$ ratio
Cache Creek above Bear Creek confluence							
Baetidae	150	7	17	6	35%	6.91	-28.71
Baetidae	150	7	16	9	58%	6.77	-28.68
Baetidae	150	7	16	12	75%	6.63	-28.73
		Means:	**16**	**9**	**56%**	**6.77**	**-28.71**
		95% Conf. Intervals:	±0.9	±3.3	±22%	±0.16	±0.03
Hydropsychidae	18	10	45	(i.s.)	(i.s.)	6.00	-24.79
Corydalidae	7	42	32	26	85%	5.54	-23.16
Bear Creek near confluence with Cache Creek							
Hydropsychidae	100	12	250	74	30%	22.64	-28.16
Corydalidae	6	45	615	652	106%	15.14	-28.2
Cache Creek 0.7 km downstream of Bear Creek confluence, following mixing in rapids							
Baetidae	100	7	32	(i.s.)	(i.s.)	13.51	-28.26
Baetidae	100	7	17	(i.s.)	(i.s.)	13.25	-28.74
Baetidae	100	7	32	18	57%	12.87	-28.76
		Means:	**27**			**13.21**	**-28.59**
		95% Conf. Intervals:	±9.5			±0.36	±0.32
Hydropsychidae	60	12	91	28	30%	13.16	-25.45
Corydalidae	6	45	32	37	115%	6.81	-23.26

Small and Juvenile Fish

Small fish were studied as additional bioindicators of potential spatial and seasonal variation in MeHg exposure. They provided a fish-based, relatively available supplement to benthic aquatic insects. Small fish were also of interest as a food item for piscivorous fish, re-human exposure, and as direct food items for piscivorous birds. A variety of locally prevalent species were sampled. Analyses and data reduction focused on ecologically similar species which could be compared across the watershed and which were representative of primary localized food items for piscivorous fish and birds. In the tributary streams, native California roach (*Hesperoleucas symmetricus*) greatly dominated the small fish fauna. California roach were replaced ecologically by other species in the main stem of Cache Creek, including native speckled dace (*Rhinichthys osculus*) at Rumsey and red shiner (*Notropis lutrensis*) further downstream. Reduced data, by site and date, are presented in Figure 17 (roach/dace/shiners). Small fish were available from a subset of the invertebrate sites and were typically collected less frequently than the invertebrates. General spatial trends in MeHg were similar to those noted among the other biotic and aqueous Hg parameters. Sites directly downstream of Hg point sources, together with mid-Bear Creek, exhibited dramatically greater small fish MeHg than all other sites, with mean wet weight concentrations of 400-600 n/g (0.40-0.60 ppm) and levels during the study period to 950 ng/g (0.95 ppm). Upper Bear Creek was again generally elevated relative to the other lower Hg sites on same dates. Variations in the apparent spatial trends between main stem Cache Creek sites, relative to those seen in the other studied parameters, may be largely a function of the three different small fish species being imperfect ecological analogs. Mean roach MeHg in upper Bear Creek and roach analog small fish in lower main stem Cache Creek ranged from 110 to 150 ng/g (0.11-0.15 ppm), with individual samples to over 180 ng/g (0.18 ppm). These concentrations were all above the 100 ng/g level suggested as a guideline for wildlife species which consume small fish, such as herons and other piscivorous birds (Eisler 1987). In contrast, roach from relative control sites Middle Creek and North Fork Cache Creek averaged 30-70 ng/g (0.03-0.07 ppm).

Methyl Hg percentage of total Hg in the whole fish composite samples is summarized for the primary study species in Figure 18. Methyl percentages were very similar in the juvenile Sacramento sucker, California roach, and speckled dace data sets, with mean levels of 87-92%. The red shiner whole body composites generated analytical results indicating over 100% MeHg (103%), likely due to slight analytical over- or under-recoveries. In any case, the whole body fish samples contained high proportions of MeHg.

In Figures 16a-16e (in the previous section), small fish time series information from the primary index sites is plotted together with invertebrate MeHg and aqueous MeHg. Within each site, the small fish data generally tracked both the invertebrate and aqueous MeHg trends. Similar to the invertebrates, the small fish exhibited fairly dramatic seasonal increases and decreases in MeHg of approximately 100% and 50% respectively. As mentioned above for the invertebrates, these apparent changes could be due to a combination of actual concentration changes in individual organisms and new recruitment under changing aqueous exposure conditions. Though the overall seasonal patterns at each site were similar to those of corresponding invertebrates, it is interesting that the small fish MeHg *concentrations* were approximately 2-3 times greater than corresponding invertebrate MeHg at the low-moderate Hg sites, while at highly Hg-elevated mid Bear Creek the invertebrate and corresponding small fish trends were nearly identical in concentration. Possible explanations include a different relative trophic level for the fish or insects at mid Bear Creek or the greater importance of direct MeHg uptake from the water at this high exposure site.

The relationships between the primary aqueous Hg fractions and small fish MeHg are summarized for the watershed as a whole in Figure 19 (log-log power regressions) and at the individual sites mid Bear Creek and Cache Creek at Rumsey in Figures 20 and 21 (linear correlations). Corresponding aqueous data were chosen as described above for the benthic invertebrates. Patterns were very similar to those discussed above for invertebrates. On a whole watershed basis, apparent correlations with small fish MeHg were seen in the ascending order of aqueous raw THg (log-log power regression r^2 of 0.25), raw MeHg (0.49), filtered THg (0.58), and filtered MeHg (0.62). Again, at individual sites, these apparent relationships completely broke down for both raw and filtered aqueous THg. Filtered MeHg, which showed the greatest overall apparent correlation with both invertebrate and small fish MeHg on a whole watershed basis, demonstrated no discernible relationship with small fish MeHg at the Cache Creek at Rumsey site, though only six data comparisons were available. At mid Bear Creek, filtered MeHg vs small fish MeHg had a higher linear r^2 of 0.38 (n=9). For both index sites, raw MeHg again demonstrated the strongest aqueous Hg relationship with same-site small fish MeHg (r^2 = 0.45 at Rumsey, n=6, and 0.89 at mid Bear Creek, n=9, $p < 0.0001$).

As seen for the benthic invertebrates, the relationship between aqueous raw MeHg and small fish MeHg was relatively consistent within each of these representative sites, but quite different between them. An aqueous MeHg exposure of 0.5 ng/L corresponded to a small fish MeHg concentration of 497 ng/g at Mid Bear Creek, while an equivalent aqueous exposure level at the Cache Creek at Rumsey site corresponded to 104 ng/g in the small fish. Again, this highlights the importance of developing site-specific relationships for waters which may exhibit widely varying mercury cycling patterns.

Figure 22 compares small fish MeHg to corresponding invertebrate MeHg from same site-samplings. A strong (0.84) power regression for the watershed as a whole must be somewhat qualified due to another bi-modal distribution, despite log-log treatment of the data. At individual sites, data were more normally distributed. General positive relationships between the two Hg bioindicator types were seen for mid Bear Creek (0.50) and Cache Creek at Rumsey (0.56), while the upper Bear Creek site demonstrated no apparent correlation. It is likely that the aquatic insects and small fish bioaccumulate (and potentially depurate) MeHg at different rates and that direct comparison of the two at identical time points may not be appropriate. The time series data (Figures 16a-e) indicate a general correspondence between aquatic insect and small fish MeHg in most cases, with apparent delays between the temporal trends of the two in others.

Figure 17.
Cache Creek watershed small fish methylmercury concentration vs. site and date.
(each bar represents the mean of three multiple-individual whole body composites)

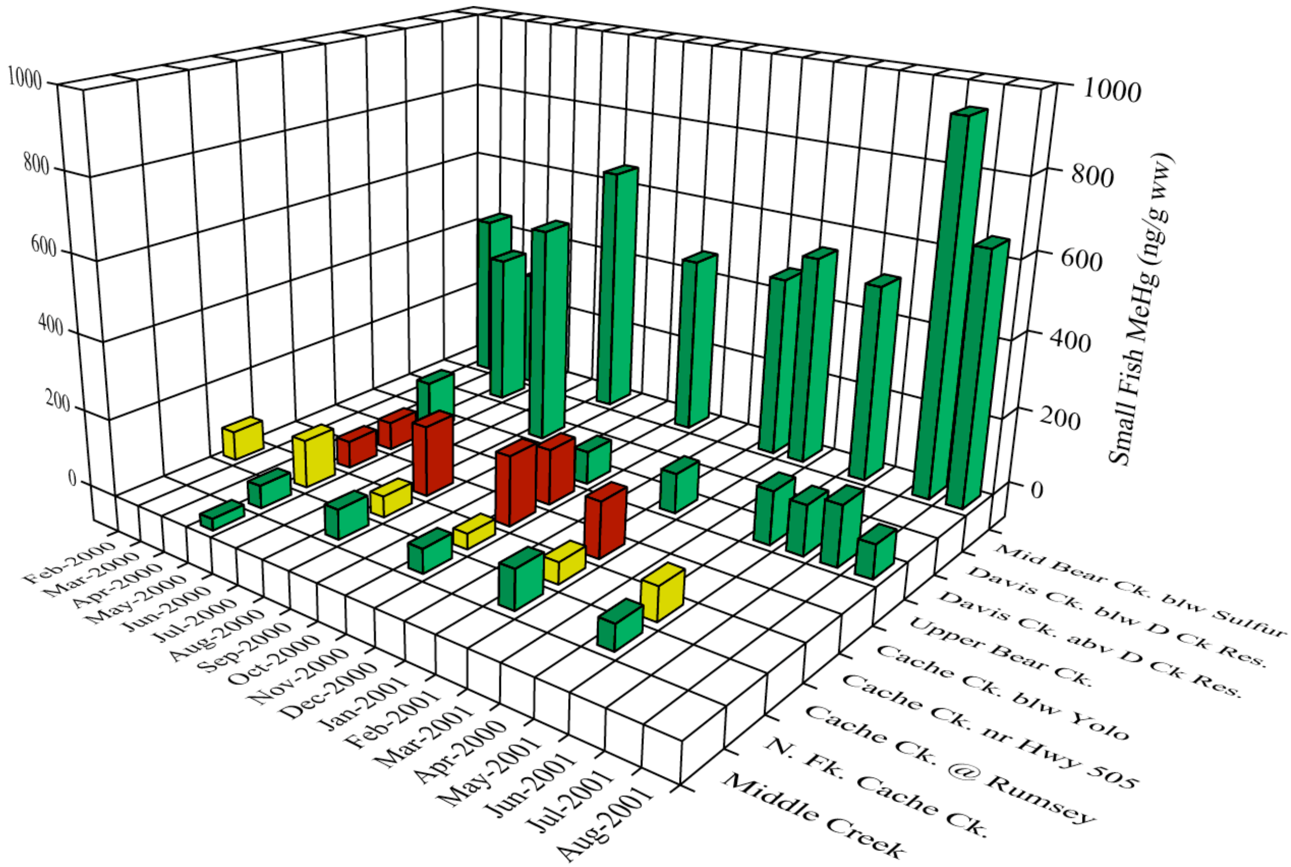


Figure 18.
Small fish methyl Hg % of total Hg in Cache Creek watershed samples.
(mean MeHg % ± 95% confidence intervals)

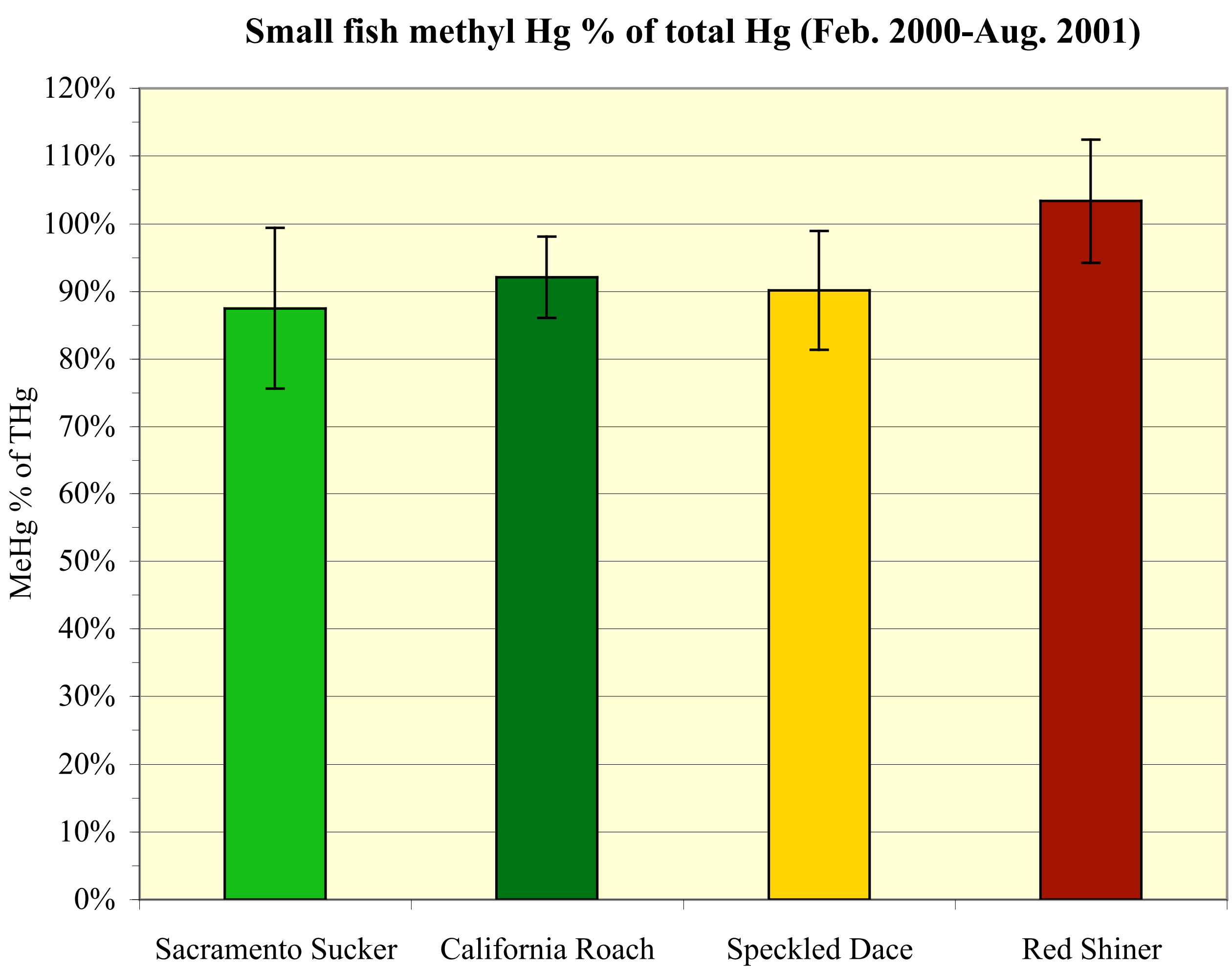


Figure 19.
Log/log (power) regressions of aqueous Hg fractions vs small fish composite MeHg.
Data from all Cache Creek watershed sites combined.
(Includes data from three species occupying similar trophic levels: California roach, speckled dace, red shiners)
(Corresponding water data from the most representative samplings prior to small fish collection)

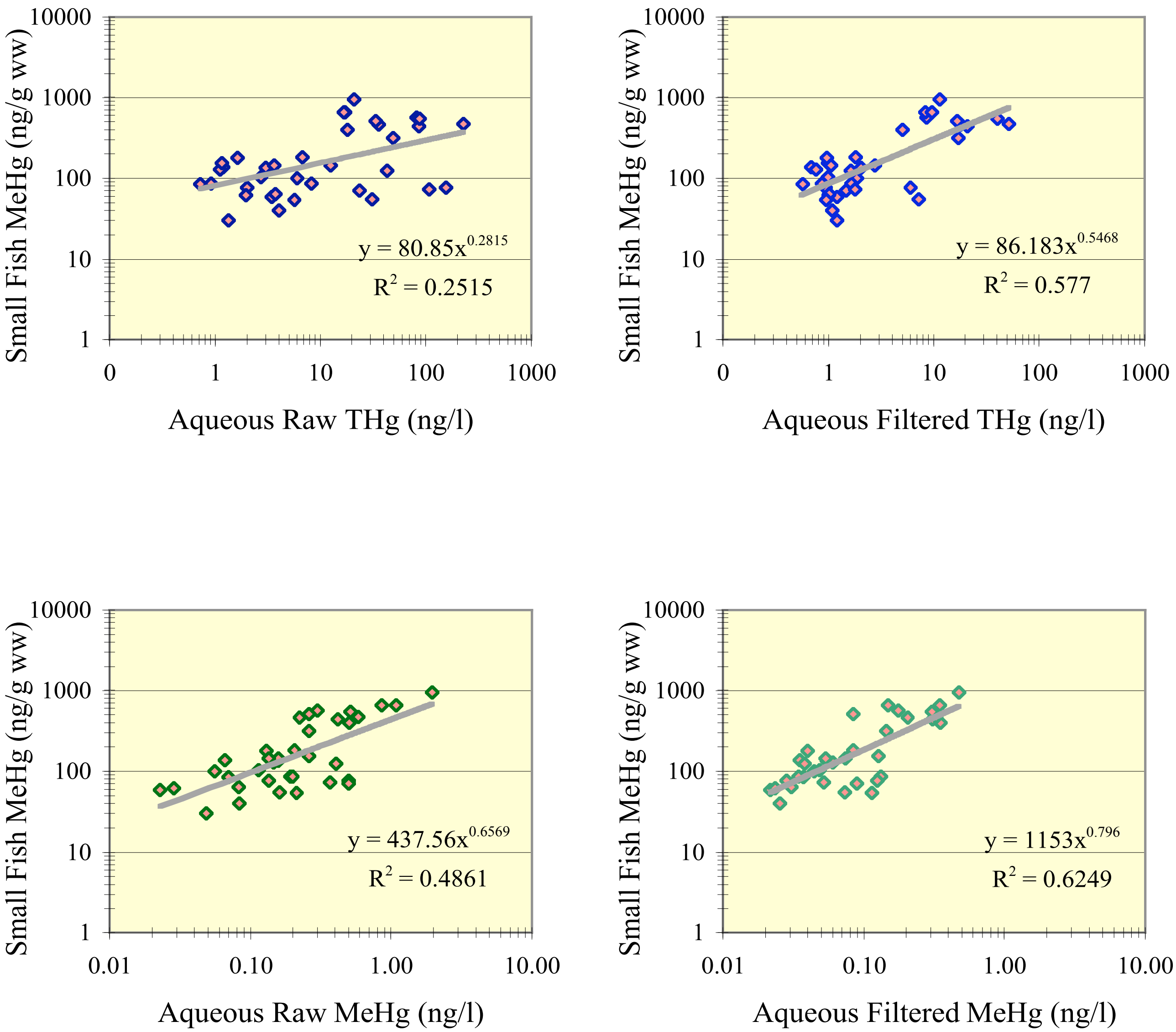


Figure 20.
Linear regressions of aqueous Hg fractions vs California roach MeHg at an individual site: Middle Bear Creek below Sulfur Creek.
(each data point represents the mean of three multiple individual whole body California roach composites)
(corresponding water data from the most representative samplings prior to small fish collection)

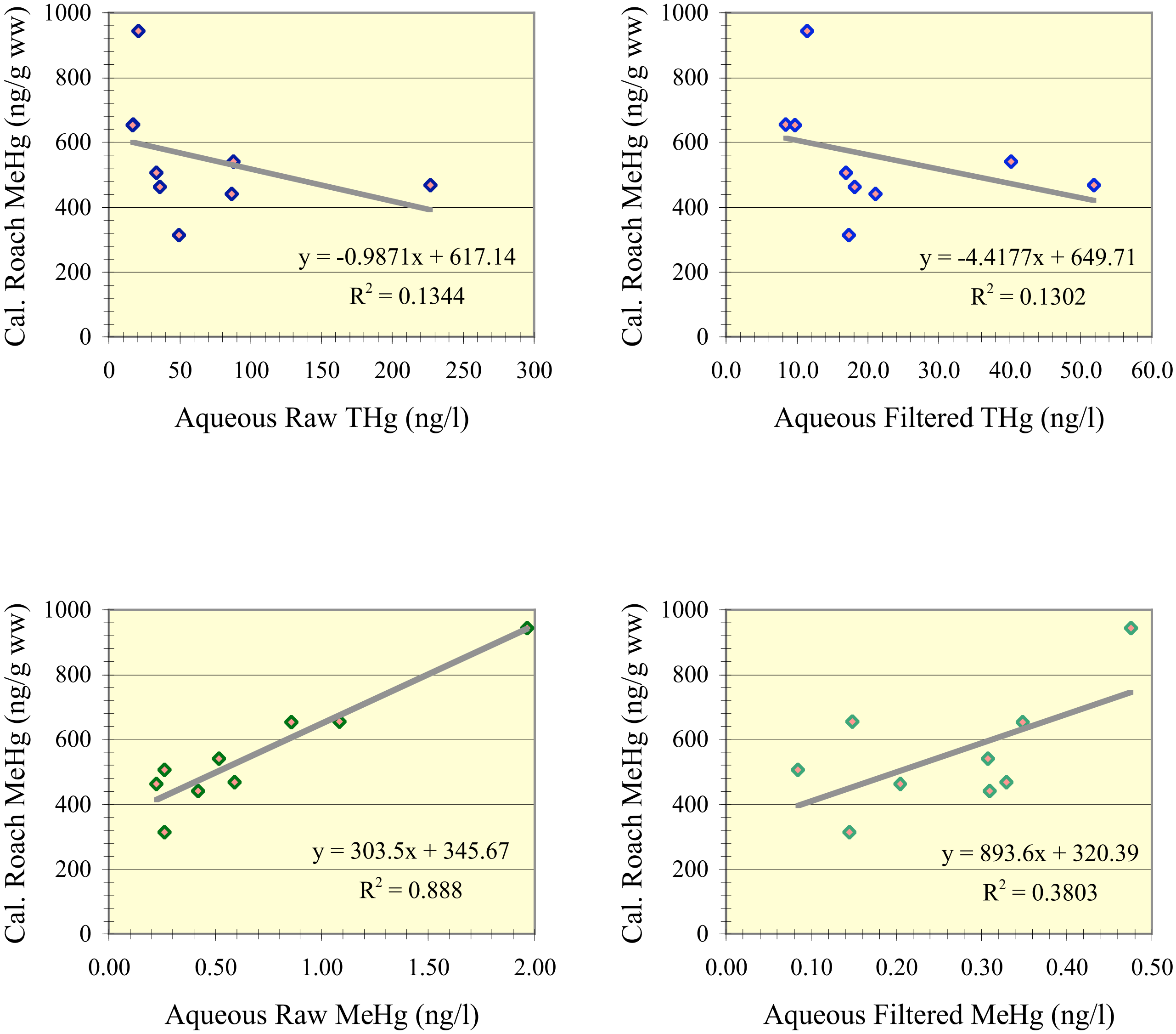


Figure 21.
Linear regressions of aqueous Hg fractions vs speckled dace MeHg at an individual site: Cache Creek at Rumsey.
(each data point represents the mean of three multiple individual whole body speckled dace composites)
(corresponding water data from the most representative samplings prior to small fish collection)

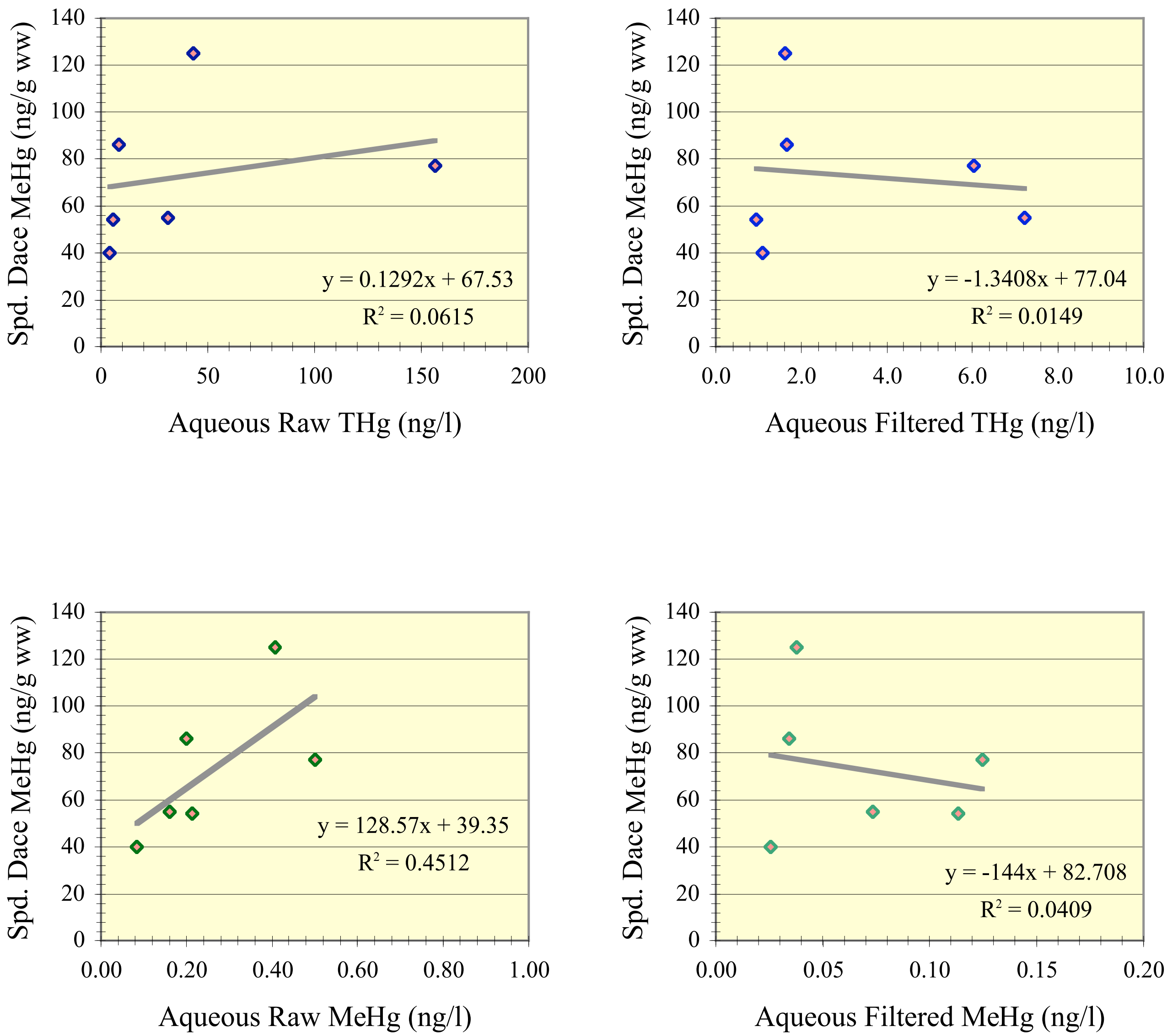
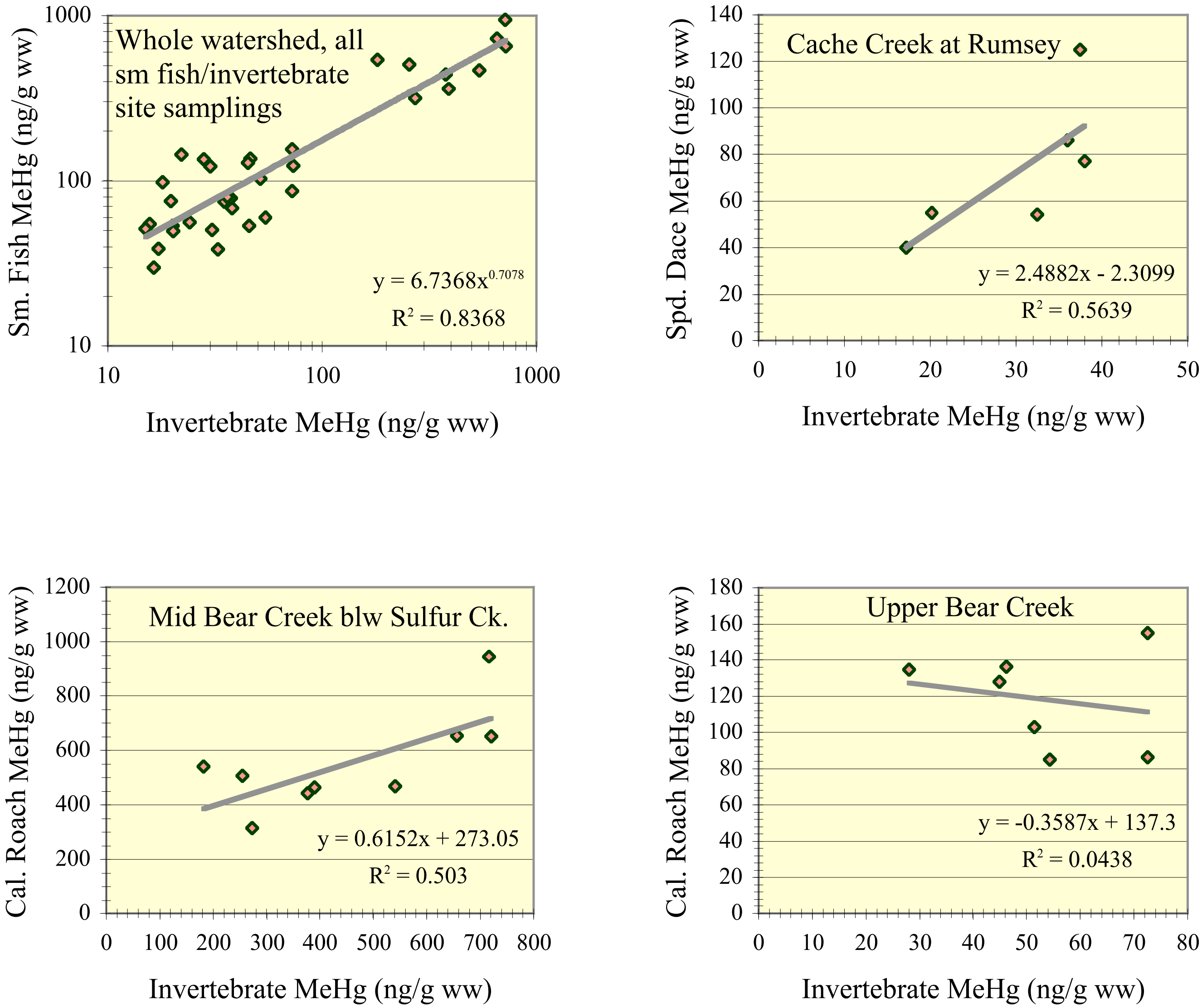


Figure 22.
Regressions of MeHg in corresponding invertebrate vs small fish bioindicator samples.
(invertebrate MeHg = mean site-sampling MeHg of Hydropsyche and predatory taxa composites)
(small fish MeHg = mean of three multiple individual whole body composites)



Pooled data approaches

In the above sections, we showed that MeHg in water and low trophic level biota varied, often dramatically, with seasonal cycles. It was possible to link these temporal changes and thus develop relationships between aqueous and bioindicator MeHg on a site-specific basis. However, a different approach was necessary to investigate potential MeHg linkages to large fish. For a number of reasons, it was neither feasible nor appropriate to sample large fish frequently enough at each sampling site, across varying exposure levels, to develop site-specific relationships. Repeat samplings would have depleted local populations. In the main stem of Cache Creek, effective sampling was only possible during brief winter periods when reservoir releases were halted and localized storm runoff was minimal. Additionally, large fish do not typically exhibit dramatic seasonal changes in muscle Hg content that would facilitate the development of site-specific relationships. Most importantly, as large fish accumulate their Hg gradually over a period of years, we felt that it was most appropriate to compare the adult fish data to aquatic insect, small fish, or aqueous MeHg data that were pooled over a substantial time period for each site. The large fish were therefore characterized for muscle Hg with single intensive collections, conducted at the midpoint of the study. For each site, size-normalized large fish data were then compared to aqueous and bioindicator MeHg data that were pooled across the entire two year project period. Temporally pooled site data are presented for aqueous unfiltered MeHg and the primary project biota in Table 6.

This approach reduces data comparisons for each site and species to single points and ratios, providing limited information for potential site-specific relationships (see BAF discussion below). However, because the sampling design incorporated sites spanning a range of MeHg exposure conditions, it was possible to estimate general predictive relationships for the watershed as a whole, using regressions of pooled data comparisons from a range of sites. The most meaningful of these are presented in Figures 23 (adult piscivorous fish), 24 (adult Sacramento suckers), and 25 (bioindicators).

The data representing each site can be temporally pooled in a number of ways, particularly for aqueous parameters. In Table 6, aqueous unfiltered MeHg is presented, pooled by site, in terms of median concentrations, overall mean concentrations, and averaged by season. The use of median concentrations was investigated as an approach to lessen the impact of potential outlier values. However, project sampling was conducted specifically to avoid outlier (event) sampling and to instead characterize general exposure conditions of each sampling period. Use of medians had the effect of removing all influence of very high-concentration data that were typical of some sites during important periods of each year. Therefore, temporal pooling with overall mean concentrations was preferred. We also investigated the possibility that exposure levels at certain times of year might be more important to bioaccumulation than exposure levels at other times of year. In particular, it has been hypothesized that adult fish MeHg bioaccumulation may be driven primarily by aqueous and dietary exposure during an "active" as opposed to "dormant" period of each year. This may indeed be the case for introduced, non-native species such as largemouth and smallmouth bass in California, which often exhibit their primary growth and activity during the warm period of the year (April-October) as compared to the cooler months of November-March. This seasonal pattern evolved in more continental regions of North America where very low winter temperatures and ice cover significantly curtail food availability and activity. Pooled aqueous MeHg data in Table 6 are therefore also presented averaged relative to these two seasonal periods. We could not justify the use of this approach, however, as (1) the bioindicator vs water studies were conducted year-round and demonstrated continuous and associated fluctuation, and (2) the majority of species utilized in this work were natives and were thus adapted to the mild California climate and year-round conditions favorable to growth and activity. In any case, all of the various pooling techniques were investigated and the strongest regressions were demonstrated using concentrations averaged across the whole, two year project period. This approach to temporal pooling of site data is used in the regression plots presented in Figures 23-25.

Sites with sufficient large fish data included most main stem Cache Creek sites, together with mid and upper Bear Creek sites. Mid Bear Creek fish Hg was so highly elevated above all of the other fish sites that its inclusion in regressions led to artificially strong correlations, independent of actual trends among the majority of sites. Therefore, mid Bear Creek was omitted from these plots so as to focus on potential trends among the majority of sites and among concentration ranges which intersected Hg criterion levels.

The pooled site data approach, using sites spanning a range of exposure levels, generated moderate to strong linkages among the previously identified primary monitoring parameters (unfiltered aqueous MeHg, bioindicator MeHg, and large fish muscle Hg). While biota vs biota relationships were more widely applicable spatially, linkages between the key aqueous parameter (unfiltered MeHg) and biota were found to be most consistent within groups of sites with similar water quality characteristics. In Figures 23-25, regressions of aqueous MeHg vs the various biota exhibited no statistical relationship when the upper Bear Creek site was included. However, substantial relationships were apparent among the remaining sites, as demonstrated in the adjacent regression plots which exclude the upper Bear Creek site in each of the figures. For this suite of sites, which includes the entire main stem and North Fork of Cache Creek, these regressions provide general predictive linkages between aqueous MeHg and bioaccumulation. These equations can be used to estimate changes in biotic Hg under potentially altered aqueous MeHg exposure regimes. They can also be used to estimate the aqueous thresholds that correspond to criterion bioaccumulation levels for this portion of the watershed. For piscivorous fish of the 270 mm normalized size, muscle Hg at the 300 ng/g USEPA criterion level corresponded to a mean annual aqueous MeHg concentration of 0.16 ng/L. Sacramento suckers, at the 290 mm normalization size used for that species, were predicted to reach the 300 ng/g criterion level with a mean aqueous MeHg level of 0.45 ng/L. It is important to note that fish of different sizes than those used for inter-site normalization would be predicted to reach the 300 ng/g criterion concentration at different underlying mean aqueous MeHg exposures. In particular, bass of legal angling size (12” = 305 mm) would intersect the criterion tissue level under lower aqueous exposure concentrations, as would larger Sacramento suckers typical of those targeted by eagles. However, the 270 mm, age 3 normalizing size used for piscivorous fish in the watershed was consistent with the nationwide pilot study conducted by USGS (Brumbaugh et al. 2001) which also focused on age 3 bass. That study developed a general, nationwide relationship which predicted the accumulation of criterion level concentrations in age 3 bass to correspond with an aqueous MeHg concentration of only 0.06 ng/L (vs 0.16 ng/L in this study). One possible explanation for the apparently weaker bioaccumulation efficiency noted in the main stem Cache Creek sites is that the USGS national pilot study relied on single date characterizations of aqueous MeHg, conducted in the summer and under minimal suspended solids loads. The main stem Cache Creek sites exhibiting relatively consistent aqueous vs biotic MeHg relationships were dominated in the warm season by reservoir releases containing high levels of biotic-based turbidity and, in the cool season, by storm runoff containing high levels of sediment-based turbidity, both of which could influence the relative efficiency of MeHg bioaccumulation. It is critical to note that very different relationships were seen between aqueous and biotic MeHg in some of the upstream tributaries, with comparable aqueous MeHg concentrations corresponding to far higher levels of bioaccumulation. This type of site-specific difference could be assessed using lower trophic level biota, as discussed below.

While pooled data approaches were necessary to estimate general linkages to large fish Hg, it is important to recognize the limitations of such highly reduced data techniques, as compared to the development of site-specific relationships. In Figure 26, previously discussed site-specific relationships between aqueous MeHg and bioindicator MeHg are superimposed for two divergent index sites, mid Bear Creek and Cache Creek at Rumsey. While aqueous concentrations overlapped between the two representative sites, biotic accumulation followed entirely different patterns. Pooled data approaches are useful for estimating general linkages to large fish Hg, particularly among sites with similar water quality characteristics, but site-specific relationships can provide a strong addition to this type of information.

The temporally-pooled reduced data also provide general relationships between the primary biotic samples. Comparable regression plots between the large fish species and the two main bioindicator types are presented below the aqueous regressions in Figures 23-24. Some strong relationships were apparent. An important distinction from the aqueous:large fish relationships was that the linkages to bioindicator organism MeHg were relatively consistent across larger regions of the watershed. Note that the regressions with bioindicators include the upper Bear Creek site, which was anomalous in the comparisons with aqueous MeHg. Aquatic invertebrate and small fish sampling may offer a useful supplement or alternative to aqueous sampling in regulatory or remediation monitoring. The relationships generated in this study indicate that criterion levels of fillet muscle Hg in age 3 piscivorous fish, in addition to linking with a mean aqueous MeHg concentration of 0.16 ng/L, may also be generally associated with mean benthic invertebrate MeHg concentrations of 27 ng/g (0.027 ppm) and mean small omnivorous fish concentrations of 56 ng/g (0.056 ppm).

Temporally-pooled site data for MeHg in aquatic insects, small fish, Sacramento sucker muscle, and piscivorous fish muscle are displayed graphically on a map of the region in Figure 27. In this figure, a general correspondence is apparent in MeHg spatial trends of co-occurring biota. Site-specific variability around the general trends are discussed below with bioaccumulation factors.

In addition to the human health-based criterion of 300 ng/g in angling-sized fish muscle, another important consideration is wildlife health relative to the consumption of fish. Because piscivorous wildlife are generally obligate fish eaters, consuming fish far more consistently than typical humans, a lower threshold MeHg exposure level is supported. While the establishment of this wildlife criterion level is currently under revision, a concentration of 100 ng/g (0.100 ppm) has been used since the 1980s as a suggested criterion level for MeHg in small fish, relative to consumption by piscivorous birds such as herons, egrets, and kingfishers (Eisler 1987). Across the relatively consistent suite of main stem and North Fork Cache Creek sites, a 100 ng/g small fish concentration corresponded to a mean aqueous MeHg concentration of 0.32 ng/L and a mean benthic invertebrate concentration of 41 ng/g. As both of these levels were higher than corresponding concentrations relative to the large fish criterion levels, management for the large fish concentrations could be expected to be protective of the wildlife guideline in this portion of the watershed, if the wildlife guideline is kept at the 100 ng g^{-1} level. Again, it is critical to note the very different relationships exhibited between aqueous and biotic MeHg in some of the upper tributaries (Fig. 26). While pooled data from the main stem suite of sites estimated an aqueous MeHg concentration of 0.32 ng/L to correspond to a small fish bioaccumulation level of 100 ng/g, this same aqueous concentration at mid Bear Creek corresponded to a small fish MeHg concentration of 443 ng/g, over four times greater and well over even the large fish muscle Hg criterion level. Localized differences in the aqueous:biotic MeHg relationship may be best addressed through the development of site-specific linkages over time, but may also be estimated with a bioaccumulation factor approach (below).

Bioaccumulation Factors (BAFs)

Another approach to pooling site data in order to indicate general relationships is the calculation of bioaccumulation factors, or BAFs, which reduce individual site data to single ratios (Table 7). Bioaccumulation factors quantify the proportion of bioaccumulated MeHg (ng/g = ppb, wet weight) relative to the corresponding aqueous concentration of MeHg (ng/g = ng/ml = µg/L = ppb), resulting in values in the range of 10,000 to over 1,000,000. Ratios have been calculated relative to both unfiltered and filtered (0.45 µm) aqueous MeHg. Ratios with aqueous filtered MeHg are included for comparison to some studies in the literature which utilized filtered aqueous MeHg. However, this study found biotic MeHg to correspond most consistently with unfiltered (raw) MeHg. Therefore, in Table 7, the calculated BAF biotic:aqueous ratios of greatest significance are those relative to unfiltered MeHg.

Consistent with standard presentations of this type of data, the values are displayed in log form, such that every integer increase of one unit represents a ten-fold increase in the concentration ratio. A BAF of 5 represents 10^5 or 100,000, a BAF of 6 represents 10^6 or 1,000,000. Corresponding expanded, non-log, arithmetic values are also included. Ratios are presented for predatory invertebrates, small omnivorous fish, and top predator fish, relative to aqueous MeHg fractions. For completeness, BAF ratios are also presented for MeHg in top predator fish relative to the two main lower trophic level bioindicator types (predatory benthic invertebrates and small omnivorous fish). Site ratios that included the large fish required the pooling of site data across the project period, as described in the previous section. Site ratios between bioindicator organisms and aqueous MeHg could be further differentiated by averaging numerous linked bioindicator and aqueous MeHg data pairs, providing mean BAF ratios together with 95% statistical confidence intervals. For context, the site data are arranged in the tables in general order of increasing absolute invertebrate MeHg. While BAF values are presented for each comparison and site containing the given parameters, it is important to recognize that these ratios are highly reduced and simplified representations of often complex relationships at each site. They should be used in close concert with the pooled and, particularly, the site-specific regression results discussed above.

The aquatic invertebrate bioaccumulation factors, relative to unfiltered aqueous MeHg, ranged between 10^5 and 10^6 (or BAF = 5.15-6.00), a seven-fold absolute difference between lowest and highest (141,000-1,000,000). This indicates that the relationship between aqueous MeHg and invertebrate MeHg was not entirely consistent among the varied sites throughout the watershed. Some of the differences were statistically significant, including ratios for mid Bear Creek (5.91 ± 0.15) vs Cache Creek at Rumsey (5.22 ± 0.18). The highest bioaccumulation factors (5.68-6.00) occurred at the near-mine, highly Hg-elevated sites of Sulfur Creek, mid Bear Creek, Davis Creek above and below Davis Creek Reservoir, and Harley Gulch. This was somewhat in contrast with results of the USGS national pilot study (Brumbaugh et al 2001), which found a general *decrease* in BAF with increasing aqueous exposure concentration. However, these results were consistent with Lawson and Mason (1998), who reported bioaccumulation factors to increase disproportionately with aqueous MeHg concentration at the upper ranges of environmental concentrations. Interestingly, mid Bear Creek showed one of the overall greatest BAFs, higher even than the more concentrated upstream Hg source area of Sulfur Creek.

Another set of relatively elevated bioaccumulation factors, though to a lesser degree (5.51-5.60), occurred at the opposite end of the aqueous Hg concentration spectrum, at the relatively clear water, low Hg control sites Middle Creek, North Fork Cache Creek, and upper Bear Creek. This phenomenon of relatively elevated BAFs at the low end of environmental aqueous MeHg concentrations has been reported in several other studies, including the USGS national pilot study. Our own work throughout California has suggested a further, positive relationship between water clarity and bioaccumulation efficiency.

In a third, somewhat distinct grouping of watershed BAFs, the four main-stem Cache Creek sites, including the Clear Lake outflow, demonstrated the lowest ratios in the watershed, similar throughout at 5.15-5.31. We note that these sites were characterized by moderate aqueous Hg levels and high turbidity, both biotic-based from the reservoir releases and sediment-based during the winter storm seasons.

The observed differences in aqueous:biotic MeHg bioaccumulation factors between these three groups of sites could be due to actual differences in bioaccumulation kinetics (e.g. Lawson and Mason 1998), differences in biomass or suspended solids which could lead to relative biodilution or scavenging (e.g. Pickhardt et al. 2002), or other factors including food web complexity (e.g. Bowles et al. 2001). In any case, the seven-fold variation in MeHg bioaccumulation factors calculated for this watershed indicates that aqueous MeHg concentrations may not, alone, be entirely predictive of corresponding bioaccumulation. This is consistent with the pooled data and site-specific regression results discussed above, which indicated distinctly different aqueous:biotic MeHg relationships between different groups of sites.

Small fish were present at a subset of the sampling locations. BAF ratios with aqueous MeHg were calculated for ten sites; five contained extensive samplings sufficient to generate statistical confidence intervals. Among the intensively sampled sites, ratios relative to unfiltered aqueous MeHg ranged from 5.41 (257,000) to 5.99 (977,000), approximately a four-fold variation. Trends were generally consistent with those described above for benthic invertebrates. Similar to the invertebrates, lowest BAF ratios for the small fish were seen at the main stem Cache Creek sites. Notably elevated BAF ratios were again seen at high mercury sites such as mid Bear Creek and upper and lower Davis Creek. However, similarly elevated ratios were also seen at the clear water, low Hg control sites North Fork Cache Creek, Upper Bear Creek, and Middle Creek. The differences were statistically significant at the 95% level of confidence for both the mid and upper Bear Creek sites vs the Cache Creek index site at Rumsey.

Large predatory fish were available in sufficient numbers from six of the sites. As they contained greater concentrations of MeHg than the corresponding aquatic invertebrates and small fish, the subsequent BAF ratios with aqueous MeHg were greater. Ratios relative to unfiltered aqueous MeHg ranged from 6.10 (1,270,000) to 6.76 (5,620,000). Trends generally followed those noted above, with main stem Cache Creek sites exhibiting the lowest BAF ratios, and notably greater ratios at the single high Hg large fish site, mid Bear Creek, as well as at the low aqueous Hg sites of upper Bear Creek and North Fork Cache Creek.

BAF ratios are also presented for the adult predatory fish relative to the two primary lower trophic level project bioindicators, predatory aquatic invertebrates and small omnivorous fish. No clear trends were apparent among the sites. In contrast with the fish:water BAFs, these ratios were very small, ranging from log values of 0.82-1.20 for large fish vs invertebrates and 0.53-0.86 vs small fish. On an absolute basis, the ratios correspond to multipliers of 6-16 for invertebrates and 3-7 for small fish--as compared to hundreds of thousands or millions relative to water. This is consistent with the ideas of Mason et al. (1995, 1996, 2000) and Pickhardt et al. (2002) that the most important step in MeHg biomagnification is the step from water into the base of the food web, with subsequent trophic transfers following somewhat predictably. However, the 2-3 fold differences noted among these broadly estimated ratios between biota indicate that localized differences in the trophic transfer of MeHg may additionally influence the ultimate concentrations of MeHg accumulated by large fish. The most widely presented explanation for variation in the trophic transfer of MeHg is site-specific differences in the number of functional trophic levels (e.g. Bowles et al. 2001, Brumbaugh et al 2001). This again indicates the importance of developing site-specific relationships for use in specific regulatory and remediation monitoring.

Table 6.
Temporally pooled site data for aqueous unfiltered MeHg and MeHg in primary project biotic samples

(aqueous site data pooled by median concentrations, overall means, and by season)
(invertebrate and small fish bioindicator data averaged across the two year project period)
(adult fish muscle Hg data size-normalized by site from mid-project collections)

	Unfiltered Aqueous MeHg (ng/l)				Biotic MeHg (ng/g ww)			
Location	Project Median	Project Mean	Dry Season Mean (Apr-Oct)	Wet Season Mean (Nov-Mar)	Invert Mean	Sm Fish Mean	Sac Sucker Norm.	Pisc. Fish Norm.
North Fork Cache Creek	0.06	0.07	0.08	0.07	22	61	75	208
Cache Creek below Clear Lake	0.12	0.14	0.20	0.08	24	32	110	172
Cache Creek at Rumsey	0.18	0.22	0.24	0.20	27	59	150	425
Cache Creek below Yolo	0.26	0.29	0.36	0.21	34	99	210	
Cache Creek below Highway 505	0.15	0.26	0.42	0.13	38	99	180	472
Upper Bear Creek	0.09	0.12	0.16	0.09	50	115	295	700
Davis Creek bel. Davis Creek Res.	0.27	0.34	0.74	0.15	232	399		
Davis Creek abv. Davis Creek Res.	0.24	0.24	0.30	0.11	262	566		
Middle Bear Ck. bel. Sulfur Creek	0.35	0.62	0.80	0.41	467	550	1650	3050
Sulfur Creek	0.92	3.98	6.57	0.96	435			
Harley Gulch	0.82	2.50	5.59	0.64	719			

Figure 23.
Linear regressions of temporally pooled site data for aqueous MeHg, invertebrate MeHg, and small fish MeHg vs piscivorous large fish Hg (270 mm normalized, fillet muscle).

(Data from all Cache Creek watershed large fish sites except mid Bear Creek, which was dramatically higher in Hg and led to artificial correlations based on a single point)
(Including and excluding Upper Bear Creek in regressions with aqueous MeHg)

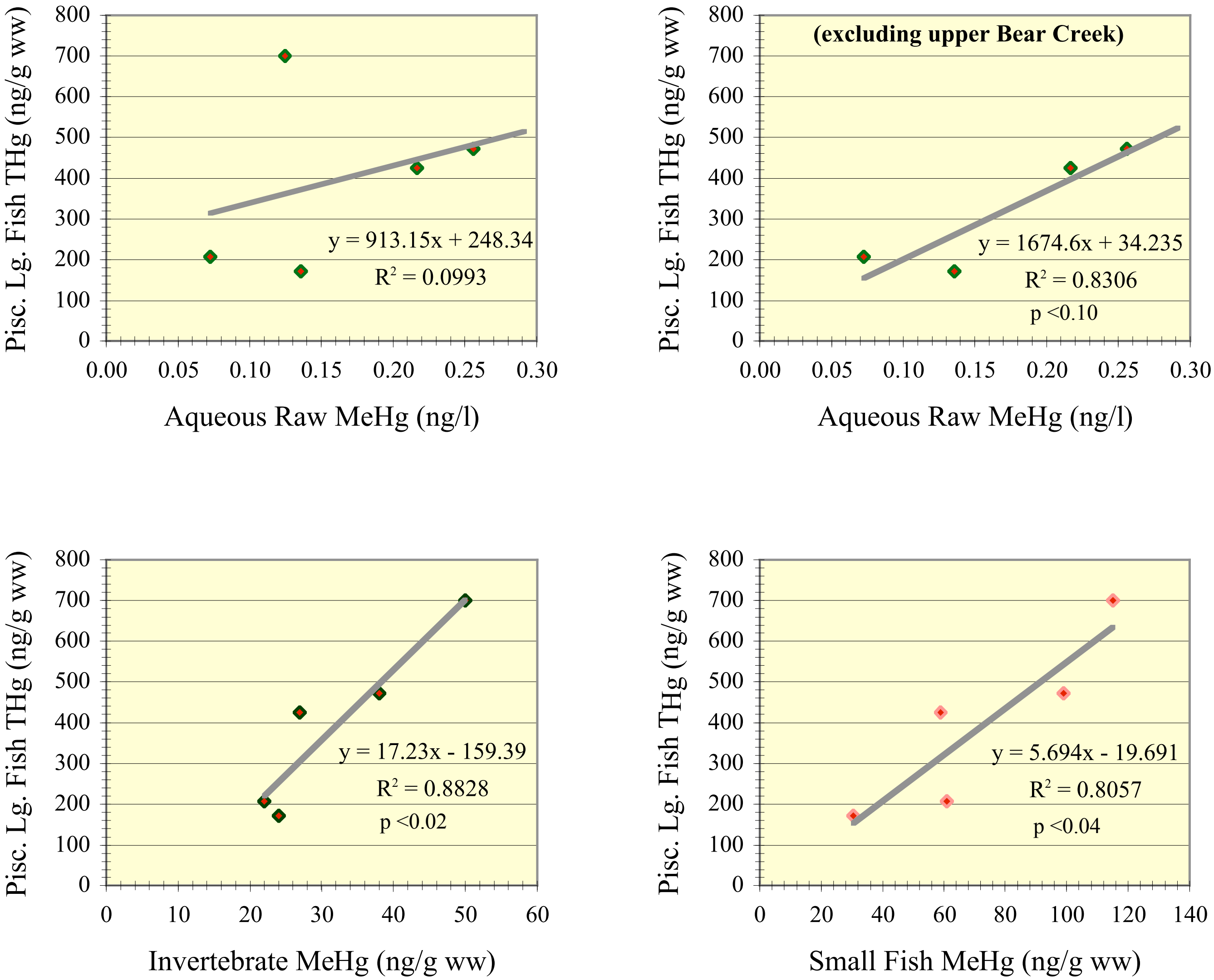


Figure 24.
Linear regressions of <u>temporally pooled site data</u> for aqueous MeHg, invertebrate MeHg, and small fish MeHg <u>vs Sacramento sucker Hg</u> (290 mm normalized, fillet muscle).

(Data from all Cache Creek watershed large fish sites except mid Bear Creek, which was dramatically higher in Hg and led to artificial correlations based on a single point)
(Including and excluding Upper Bear Creek in regressions with aqueous MeHg)

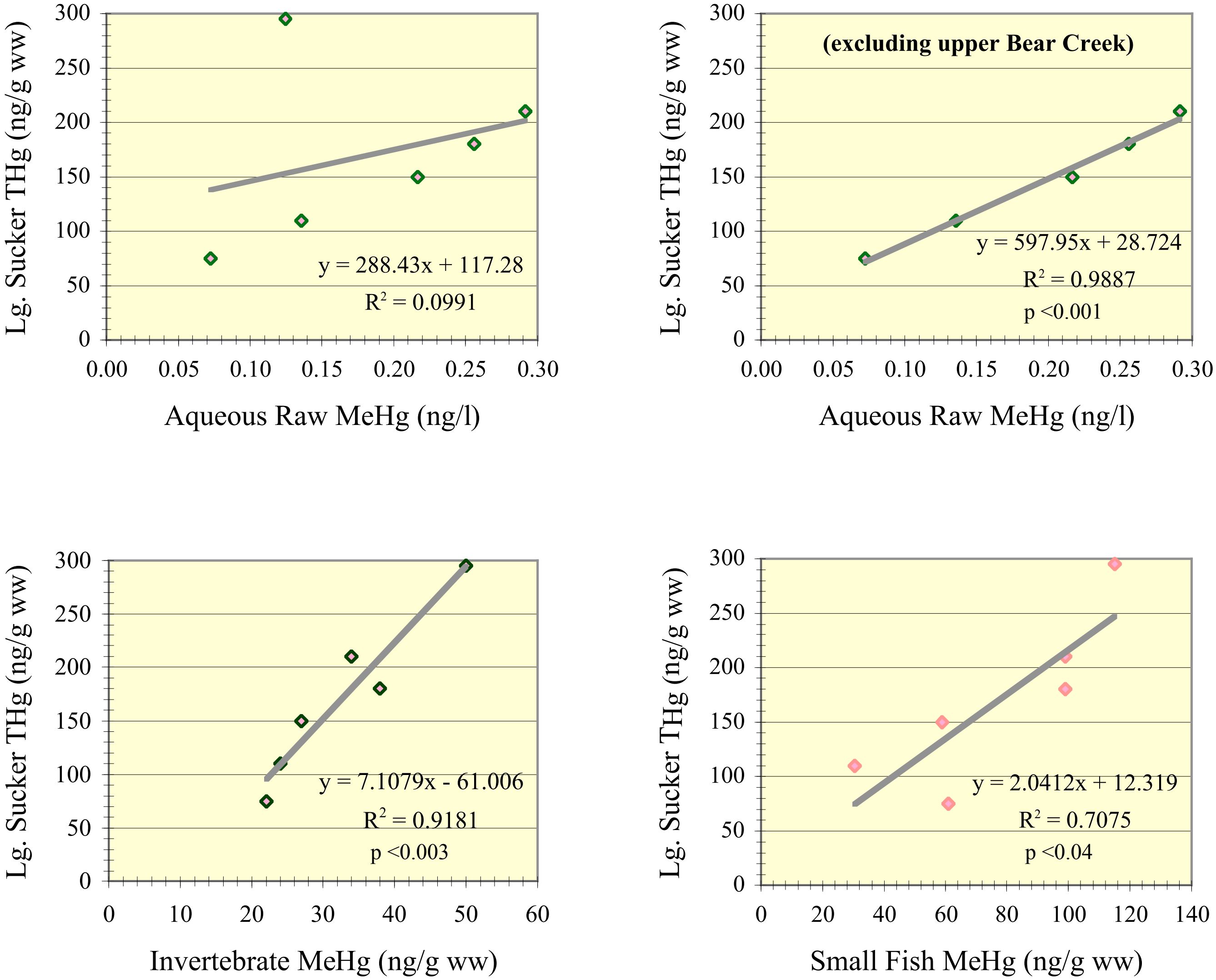


Figure 25.
Linear regressions of temporally pooled site data for aqueous MeHg vs invertebrate MeHg and small fish MeHg.

(Data from all Cache Creek watershed large fish sites except mid Bear Creek, which was dramatically higher in Hg and led to artificial correlations based on a single point)
(Including and excluding Upper Bear Creek in regressions with aqueous MeHg)

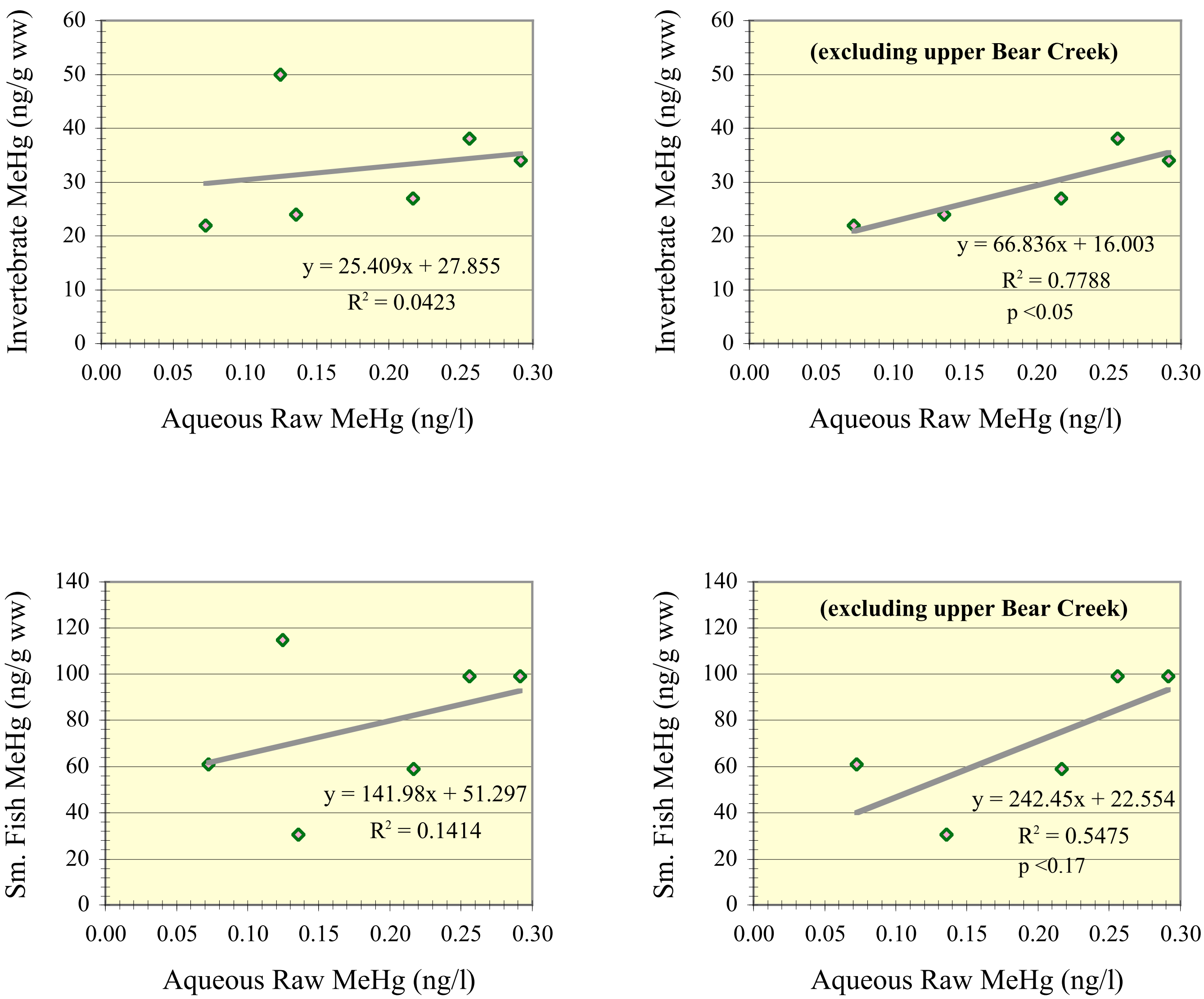
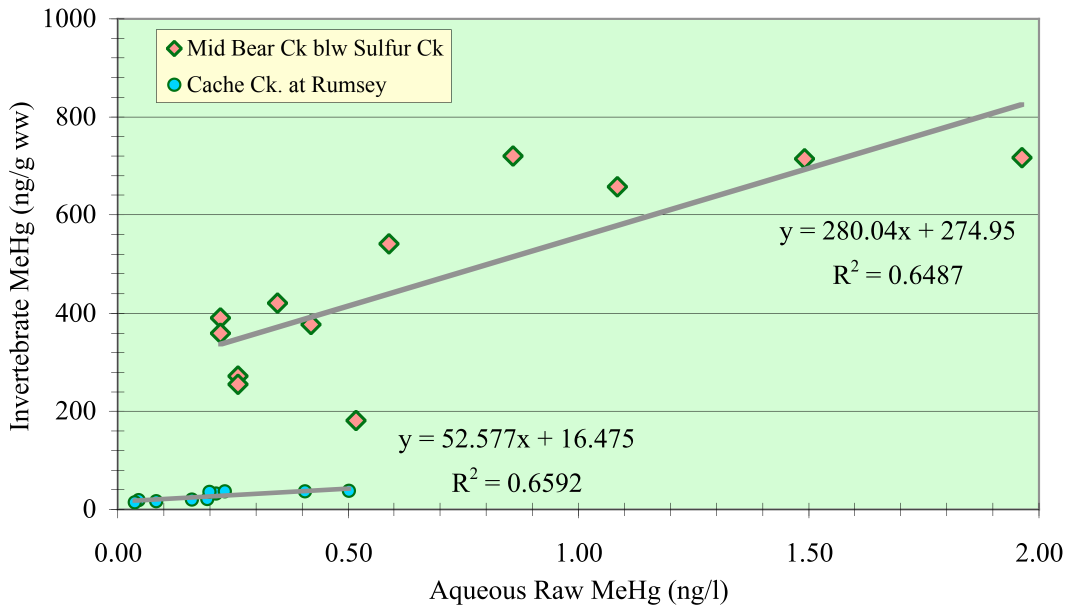


Figure 26.
<u>Comparative site-specific relationships</u> between aqueous raw MeHg and bioindicator organism MeHg at two sites representative of different regimes in the watershed.
(a) mixed predatory invertebrates
(b) small omnivorous fish



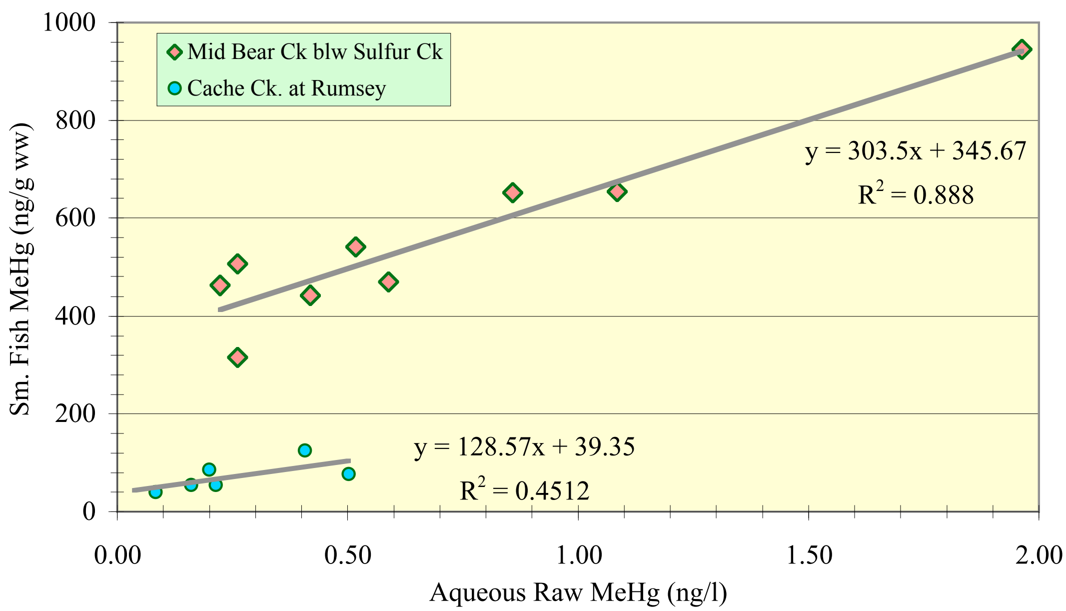


Figure 27.
Spatial distributions of condensed project MeHg biotic data for invertebrates, small fish, normalized Sacramento suckers, and normalized piscivorous fish.

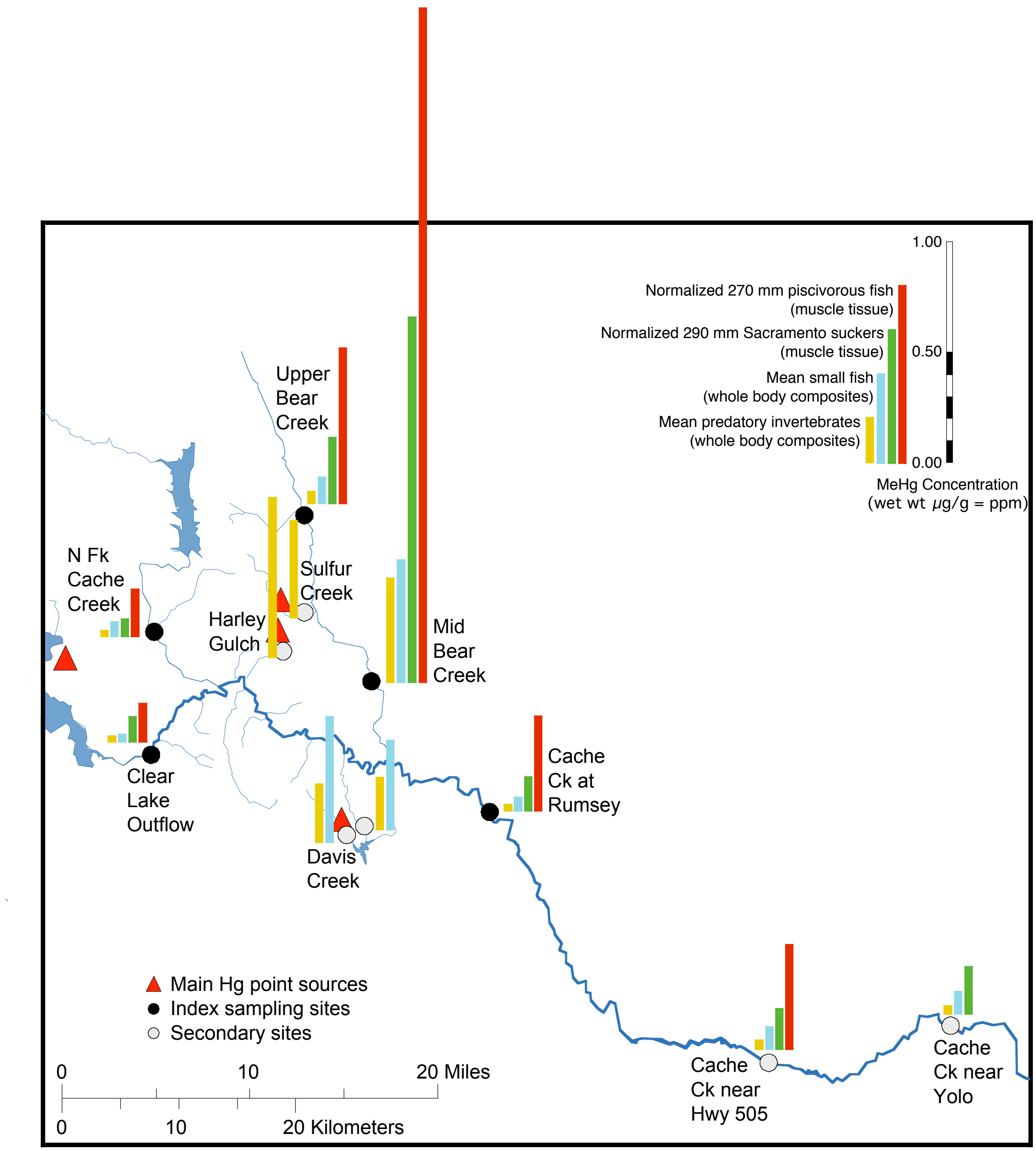


Table 7.
Summary Bioaccumulation Factors (BAFs) comparing biotic MeHg to corresponding aqueous MeHg and lower trophic level MeHg

(log and non-log ratios of biotic MeHg concentration to corresponding aqueous or lower trophic level concentration)
(sites arranged in order of increasing mean invertebrate MeHg)
(for invertebrates and small fish, corresponding aqueous data were averaged from water collected between 9 weeks prior to and 3 weeks following the corresponding biotic collection)

Location	Aquatic Invertebrate vs. Unfiltered MeHg BAF Log(Ci/Cw) ± 95% CI	Corresponding Non-Log Mean Ratio	Aquatic Invertebrate vs. Filtered MeHg BAF Log(Ci/Cw) ± 95% CI	Corresponding Non-Log Mean Ratio
Middle Creek	5.53	339,000		
North Fork Cache Creek	5.51 ± 0.33	324,000	5.81 ± 0.18	646,000
Cache Creek below Clear Lake	5.31 ± 0.14	204,000	5.67 ± 0.12	468,000
Cache Creek at Rumsey	5.22 ± 0.18	166,000	5.69 ± 0.16	490,000
Cache Creek below Highway 505	5.19 ± 0.54	155,000	5.68± 0.42	479,000
Cache Creek below Yolo	5.15	141,000	5.78	603,000
Upper Bear Creek	5.60 ± 0.16	398,000	5.93 ± 0.13	851,000
Davis Creek bel. Davis Creek Res.	5.66	457,000	5.82	661,000
Davis Creek abv. Davis Creek Res.	6.00 ± 1.37	1,000,000	6.18 ± 0.98	1,510,000
Middle Bear Ck. bel. Sulfur Creek	5.91 ± 0.15	813,000	6.29 ± 0.14	1,950,000
Sulfur Creek	5.68 ± 0.32	479,000	6.19 ± 0.36	1,550,000
Harley Gulch	5.77 ± 0.89	589,000	6.22 ± 0.48	1,660,000

Location	Small Fish vs. Unfiltered MeHg BAF Log(Csf/Cw) ± 95% CI	Corresponding Non-Log Mean Ratio	Small Fish vs. Filtered MeHg BAF Log(Csf/Cw) ± 95% CI	Corresponding Non-Log Mean Ratio
Middle Creek	5.79	617,000		
North Fork Cache Creek	5.97 ± 0.35	933,000	6.19 ± 0.29	1,550,000
Cache Creek below Clear Lake	5.07	117,000	5.760	575,000
Cache Creek at Rumsey	5.41 ± 0.22	257,000	6.00 ± 0.36	1,000,000
Cache Creek below Highway 505	5.64± 0.69	437,000	6.13 ± 0.36	1,350,000
Cache Creek below Yolo	5.59	389,000	6.22	1,660,000
Upper Bear Creek	5.93 ± 0.19	851,000	6.25 ± 0.23	1,780,000
Davis Creek bel. Davis Creek Res.	5.90	794,000	6.05	1,120,000
Davis Creek abv. Davis Creek Res.	6.27	1,860,000	6.51	3,240,000
Middle Bear Ck. bel. Sulfur Creek	5.99 ± 0.15	977,000	6.35 ± 0.18	2,240,000

(continued)

Table 7. *(continued)*

(for piscivorous large fish muscle Hg comparisons, corresponding aqueous and lower trophic level biota data were averaged across the entire project sampling period, 1/00 – 8/01)

Location	Norm. Pisc. Fish vs. Unfiltered MeHg BAF Log(Cf/Cw)	Corresponding Non-Log Ratio	Norm. Pisc. Fish vs. Filtered MeHg BAF Log(Cf/Cw)	Corresponding Non-Log Ratio
North Fork Cache Creek	6.459	2,880,000	6.695	4,950,000
Cache Creek below Clear Lake	6.103	1,270,000	6.538	3,450,000
Cache Creek at Rumsey	6.292	1,960,000	6.791	6,180,000
Cache Creek below Highway 505	6.209	1,620,000	6.871	7,430,000
Upper Bear Creek	6.750	5,620,000	7.063	11,600,000
Middle Bear Ck. bel. Sulfur Creek	6.691	4,910,000	7.096	12,500,000

Location	Norm. Pisc. Fish vs. Invertebrate BAF Log(Cf/Ci)	Corresponding Non-Log Ratio	Norm. Pisc. Fish vs. Small Fish BAF Log(Cf/Csf)	Corresponding Non-Log Ratio
North Fork Cache Creek	0.976	9.5	0.533	3.4
Cache Creek below Clear Lake	0.855	7.2	0.730	5.4
Cache Creek at Rumsey	1.197	15.7	0.858	7.2
Cache Creek below Highway 505	1.094	12.4	0.678	4.8
Upper Bear Creek	1.146	14.0	0.784	6.1
Middle Bear Ck. bel. Sulfur Creek	0.815	6.5	0.744	5.5

Summary and Conclusions

In this work, the Turkey Run/Abbott complex of Hg mines and the Sulfur Creek complex of Hg mines and geothermal springs were confirmed to constitute important point sources of elevated THg, MeHg, and MeHg bioaccumulation in the Cache Creek watershed. Results of this study indicate that remediation of Hg discharges from these regions may be expected to significantly reduce MeHg bioaccumulation levels, both locally and potentially more widely in the watershed. Piscivorous fish at mid Bear Creek, 10 km downstream of Sulfur Creek, demonstrated among the highest muscle Hg levels found to date in California, with most wet weight concentrations at 2.00-4.00 µg/g, ranging to over 6.00 µg/g, all in fish of only 0.6 kg size and smaller. These concentrations were multiple times greater than state, national, or international consumption guidelines. Even detritivorous Sacramento suckers demonstrated concentrations well above the US FDA 1.00 µg/g action guideline in 83% of samples from this area. At the Harley Gulch location, downstream of the other primary point source region, fish were not present for monitoring but aquatic invertebrates exhibited accumulations of MeHg more elevated than at any other site sampled in the watershed.

While Hg concentrations of all measured parameters were most elevated in the tributary streams draining the point source regions, effects were also apparent well downstream. In the main stem of Cache Creek, MeHg bioaccumulation was shown to increase downstream of the identified point source inflows by approximately 100% in the larger fishes, continuing to the outlet of the creek. While absolute concentrations in the main stem were far less elevated than in tributaries nearer the point sources, piscivorous fish in the effected reaches were well above the EPA 0.30 µg/g criterion in all but young-of-year individuals, with concentrations to approximately 1.50 µg/g. Detritivorous Sacramento suckers were above the EPA criterion in individuals over approximately 400 mm. Minnows and other small fishes contained MeHg above the 0.10 µg/g level recommended for protection of piscivorous wildlife at Cache Creek sites downstream of the identified point source inflows, with dramatically greater concentrations in tributaries nearer the Hg point sources. Comparative large fish muscle and small fish whole body MeHg were generally below the respective guideline levels at upstream control sites, though the Upper Bear Creek site was a notable exception.

The large range of aqueous and biotic Hg concentrations in the Cache Creek watershed allowed us to examine potential relationships among and between aqueous and biotic parameters. A number of general predictive relationships could be described and others dismissed by project results. A recurring conclusion was the importance of examining linkages between Hg parameters on a site-specific basis when possible.

The point source distribution of Hg resulted in a somewhat bi-modal range of exposure and bioaccumulation levels across the watershed, leading to apparent general Hg correlations among and between all of the primary sample types. When examined on an individual site basis, though, aqueous raw MeHg alone was found to be predictive of corresponding MeHg bioaccumulation in low trophic level bioindicator organisms. When aqueous Hg parameters were compared to each other on a site-specific basis, a strong correspondence remained between unfiltered (raw) and filtered THg and between unfiltered and filtered MeHg. Suspended solids

(TSS) were found to be generally predictive of aqueous raw THg on a site-specific basis, but not of MeHg. It was not possible to establish the potentially more critical linkage between TSS or THg (re loading) and MeHg. During portions of each year, particularly in the tributary locations, aqueous MeHg became highly (and variably) elevated relative to corresponding aqueous THg, indicating variably enhanced localized net MeHg production.

It was possible to investigate site-specific relationships by focusing on water and low trophic level biota, both of which varied dynamically with the seasons. For linkages to large fish Hg, it was necessary to average aqueous and low trophic level biotic Hg data by site and then broadly compare site ratios. Largely due to the presence of a range of exposure conditions across the watershed, this approach yielded some surprisingly strong general MeHg relationships between bioindicators and large fish, as well as directly between aqueous MeHg and all of the monitored biota, including large fish. Relatively consistent linkages between water and biota were limited to sites with similar water quality characteristics, while inter-trophic biotic MeHg linkages were more broadly applicable in the watershed. These general relationships may be used to estimate reductions in fish Hg corresponding to various projected declines in aqueous MeHg, in relation to regulatory and remediation management. However, while pooled data approaches were necessary to estimate general linkages to large fish Hg, it is important to recognize the limitations of such highly reduced data techniques, as compared to the development of site-specific relationships. This watershed was shown to contain some very different site-specific relationships.

Results of this study indicate that the most useful environmental samples for regulatory and remediation monitoring for Hg include unfiltered aqueous MeHg and short-lived, relatively easily obtainable, low trophic level biota, in addition to larger fish of human health concern. To be most useful predictively, water must be characterized across annual cycles with numerous samplings. Benthic invertebrates and young-of-year small fish may offer more integrative seasonal measures of relative MeHg exposure. Large fish integrate their Hg bioaccumulation over periods of years. Seasonal comparisons of water vs low trophic level bioindicators at individual sites can provide direct linkages between aqueous MeHg and localized bioaccumulation into the aquatic food web. Bioindicator MeHg may additionally provide a dynamic, readily assessed, integrative measure of relative MeHg exposure and bioaccumulation that can be linked to ultimate concentrations in co-occurring large fish.

These techniques should be useful in future Hg monitoring of the Cache Creek watershed in particular, as well as other watersheds in general.

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References Cited

Benoit, J.M., C.C. Gilmour, R.P. Mason, and A. Heyes. 1999. Sulfide controls on mercury speciation and bioavailability to methylating bacteria in sediment pore waters. *Environ. Sci. & Technol.*, 33(6): 951-957.

Bonzongo, J.C., K.J. Heim, J.J. Warwick, and W.B. Lyons, 1996. Mercury levels in surface waters of the Carson River-Lahontan Reservoir system, Nevada: Influence of historic mining activities. *Environmental Pollution*, 92(2): 193-201.

Bowles, K.C., S.C. Apte, W.A. Maher, M. Kawei, and R. Smith. 2001. Bioaccumulation and biomagnification of mercury in Lake Murray, Papua New Guinea. *Can. J. Fish. Aquat. Sci.*, 58: 888-897.

Brumbaugh, W.G., D.P. Krabbenhoft, D.R. Helsel, J.G. Wiener, and K.R. Echols, 2001. A national pilot study of mercury contamination of aquatic ecosystems along multiple gradients: bioaccumulation in fish. USGS/BRD/BSR-2001-0009, iii + 25 pp.

Cleckner, L.B., P.J. Garrison, J.P. Hurley, M.L. Olson, and D.P. Krabbenhoft. 1998. Trophic transfer of methyl mercury in the northern Florida Everglades. *Biogeochemistr*y, 40(2-3): 347-361.

Cleckner, L.B., C.C. Gilmour, J.P. Hurley, and D.P. Krabbenhoft. 1999. Mercury methylation in periphyton of the Florida Everglades. *Limnology & Oceanograph*y, 44(7): 1815-1825.

Domagalski, J.L 2001. Mercury and methylmercury in water and sediment of the Sacramento River basin, California: *Applied Geochemistry*, vol. 16/15, pp. 1677-1691.

Domagalski, J.L., C.N. Alpers, D.G. Slotton, S.M. Ayers, and T.H. Suchanek. 2002. Mercury and methylmercury concentrations and loads within the Cache Creek Watershed, California, January 2000 through May 2001. *Draft report to CALFED*, August 2002.

Eisler R., 1987. Mercury hazards to fish, wildlife and invertebrates: a synoptic review. *US Fish and Wildlife Service, Biological Report*, 85(1.10), Patuxent Wildlife Research Center Laurel, Maryland. 90 pp.

Foe, C., and W. Croyle. 1998. Mercury concentrations and loads from the Sacramento River and from Cache Creek to the Sacramento-San Joaquin Delta estuary. *Central Valley Regional Water Quality Control Board staff report*, 101 pp.

Ganguli, P.M., R.P. Mason, K.E. Abu-Saba, R.S. Anderson, and A.R. Flegal. 2000. Mercury speciation in drainage from the New Idria mercury mine, California. *Environmental Science & Technology*, 34: 4773-4779.

Gilmour, C.C., E.A. Henry, and R. Mitchell. 1992. Sulfate stimulation of mercury methylation in freshwater sediments. *Environmental Science & Technology.* 26(11). 1992. 2281-2287.

Gray, J.E., P.M. Theodorakos, E.A. Bailey, and R.R. Turner. 2000. Distribution, speciation, and transport of mercury in stream-sediment, stream-water, and fish collected near abandoned mercury mines in southwestern Alaska. *Science of the Total Environment*, 260: 21-33.

Hill, W.R., A.J. Stewart, and G.E. Napolitano. 1996. Mercury speciation and bioaccumulation in lotic primary producers and primary consumers. *Can. J. Fish. Aquat. Sci.* 53: 812-819.

Hines, M.E., M. Horvat, J. Faganeli, J.J. Bonzongo, T. Barkay, E.B. Major, K.J. Scott, E.A. Bailey, J.J. Warwick, and W.B. Lyons. 2000. Mercury biogeochemistry in the Idrija River, Slovenia, from above the mine into the Gulf of Trieste. *Environ. Research*, 83: 129-139.

Krabbenhoft, D. P., J. G. Wiener, W. G. Brumbaugh, M. L. Olson, J. F. DeWild, and T. J. Sabin (1999). A national pilot study of mercury contamination of aquatic ecostystems along multiple gradients. *In:* U.S. Geological Survey Toxic Substances Hydrology Program-Proceedings of the Technical Meeting, Charleston, South Carolina, D. W. Morganwalp and H. T. Buxton, editors, pp. 147-160.

Lawson, N.M., and R.P. Mason. 1998. Accumulation of mercury in estuarine food chains. *Biogeochemistry*, 40(2-3): 235-247.

Mason, R.P., J.R. Reinfelder, and F.M.M. Morel. 1995. Bioaccumulation of mercury and methylmercury. *Water, Air, Soil Pollut.,* 80(1-4): 915-921.

Mason, R.P., J.R. Reinfelder, and F.M.M. Morel. 1996. Uptake, toxicity, and trophic transfer of mercury in a coastal diatom. *Environ. Sci. & Technol.*, 30(6): 1835-1845.

Mason, R.P., J.M. Laporte, and S. Andres. 2000. Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish. *Arch. Environ. Contam. Toxicol.*, 38: 283-297.

Maury-Brachet, R., F. Ribeyre, A. Boudou. 1990. Actions and interactions of temperature and photoperiod on mercury accumulation by *Elodea densa* from sediment source. *Ecotoxicology & Environ.l Safety,* 20(2): 141-155.

Moyle, P.B. 1976. *Inland fishes of California*. University of California Press, Berkeley, Los Angeles, London. 405 pp.

Moyle, P.B. 2002. *Inland fishes of California (revised and expanded)*. University of California Press, Berkeley, Los Angeles, London. 502 pp.

Odin, M., A. Feurtet-Mazel, F. Ribeyre, A. Boudou. 1994. Actions and interactions of temperature, pH and photoperiod on mercury bioaccumulation by nymphs of the burrowing mayfly *Hexagenia rigida*, from the sediment contamination source. *Environ. Toxicol. Chem.,* 13(8): 1291-1302.

Pickhardt, P.C., C.L. Folt, C.Y. Chen, B. Klaue, and J.D. Blum. 2002. Algal blooms reduce the uptake of toxic methylmercury in freshwater food webs. *Proc. Natl. Acad. Sci.* 99(7): 4419-4423.

Puckett, H.M. and B.H. van Buuren. 2000. *Quality Assurance Project Plan for the CALFED Project: An assessment of ecological and human health impacts of mercury in the bay-delta watershed*, available on the Internet at: http://loer.tamug.tamu.edu/calfed/QA.htm.

Rytuba, J.J. 2000. Mercury mine drainage and processes that control its environmental impact. *Science of the Total Environment*, 260: 57-71.

Schetagne, R., J.F. Doyon, and J.J. Fournier. 2000. Export of mercury downstream from reservoirs. *Science of the Total Environment,* 260(1-3): 135-145.

Sellers, P., C.A. Kelly, J.W.M. Rudd, A.R. Machutchon. 1996. Photodegradation of methylmercury in lakes. *Nature,* 380(6576): 694-697.

Slotton, D.G., S.M. Ayers, J.E. Reuter, and C.R. Goldman. 1995. Gold mining impacts on food chain mercury in northwestern Sierra Nevada streams. *Technical Completion Report for* *the University of California Water Resources Center, Project W-816*, August 1995, 46 pp.

Slotton, D.G., S.M. Ayers, J.E. Reuter, and C.R. Goldman. 1997. Cache Creek watershed preliminary mercury assessment, using benthic macro-invertebrates. *Final Report for the Central Valley Regional Water Quality Control Board and the National Science Foundation*, June 1997, 36 pp.

Slotton, D.G., T.H. Suchanek, and S.M. Ayers 2000. Delta wetlands restoration and the mercury question. *Interagency Ecological Program Newsletter*, 13(4):40-50.

Southworth, G.R., R.R. Turner, M.J. Peterson, and M.A. Bogle. 1995. Form of mercury in stream fish exposed to high concentrations of dissolved inorganic mercury. *Chemosphere.* 30(4): 779-787.

Southworth, G.R., R.R. Turner, M.J. Peterson, M.A. Bogle, and M.G. Ryon. 2000. Response of mercury contamination in fish to decreased aqueous concentrations and loading of inorganic mercury in a small stream. *Environmental Monitoring and Assessment*, 63: 481-494.

Suchanek, T.H., P.J. Richerson, L.J. Mullen, L.L. Brister, J.C. Becker, A. Maxson, and D.G. Slotton. 1997. The role of the Sulphur Bank Mercury Mine site (and associated hydrogeological processes) in the dynamics of mercury transport and bioaccumulation within the Clear Lake aquatic ecosystem. *A report prepared for the US EPA, Region IX Superfund Program*. 479 pp.

Tremblay, A., M. Lucotte, and I. Rheault. 1996. Methylmercury in a benthic food web of two hydroelectric reservoirs and a natural lake of northern Quebec (Canada). *Water, Air, Soil Pollut,* 91(3-4): 255-269.

Ullrich, S.M., T.W. Tanton, S.A. Abdrashitova. 2001. Mercury in the aquatic environment: A review of factors affecting methylation. *Critical Reviews in Environmental Science & Technology*, 31(3): 241-293.

US EPA. 2001. Water Quality Criterion for the Protection of Human Health: Methylmercury. *Federal document*: EPA-823-R-01-001. Office of Science and Technology, Office of Water, U.S. Environmental Protection Agency Washington, DC 20460.

See accompanying Data Appendix for compiled raw data for water, invertebrates, small fish, and large fish used in this report, together with QA/QC information