Metal exposure to a benthic invertebrate, *Hydropsyche californica*, in the Sacramento River downstream of Keswick Reservoir, California

By Daniel J. Cain, James L. Carter, Steven V. Fend, Samuel N. Luoma, Charles N. Alpers and Howard E. Taylor

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Bruce Babbitt, Secretary

U.S. Geological Survey
Charles G. Groat, Director

For additional information write to:

Daniel J. Cain
U.S. Geological Survey
Water Resources Division, MS 465
Menlo Park, CA 94025

Copies of this report can be obtained from the authors or:

U. S. Geological Survey
Information Services
Box 25286, MS 517
Denver Federal Center
Denver, CO 80225

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Abstract

Metals (Al, Cd, Cu, Fe, Hg, Pb, and Zn) were determined in a resident, invertebrate species to assess the occurrence and distribution of biologically available metal along a 111 km section of the upper Sacramento River affected by acid mine drainage. Samples of the filter-feeding caddisfly *Hydropsyche californica* (Insecta: Trichoptera) were collected from five stations in the Sacramento River between Redding and Tehama, California. Metal concentrations in these samples were referenced to samples from an uncontaminated tributary, Cottonwood Creek. Aluminum, Cd, Cu, Fe, Pb, and Zn in the body of the insect were separated into cytosolic (soluble) and particulate components. The cytosol is an intracellular metal fraction and therefore, an indicator of metal bioavailability. The concentrations and the relative proportions of metals in the cytosol identified differences in bioaccumulation among metals and characterized spatial patterns of bioavailable metals. Total Hg was determined in the whole body, but not the cytosol. Results showed that concentrations of Cd, Cu, Pb, and Zn in the whole body, and both the particulate and cytosolic fractions of *Hydropsyche* from the Sacramento River were significantly greater than in those from the reference site. Enrichment of Cd was greater than the other metals, exceeding the Cd reference concentration by nearly 20 fold at the most upstream station. Concentrations of Hg and Fe in caddisflies were not elevated. Aluminum concentrations were elevated in the cytosol, only. This enrichment was not evident in the whole body because cytosolic Al accounted for less than 1 percent of the total body burden. The vast majority of Al was associated with undigested gut content, the exoskeleton, and other structures associated with the particulate fraction. Concentrations in this fraction were not enriched. Likewise, only a minor fraction of the total Fe and Pb were associated with the cytosol. In contrast, a high proportion of the Cd, Cu, and Zn in *Hydropsyche* sp. were contained in the cytosol, indicating that these metals were biologically available. Maximum concentrations of cytosolic Cd, Cu, Pb, and Al occurred at the three most upstream stations (river km 479-444) and decreased downstream to Tehama (river km 368), although a small increase in bioavailable Cd and Cu was observed at Bend Bridge (river km 415). Except for a small increase in cytosolic Zn at Bend Bridge, Zn concentrations did not attenuate downstream, but instead were uniformly elevated throughout the 111 km section. At Tehama, concentrations of cytosolic Al, Cd, Cu, Pb, and Zn were 1.5 to 5 times greater than reference concentrations from Cottonwood Creek, suggesting that transport of bioavailable forms of these metals extended downstream of Tehama. The results indicate that *Hydropsyche* in the upper Sacramento River were exposed to elevated concentrations of bioavailable Al, Cd, Cu, Pb, and Zn. Distribution patterns of bioavailable Al, Cd, Cu, and Pb were consistent with documented sources of metal from the East Shasta and West Shasta mining districts, upstream of Redding.

Introduction

Drainage from base-metal mines at Iron Mountain in northern California is a source of metals to the Sacramento River that has threatened resident fauna (Shaw, 1940; Finlayson and Ashuckian, 1979; Finlayson and Wilson, 1979; Finlayson and Verrue, 1980; NOAA, 1989). Recurring fish kills (USEPA, 1992) prompted the construction of the Spring Creek Debris Dam

(SCDD) in 1963 to abate the discharge of metal-laden acid mine water from Spring Creek into the Sacramento River (fig. 1). In addition, water is treated with lime to precipitate metals. A temporary lime neutralization plant operated 3 to 4 months per year during 1989-1993. Since July 1994, there has been continuous, year-round, treatment. Treatment has reduced annual metal loadings of Cu by about 80-85 percent and Zn (and probably cadmium also) by about 90 percent. Prior to lime neutralization treatment, about 90 percent of the Cu loading to the Sacramento River at Keswick Dam could be attributed to Spring Creek and the Iron Mountain mine drainage (D. Heiman, State of California Regional Water Quality Control Board, Sacramento, CA, unpublished data). Since 1994, this component of the overall Cu loading has been reduced to about 50 percent. The remainder is predominantly from other mines in the West Shasta mining district that drain into Shasta Lake via Little Backbone Creek and West Squaw Creek (D. Heiman, unpublished data).

Flows from the SCDD are regulated by the Bureau of Reclamation, together with dilution flows from Shasta Lake and Whiskeytown Lake (via the Spring Creek Power Plant), to meet water-quality criteria for the protection of resident fish at a compliance point below Keswick Dam (fig. 1). Nevertheless, these water-quality criteria are sometimes exceeded during times of heavy rainfall when water in the Spring Creek Reservoir overtops the SCDD and insufficient dilution flows are available. In addition, the operation of the SCDD increases the total duration of exposure of fish to metals at chronically toxic concentrations (USEPA, 1992).

The aquatic habitat in the section of the Sacramento River between Keswick Dam and Red Bluff is of special concern because it includes spawning ground for several salmonid fish species, including four distinct runs of chinook salmon (*Oncoryhnchus tshawytscha*), plus steelhead trout and resident rainbow trout (*O. mykiss*) (USEPA, 1992). The winter-run chinook salmon is a Federally listed endangered species, and the steelhead trout and one or more of the other chinook salmon runs recently have been listed as threatened species (NOAA, 1994; 1997). A study of metals in livers from trout below Keswick Dam (Wilson and others, 1981) showed elevated concentrations of Cd, Cu, and Zn, at possibly harmful levels. A more recent survey (1990) indicated a general contamination of the aquatic food web in the Sacramento River downstream of Keswick Reservoir to Jelly's Ferry, located above Red Bluff (Saiki and others, 1995). However, the downstream extent of contamination has not been fully resolved, and little is known specifically about the biological availability of metals within the river.

Benthic insects are one group of organisms used to monitor metal exposures and assess biological effects in freshwaters (Cain and others, 1992; Hare, 1992; Rosenberg and Resh, 1993). Assessments of biological risk associated with exposure are strengthened when methods allow distinction between metal that is taken up and accumulated within cells from metal that occurs extracellularly. The latter includes a variety of forms that probably pose little toxic risk (e.g. metals or metal-bearing particles on external body parts and metals retained with undigested material in the gut of the animal). Recently, Cain and Luoma (1998) evaluated metal exposures in a mining-impacted river by determining metal concentrations of the cytosol (the soluble portion of the cell cytoplasm) in an aquatic insect.

Metal analysis of the cytosol and/or other intracellular components provides an unambiguous indicator of metal bioavailability. The cytosol appears to be an important accumulation site for essential metals such as Cu and Zn and certain non-essential elements, including Cd (Seidman and others, 1986; Suzuki and others; 1988; Cain and Luoma, 1998). Furthermore, sublethal effects have been shown to coincide with accumulation of Cd in the cytosol and redistribution of Cd among cytosolic ligands (Jenkins and Mason, 1998). Therefore, concentrations of cytosolic metals reflect intracellular dose, and may be a better diagnostic of toxicity than either whole-body or whole-tissue concentrations (Roesijadi, 1994; Thorp and Costlow, 1989).

Saiki and others (1995) showed that metals contaminate fish and some of their prey species in the Sacramento River, implying that food was a potential source of metals to the fish. The transfer of metals

through food webs may be influenced by how metals are constituted in the body of prey species. Cytosolic metal appears to be a biologically available component of food. Reinfelder and Fisher (1991) demonstrated that the efficiency of metal absorption by copepods fed metal-contaminated algae was directly proportional to metal in the cytosolic fraction of the algae. Although such simple relationships are not always observed (Lee and Luoma, 1998), studies with predators and their prey suggest that the cytosolic metal fraction is one component of dietary exposure (Reinfelder and Fisher, 1994; Wallace and Lopez, 1997). In contrast, metals that are encased in intracellular inclusions, such as calcium or phosphate-rich granules, are not efficiently digested by predators, and are passed intact through the digestive tract (Nott and Nicolaidou 1990). Similarly, other particulate forms of metal that reside outside the cell, such as those sorbed to external body parts and bound to undigested gut content, may be largely unavailable to higher trophic organisms. Thus, an analysis of metal partitioning in prey species may help identify metals most likely to be accumulated from food.

This study was conducted in conjunction with a broader study of the distribution, geochemistry, and transport of metals in the Sacramento River (Alpers and others, 2000a; 2000b). The principal objective of this study was to assess the occurrence and distribution of biologically available metals in the upper Sacramento River, below Keswick Reservoir. Also, because of the concern for resident fish, there was a need for data that were at least broadly indicative of dietary metal exposure to fish in the upper Sacramento River.

Metal contamination and bioavailability was assessed by comparative analysis of metal concentrations in the hydropsychid caddisfly, *Hydropsyche* (Insecta: Trichoptera). This taxon has been successfully used in metal contamination studies (Cain and others, 1992; Axtmann and others, 1997; Vuori and Kukkonen 1996). The larva is a filter-feeding organism that uses a silken mesh to sieve suspended particles from solution. The larva has a sedentary life stage of about 1-year, is relatively metal tolerant (Clements and others, 1992), and is widely distributed and abundant in many rivers, including the Sacramento River. Partitioning of metals between the cytosol and the whole body was useful in identifying differences in bioavailability among metals. The concentrations and spatial distributions of bioavailable metals were evaluated from the cytosol of *Hydropsyche* sampled from five stations in the Sacramento River and a reference station on Cottonwood Creek, a tributary to the Sacramento River near Redding (fig. 1). Total Hg concentrations were determined in the whole body, but not the cytosol, of *Hydropsyche* to assess the occurrence of Hg contamination in the upper Sacramento River. Consumption of metal-contaminated benthic macroinvertebrates including *Hydropsyche* can be a significant cause of chronic metal contamination in resident fish (Woodward and others, 1995; Farag and others, 1995). Therefore, dietary exposure to fish was assessed from metals concentrations in *Hydropsyche*, especially those associated with the cytosol.

Methods

Sample Collection

Samples of *Hydropsyche* larvae were collected at five stations in the Sacramento River within a 111 km section between Rodeo Park near Redding (river km 479) and Tehama (river km 368) (fig. 1; appendix 1). Stations were sampled during a period of low-flow conditions on October 21-23, 1996. In addition, a sample was collected from Cottonwood Creek, near Cottonwood (river km 439) (fig. 1; appendix 1). This sample was used as a local reference to evaluate metal levels in samples from the Sacramento River. Samples were not collected upstream of Keswick Reservoir, downstream of Tehama or during other times of the year because of the scarcity of habitat that could be sampled using the methods we employed. *Hydropsyche* larvae were collected with large kick nets and by hand from a single, wadeable (approximately 0.3 meter deep) riffle at each station.

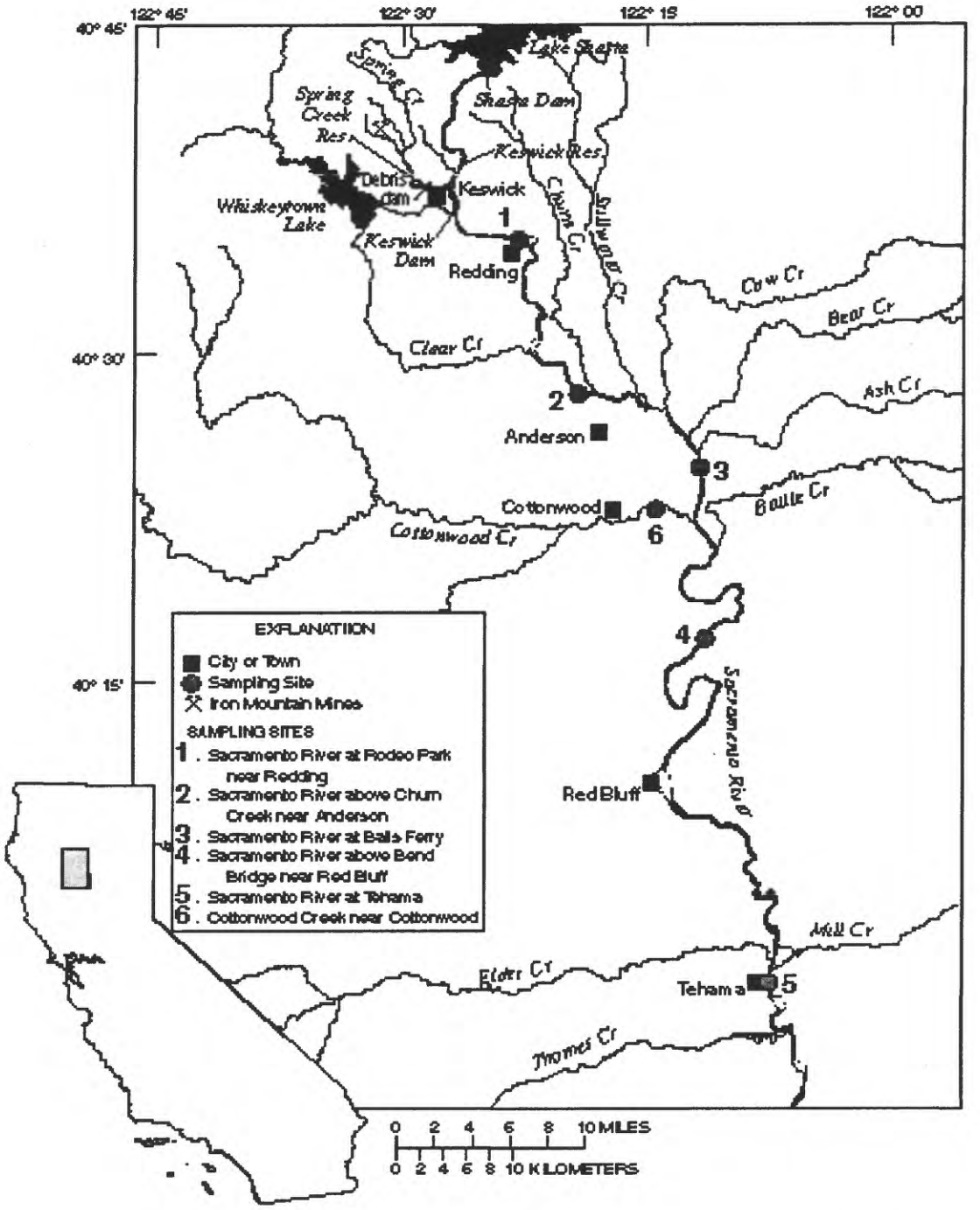

Figure 1. Map of the study area names, numerical designations, and locations of stations in the upper Sacramento River and Cottonwood Creek where *Hydropsyche californica* were collected in October 1996.

Specimens were picked from the net with nylon forceps and placed into plastic trays with stream water (forceps and trays were previously acid washed). Water in the trays was freshened periodically. Specimens were transferred from the trays to plastic, sealed bags and frozen on dry ice in a small volume of river water within 1 hour of collection. The samples were moved to the laboratory where they were stored at -70° C until sample preparation. Additional specimens for taxonomic identification were preserved in 10 percent formalin in the field and transferred to 75 percent ethanol in the laboratory. Samples were dominated by a single species, *Hydropsyche californica*.

Sample Preparation

Samples for the determination of aluminum (Al), cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), and zinc (Zn) were prepared following the method described by Cain and Luoma (1998). Specimens were partially thawed in batches, rinsed with cold deionized water to remove sediment and detritus, and then transferred to a glass, sorting dish that was placed on a bed of ice. The animals were immersed in a small amount of water and viewed individually under a stereomicroscope for identification and further cleaning. Instars of *H. californica* were not sorted, although smaller specimens that could not be identified were discarded. Specimens were then transferred to a cooler. When the entire sample had been sorted and cleaned, the animals were, blotted dry with tissue paper, pooled into replicate subsamples (n=4-6) of approximately the same wet weight, and then temporarily refrigerated.

Cold 0.05 M Tris-HCl buffer (pH 7.4, previously degassed by vacuum and then bubbled with N_2) was added to each subsample at a ratio of 8:1 (milliliters Tris:gram wet sample). Subsamples were homogenized with a stainless-steel high-speed tissue homogenizer under a nitrogen atmosphere for one minute. The homogenate was split into two fractions: one for the whole-body metal analysis and the other for the cytosolic metals. The cytosol was isolated by centrifuging the homogenate at 100,000 x g for 1 hour at 5° C. The supernatant (cytosol) and pellet were collected and transferred to separate screw-cap glass vials. Samples were kept cold throughout the procedure. Sample fractions were frozen at -20° C as they were prepared. Later, they were freeze-dried, weighed, digested by reflux in hot, isopiestically distilled (Kuehner and others, 1972) 16 N HNO_3. When the digestion was complete, the samples were evaporated to dryness.

Prior to analysis, sample residues were reconstituted by the addition of 10 milliliters (ml) of 1 percent high-purity HNO_3. Five ml of this solution was diluted to 50 ml for trace metal analysis.

All plastic and glassware used for the preparation and digestion was cleaned by soaking overnight in a Micro® solution, rinsed with deionized H_2O, then washed in 5 percent HCl and rinsed with deionized H_2O. The tissue homogenizer was cleaned by soaking overnight in a solution of RBS® and rinsed in deionized H_2O.

Additional samples from all stations were sorted and cleaned as described above for the determination of mercury (Hg). Samples were composed of single or duplicate subsamples, which were frozen at –20° C immediately. The subsamples were freeze dried and then homogenized with a mixer-mill using 125 ml polycarbonate jars and methacrylate balls. Samples were digested following the procedure described by Elrick and Horowitz (1986).

Metals Analysis

Aluminum, Cd, Cu, Pb and Zn in the digested samples were determined by inductively coupled plasma-mass spectrometry (ICP-MS), using a modification of a direct analysis procedure reported by Taylor and Garbarino (1991) and Garbarino and Taylor (1994). All of these determinations were performed with a Perkin-Elmer Sciex, Model 5000 inductively coupled plasma-mass spectrometer fitted with a gem-cone® pneumatic nebulizer. Instrument operating conditions and specific isotopes used for measurement are listed in appendix 2.

Iron was determined by a modified inductively coupled plasma-atomic emission spectrometric (ICP-AES) technique (Taylor and Garbarino, 1985) at a wavelength of 259.94 nm. Measurements were made with a Thermal Jarrell-Ash model 975 atomic emission spectrometer using a U.S.

Geological Survey designed Babington-type pneumatic nebulizer (Garbarino and Taylor, 1980).

Mercury was determined by cold vapor atomic absorption spectrophotometry using conditions described by Elrick and Horowitz (1986).

Quality Assurance

In addition to the previously described, independently processed subsamples collected at each location, laboratory determinations for Al, Cd, Cu, Fe, Pb, and Zn were performed in triplicate. Single or duplicate determinations of Hg were performed. Standard deviations reported for sample concentrations represent the combined precisions associated with sample collection, processing, and analysis.

Accuracy was established by the analysis of Standard Reference Materials obtained from the National Institute of Standards and Technology (NIST) and the National Research Council Canada (NRC). Four materials were selected to simulate invertebrate tissue: NIST SRM 1566a oyster tissue, NIST RM50, albacore tuna, NRC Tort –2, lobster hepatopancreas, and NRC Dorm – 2, dogfish muscle. Standards were processed in a manner identical to the procedure used for the invertebrate specimens. Results obtained for the analysis of these reference materials are given in appendix 3. In addition, selected representative samples were spiked with a standard containing Cd, Cu, Pb, and Zn prior to sample processing to establish their recovery during sample handling and analysis. The median (and range) of spike recoveries was 98 (95 to 102) percent for Cd, 94 (82 to 100) percent for Cu, 93 (87 to 98) percent for Pb, and 96 (93 to 108) percent for Zn.

Procedural and reagent blanks were analyzed to evaluate potential contamination problems during sample processing and analysis. Appropriate reagent blank concentration values were used to correct the chemical analyses where necessary.

Data Analysis

The mean, standard deviation, and standard error of composite caddisfly samples (n=4-6 for all samples except those analyzed for Hg n=1-2) from each station were calculated. The number of replicates (n) generally reflected the abundance of *H. californica* at each station. Enrichment factors were calculated by dividing the mean metal concentrations of stations in the Sacramento River by the mean metal concentrations of the sample from Cottonwood Creek. The percent of metal recovered in the cytosol was calculated by dividing the metal concentration of the cytosol by the whole-body metal concentration and multiplying the result by 100. Differences in metal concentrations among stations were determined by single classification ANOVA, after the data were log transformed to correct for heteroscedasticity. Specific station comparisons were analyzed by the Turkey honest significant differences test for unequal sample sizes. Data that were not corrected by log transformation were analyzed by the Kruscal-Wallis ANOVA. Pearson product-moment correlations were determined between whole-body and cytosolic metal concentrations using the mean sample concentrations to avoid any bias due to unequal sample sizes (even though within station variance was much less that among station variance). Results of statistical tests were considered significant if $\alpha < 0.05$.

Results

Metal Enrichment in the Sacramento River

Mean concentrations of Cd, Cu, Pb, and Zn in the whole body, pellet, and the cytosol of *Hydropsyche* from all stations in the Sacramento River were significantly greater than in those from Cottonwood Creek, the reference site (table 1). Aluminum concentrations in the cytosol also were significantly higher in samples from the Sacramento River; however, concentrations in the whole body (and pellet) were significantly different than Cottonwood Creek at Bend Bridge and Tehama, only. Iron concentrations of all body fractions in Sacramento Rive samples were variable, but were not either uniformly higher or lower than concentrations at Cottonwood Creek. Mercury concentrations in samples from the Sacramento River were ≤0.06 µg/g, and lower than the concentration of the sample from Cottonwood Creek. Among the metals analyzed, Cd showed the greatest degree of

enrichment (Sacramento River / Cottonwood Creek). Enrichment factors for other metals in the whole body and the pellet followed the order Cu > Pb > Zn > Al. Relative to Cottonwood Creek, Al and Pb were more enriched in the cytosol than in the whole body. Therefore, the order of enrichment in the cytosol was Cd > Pb > Al ≥ Cu > Zn.

Table 1. Metal concentrations in *Hydropsyche californica* collected from the Sacramento River and Cottonwood Cr. during October 21-23, 1996. Values are the mean ± 1 standard deviation (n=1-6; units are µg/g dry weight). Metal enrichment factors (EF) in the Sacramento River (metal concentration of Sacramento River station/metal concentration in Cottonwood Cr.) are shown. Ne designates "not enriched" (i.e. enrichment factor was less than 1).

Element	Body Fraction	Station						EF
		Rodeo Park	Churn Cr.	Balls Ferry	Bend Br.	Tehama	Cottonwood Cr.	
Al	Whole body	1240±50	1350±60	1300±40	1940±40	2110±110	1360±160	0.9-1.6
	Cytosol	10±1	8±0.4	11±2	6±1	6±1	3±0.4	2.0-3.7
	Pellet	960±150	1420±87	1270±80	1720±130	1710±80	1130±110	0.8-1.5
Cd	Whole body	2.16±0.10	0.96±0.05	0.77±0.08	1.14±0.09	0.66±0.02	0.06±0.02	11-36
	Cytosol	1.27±0.09	0.55±0.04	0.52±0.14	0.73±0.05	0.36±0.02	0.07±0.04	5.1-18
	Pellet	0.94±0.23	0.41±0.02	0.33±0.04	0.52±0.04	0.30±0.02	≤0.02	≥15-47
Cu	Whole body	37.5±3.2	37.7±1.6	25.0±1.3	30.8±2.5	25.6±1.2	14.5±0.4	1.7-2.6
	Cytosol	20.7±1.1	20.8±1.0	14.1±0.3	16.8±1.3	12.1±0.9	6.9 ±0.4	1.8-3.0
	Pellet	15.3±6.0	18.4±1.3	12.1±0.8	16.2±1.3	14.0±0.6	7.8±0.6	1.6-2.4
Fe	Whole body	1460±150	2070±50	1340±320	1970±590	2830±190	1860±200	0.7-1.5
	Cytosol	45±2	57±9	65±5	78±8	55±7	69±10	0.7-1.1
	Pellet	1360±390	1990±70	1470±160	1880±360	2740±240	1880±80	0.7-1.5
Hg	Whole body	0.040	0.060	0.05±0.01	0.045±0.001	0.03±0.01	0.08	Ne
Pb	Whole body	1.26±0.05	1.26±0.04	0.93±0.10	1.07±0.05	1.23±0.08	0.59±0.05	1.6-2.1
	Cytosol	0.25±0.02	0.15±0.02	0.18±0.05	0.18±0.07	0.15±0.03	0.05±0.04	3.0-5.0
	Pellet	1.06±0.46	1.08±0.07	0.88±0.19	1.02±0.05	1.05±0.05	0.52±0.02	1.7-2.1
Zn	Whole body	169±9	160±4	171±4	208±6	160±5	113±6	1.4-1.8
	Cytosol	82±3	96±5	95±3	104±4	80±5	59±5	1.4-1.8
	Pellet	87±17	101±3	91±8	108±5	94±3	58±5	1.5-1.9

Metal Partitioning in the Insect

Whole-body concentrations represent the accumulation of both cytosolic and non-cytosolic, particulate metal forms. (Particulate metal forms are operationally defined as metal retained in the pellet after ultracentrifugation.) Partitioning between cytosolic and particulate forms differed among different groups of elements. Cytosolic Al, Fe, and Pb accounted for small percentages of the total body burden (table 2). Instead, those elements were primarily associated with particulate form(s) that represented more 99 percent of the total Al body burden and at least 85 percent of the total Fe body burden. The proportion of particulate Pb ranged between 80 and 92 percent. In contrast, the cytosol was an important accumulation site for Cd, Cu, and Zn, accounting for approximately 50 to 100 percent of the total body burden of these elements (table 2).

As might be expected, the results of correlations between whole-body and cytosolic metal concentrations were affected by the proportional accumulation of metal in the cytosol. Because the cytosol was a major accumulation site for Cd, Cu and Zn, cytosolic concentrations of these metals correlated strongly with whole-body concentrations (fig. 2). In contrast, correlations for Al, Fe, and Pb were weak, reflecting the dominance of non-cytosolic metal forms. Whole-body and cytosolic concentrations of Al generally showed opposite patterns of accumulation among stations in the Sacramento River (a significant negative correlation ($p = 0.04$) when just stations within the Sacramento River were analyzed) (fig 2). Cytosolic Fe concentrations were independent of whole-body concentrations. A positive relationship between whole body and cytosolic Pb was influenced by the low concentrations in Cottonwood Creek (fig. 2). Among samples from the Sacramento River, whole-body Pb concentrations were not predictive of cytosolic Pb concentrations (fig. 2).

Table 2. The percent of total metal body burden recovered in the cytosol (calculated as cytosolic metal concentration/whole-body metal concentration X 100).

Element	Station					
	Rodeo Park	Churn Cr.	Balls Ferry	Bend Br.	Tehama	Cottonwood Cr.
Al	0.8	0.6	0.8	0.3	0.3	0.2
Cd	59	57	68	64	55	100
Cu	55	55	56	55	47	48
Fe	3	3	5	4	2	4
Pb	20	12	19	17	12	8
Zn	49	60	56	50	50	52

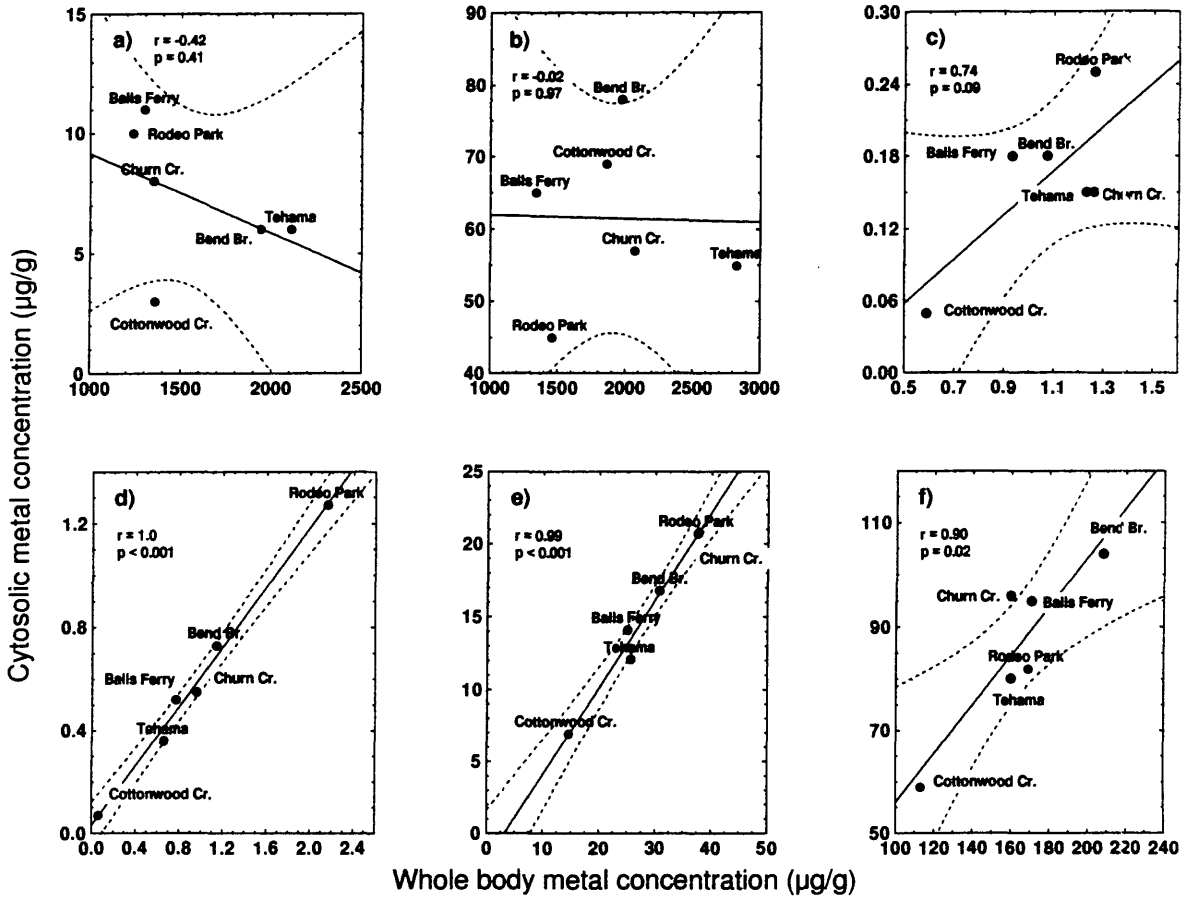

Figure 2. Mean cytosolic metal concentrations plotted with mean whole-body concentrations in *Hydropsyche californica*. Data are identified by station and correlated using the Pearson product - moment correlation. The correlation coefficient r and the probability level p are given for each element. Dotted lines are the 95% confidence intervals. a) Al; b) Fe; c) Pb; d) Cd; e) Cu; f) Zn.

Spatial patterns in Whole-Body Metal Concentrations

As suggested above, spatial patterns in the concentrations of Al, Fe, and Pb in the whole body (and the particulate fraction) were distinctly different from those displayed in the cytosol. Aluminum and Fe exhibited similarities in their longitudinal distribution within the Sacramento River that differed from the distributions of other elements. Whole-body concentrations of Al and Fe between Rodeo Park (station 1) and Balls Ferry (station 3) were either equal to or slightly lower than the reference concentrations in Cottonwood Creek (station 6) (fig. 3; table 1); however, concentrations increased significantly downstream of Balls Ferry. Concentrations were greater than reference concentrations at Bend Bridge (station 4) (Al only) and Tehama (station 5). Concentrations of Pb were highest at Rodeo Park, Churn Creek (station 2), and Tehama (fig. 3). Thus, there was no net change in Pb concentration between the most upstream and downstream stations, but concentrations decreased significantly between Churn Creek and Balls Ferry, then progressively increased downstream to Tehama. The concentration pattern of Pb between Balls Ferry and Tehama resembled that of Fe.

Mercury concentrations in samples collected from the Sacramento River were slightly less than at Cottonwood Creek, and did not exhibit any organized spatial pattern (table 1).

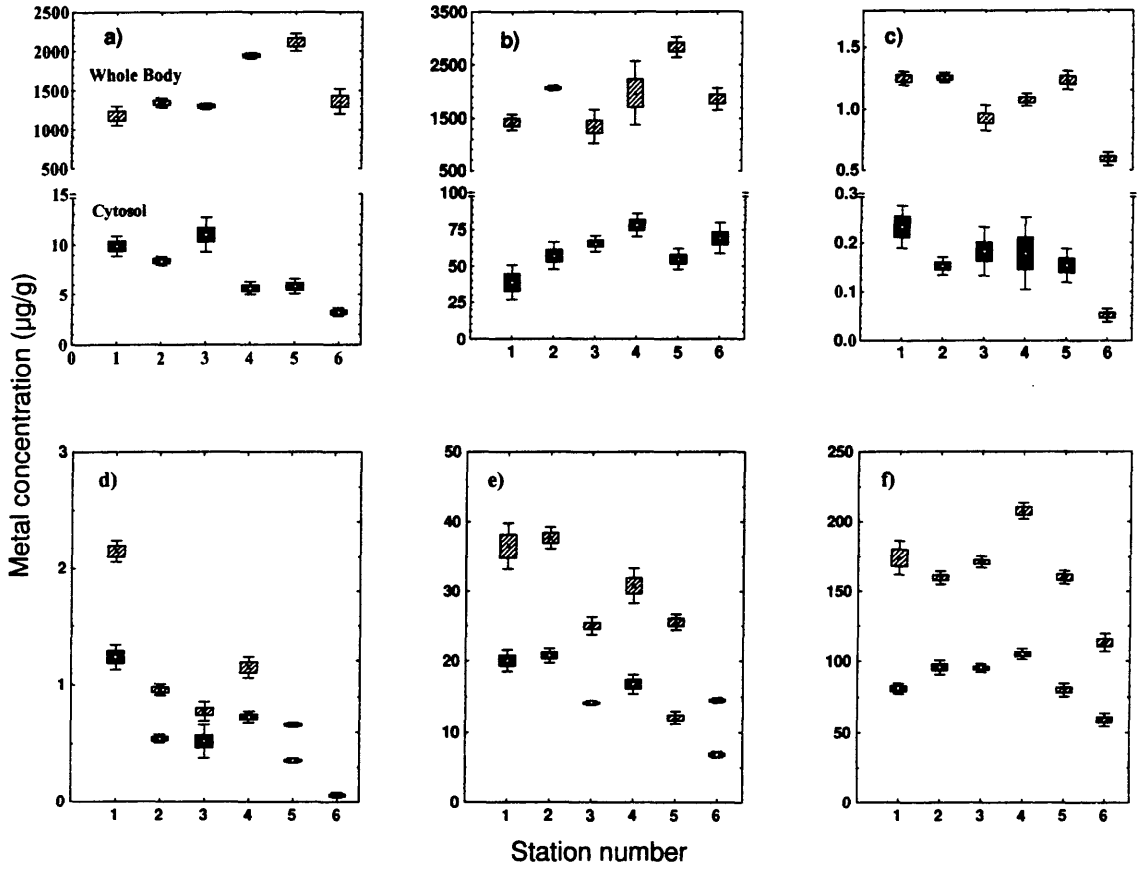

Figure 3. Metal concentrations in the whole body and the cytosol of *H. californica* from stations in the Sacramento River and Cottonwood Creek. Stations in the Sacramento River are numbered sequentially (1-5) from upstream to downstream, as described in Figure 1 and Appendix 1. Station 6 is Cottonwood Creek. a) Al; b) Fe; c) Pb; d) Cd; e) Cu; f) Zn. Note the scale breaks for the concentrations of Al, Fe, and Pb. Values are the mean ± 1 standard error (box) and ± 1 standard deviation (whiskers).

As discussed earlier, the cytosol represented a fairly large proportion of the Cd, Cu, and Zn whole-body concentrations. Therefore, spatial changes in whole-body concentrations of these metals closely followed cytosolic metal concentrations.

Spatial Patterns in Enriched, Cytosolic Metals

Maximum concentrations of Cd, Cu, Pb, and Al occurred in the samples from the three most upstream stations (the Sacramento River at Rodeo Park, Churn Creek, and Balls Ferry). Cytosolic concentrations of Cd, Cu, and Pb generally decreased downstream of these stations to Tehama, although the details in attenuation patterns differed in some aspects. The greatest decrease in Cd occurred between Rodeo Park at Redding and Churn Creek (near Anderson) (fig. 3). Copper concentrations were stable through that reach then declined between Churn Creek and Balls Ferry. There was little overall change in concentrations between Balls Ferry and Tehama. However, both Cd and Cu exhibited a small, but significant increase in concentration at Bend Bridge. Lead concentrations were not significantly different among stations in the Sacramento River, although concentrations declined by 40 percent between Rodeo Park and Churn Creek (fig. 3). Cytosolic Al concentrations ranged between 8 and 11 µg/g in the reach between Rodeo Park and Balls Ferry, and then declined significantly to 6 µg/g at Bend

Bridge and Tehama. The distribution pattern of Zn contrasted to that of the other metals. Zinc concentrations at Rodeo Park and Tehama were the same and significantly lower than concentrations at Churn Creek, Balls Ferry, and Bend Bridge. The highest Zn concentration occurred at Bend Bridge.

Discussion

Metal concentrations of *Hydropsyche* samples taken in October 1996 from the Sacramento River indicated the presence of metal contamination between Redding (Rodeo Park) and Tehama (368 km from river mouth). Tehama is approximately 120 km downstream of the Keswick Dam. Furthermore, analysis of metal accumulation in the cytosol of *Hydropsyche* verified that Al, Cd, Cu, Pb, and Zn were present in biologically available forms. Only Fe and Hg were not consistently higher in Sacramento River samples than samples from the reference site, Cottonwood Creek.

Whole-body concentrations comprise a variety of metal forms accumulated by different processes. Not all of these forms may represent metal that is biologically available (i.e. transported across cell membranes). Differences in the partitioning of different metals between the cytosol and particulate (pellet) fractions in this study were consistent with results for the same genus reported by Cain and Luoma (1998) and are probably indicative of the location and form of metal within the animal. For example, the majority of Al, Fe, and Pb occurred with the pellet fraction. Although the pellet was not further characterized, its content would include the exoskeleton, undigested gut content, cell membranes, larger intracellular organelles and insoluble intracellular granules. Other studies have shown that substantial quantities of Al and Fe may be sorbed to external body surfaces (Cain and others, 1992; Boggs, 1994), where they might provide binding sites for other metals. Lead and Fe occur together as external contaminants (Krantzberg and Stokes, 1988; Hare and others, 1989; 1991b). In undepurated samples, the undigested gut content can also contribute variable amounts of metal to the body (Smock, 1983; Gower and Darlington, 1990; Hare and others, 1989; 1991b; Cain and others, 1995). This material can be rich in Al and Fe due to the inadvertent ingestion of sediment particles. Trace metals may also be associated with gut content. However, a large portion of the total Cd, Cu, and Zn appears to be stored either on cell membranes or intracellularly (Timmermans and Walker, 1989; Gower and Darlington, 1990; Cain and Luoma, 1998). The gut epithelium, malphigan tubules, fat body, anal papillae, and cytosol are major accumulation sites for Cd, Cu and Zn in insects (Sohal and others, 1976; Marshall, 1983; Suzuki and others, 1984; 1988; Seidman and others, 1986; Krantzberg and Stokes, 1990; Hare and others, 1991a). Metals may also be incorporated into intracellular granules as a product of metal detoxification (Darlington and Gower, 1990).

Comparative analysis of metal concentrations in the whole body, cytosol, and pellet resolved ambiguities in assessing metal bioavailability. There was no correspondence between concentrations of Al, Fe, and Pb in the whole body (and particulate fraction) and in the cytosol. Whole-body concentrations of Pb were generally indicative of differences in cytosolic Pb between uncontaminated (Cottonwood Creek) and contaminated (Sacramento River) samples, but not among samples in the Sacramento River. It is possible the relatively narrow range of low concentrations in the Sacramento River influenced this relationship. Significant correlations between whole-body and cytosolic Pb have been observed where Pb contamination is greater than in the Sacramento River (Cain and Luoma 1998). Whole-body concentrations of Al, Fe, and Pb, reflecting metal associated with the particulate fraction, tended to either increase downstream or showed no spatial trend. On the other hand, the small fraction of the total Pb and Al associated with the cytosol exhibited some downstream attenuation, more like the spatial patterns of Cd and Cu. Whole-body concentrations of Cd, Cu, and Zn were generally indicative of spatial patterns of metal bioavailability because a large proportion of the total body burden was accumulated in the cytosol.

Differences in the biological availability of metals indicated by *Hydropsyche* appear consistent with water chemistry data. In the upper section of the

Sacramento River (below Shasta Dam to Bend Bridge), Cd, Cu, and Zn are transported largely in dissolved phase, whereas, Al, Fe, and Pb are transported principally as colloids (Alpers and others 2000a; 200b). Uptake of dissolved Cd, Cu, and Zn could explain the accumulation of these metals in the cytosol, although it does not exclude the possibility of uptake from food, also. The low concentrations of Al, Fe, and Pb in the cytosol relative to the whole body, and similarities in spatial patterns of whole-body concentrations of these elements, particularly downstream of Balls Ferry, suggests that these elements accumulated by similar processes, possibly by sorption of colloidal metals to external body parts and/or inadvertent ingestion of colloids and sediment.

Metals enriched in the cytosol of samples from the Sacramento River differed with respect to the degree of enrichment and downstream attenuation pattern. Cadmium showed the highest degree of enrichment in both the whole body and cytosol. At Rodeo Park (Redding), cytosolic Cd was 18 times greater than the reference concentration. Concentrations decreased by about 70 percent between Rodeo Park and Tehama; however, at Tehama, concentrations were still 5 times greater than at Cottonwood Creek, suggesting the transport of bioavailable Cd extended farther downstream than Tehama. The bioaccumulation pattern of Zn differed from the other elements considered. Cytosolic (and whole body) Zn did not attenuate downstream, although concentrations in samples from all five Sacramento River sites were consistently higher than in the sample from Cottonwood Creek. A similar pattern in cytosolic Zn has been observed in *Hydropsyche* in the Clark Fork river, Montana (Cain and Luoma, 1998). Differences in metal accumulation patterns reflect the combination of geochemical conditions affecting metal transport and bioavailability, and biological processes of metal uptake, distribution among tissues in the body, and loss.

Saiki and others (1995) previously showed that Cd, Cu, and Zn contaminated sediments, detritus, plants, benthic insects, and fish in the reach between Redding and Bend Bridge. Although that study did not sample *Hydropsyche*, the general pattern of metal contamination in other benthic invertebrates that were collected were similar to our results. The distribution patterns of Cd, Cu, and (cytosolic) Pb and Al in *Hydropsyche* were consistent with known sources of metals from the East Shasta and West Shasta mining districts, upstream of Redding (Nordstrom and others, 1977; Alpers and others 2000a; 2000b). It is possible that unmeasured urban sources at Redding could affect the observed distribution pattern.

Biological and water chemistry data indicate a secondary, unidentified source(s) of metal to the Sacramento River downstream of Keswick Reservoir. In this study, concentrations of Cd, Cu, and Zn increased in caddisflies between Balls Ferry (river km 444) and Bend Bridge (river km 415). Saiki and others (1995) also noted increases in Cd and Zn concentrations in aquatic plants, mayflies and midges (Zn only) from Lake Redding (near our station at Rodeo Park) to a station located between Balls Ferry and Bend Bridge. Sediment and detritus, however, did not show this pattern. Water samples collected on six occasions between July 1996 and June 1997 below Keswick Dam, at Bend Bridge, and at several other locations along the Sacramento River also indicated likely sources of Cd, Cu, and Zn loading to the Sacramento River between Keswick Dam and Bend Bridge (Alpers and others, 2000a; 2000b). Several tributaries, including Cottonwood Creek, Battle Creek, Mill Creek, Churn Creek, and Cow Creek, enter the Sacramento River below Keswick Reservoir (fig. 1). Metal loads to the Sacramento River from these tributaries have not been quantified, so it is not possible to determine if any of them contributed to metal exposures to *Hydropsyche* in the Sacramento River downstream of Balls Ferry. However, it seems unlikely that either Cottonwood Creek or Battle Creek - the only tributaries that enter the Sacramento River downstream of Balls Ferry – would be significant sources of metals since neither showed any metal enrichment in sediment and biological samples (Saiki and others, 1995; Alpers and others, 2000a; 2000b; this study).

The increase in trace metal concentrations in *Hydropsyche* at Bend Bridge might also be related to increases in

metal-enriched suspended particles in the reach of the Sacramento River between Keswick Reservoir and Bend Bridge. Suspended colloids were relatively abundant in whole-water samples taken at Bend Bridge. As discussed previously, these colloids represent a dominant form of Al, Fe, and Pb in the water column, and also contain Cu, Zn, and Cd (Alpers and others, 2000a; 2000b). Coincidentally, the body surfaces of *Hydropsyche* collected from Bend Bridge appeared to be fouled with fine particles and detritus. Contamination of the insect exoskeleton with colloids could explain increases in whole-body and particulate metal concentrations downstream of Balls Ferry. The increase in cytosolic metals is harder to understand. Colloids ingested with larger food particles might contribute to cytosolic Cd, Cu, and Zn if some fraction of the colloidal metal was assimilated during digestion. Sequential chemical extraction of colloids collected from the mainstem of the Sacramento River in May-June 1997 indicated that Cd was mainly associated with a reducible (iron-manganese oxide) phase, and copper and Zn were about evenly distributed between the reducible and a residual (refractory) phase, with a minor fraction present in an oxidizable phase (Alpers and others, 2000a; 2000b). If the reducible phase is more bioavailable, this could explain why the increase in cytosolic Cd was relatively greater than Cu and Zn.

Metal exposures to *Hydropsyche* in the Sacramento River can be placed into a general context by comparison to rivers in other basins. A fairly extensive data set is available from the Clark Fork, a mining-impacted river in Montana. Over a 7-year period, annual Cd concentrations (whole body) in *Hydropsyche* sp. from the most heavily contaminated reach of the Clark Fork varied from approximately 1.5 to 3 µg/g (Hornberger and others, 1997). Cytosolic Cd concentrations in this same area ranged from approximately 0.25 µg/g to 1.5 µg/g (Cain and Luoma, 1998). In the Sacramento River at Rodeo Park near Redding, Cd concentrations of the whole body were 2.16 µg/g, of which 59 percent (1.27 µg/g) was recovered in the cytosol. Thus, Cd concentrations at Rodeo Park were similar to some of the highest concentrations observed in the Clark Fork. Concentrations of Cu, Pb and Zn in the Sacramento River appear to be indicative of moderate contamination. Whole-body concentrations were less than three times greater than those from Cottonwood Creek, but were lower than the upper ranges for whole-body concentrations in the Clark Fork (200-300 µg/g Zn, 150-200 µg/g Cu, and 5-10 µg/g Pb) (Cain and others, 1992; Lambing and others, 1995; Hornberger and others, 1997).

Cadmium, Cu, Pb, and Zn concentrations in samples from Cottonwood Creek are representative of concentrations reported for uncontaminated rivers. For example, typical whole-body concentrations in *Hydropsyche* spp. from uncontaminated tributaries of the Clark Fork were 0.2 µg/g of Cd, 10-20 µg/g of Cu, 2 µg/g of Pb, and 120 µg/g of Zn (Cain and others, 1992; Lambing and others, 1995). Whole-body concentrations in *Hydropsyche californica* for unpolluted tributaries in the Yakima River Basin did not exceed 0.12 µg/g of Cd, 14 µg/g of Cu, 1 µg/g of Pb, and 140 µg/g of Zn (Fuhrer and others, 1994).

Mercury concentrations in *Hydropsyche* in the upper Sacramento River were low (< 0.1 µg/g) compared to other locations in California. In streams in the coastal mountains and Sierra Nevada foothills where Hg was either mined or used for gold mining, Hg concentrations exceed 0.1 µg/g in hydropsychid caddisflies (Slotton and others, 1997a; 1997b). Mercury levels in *Hydropsyche* in the upper Sacramento River appear to reflect the absence of significant Hg sources in the upper drainage of the river. Roth and others (U.S. Geological Survey, written commun., 1999) concluded that Hg sources upstream of Red Bluff, including drainage from Iron Mountain Mine, contributed only a minor fraction of the total Hg load to the lower Sacramento River. The major Hg sources appeared to occur downstream of the section that we sampled.

Characterizing chemical exposures is one step in identifying potential risks to biological resources. Studies to date have indicated that metals contaminate a large segment of the aquatic community in the upper Sacramento River (Saiki and others 1995; this study). Cadmium, Cu, Pb, and Zn all showed some degree of enrichment in biological samples. As discussed above, concentrations of Cu, Pb, and Zn in *Hydropsyche* sp. appear to be moderately high, but Cd enrichment exceeded

representative reference concentrations by nearly 20 fold at Rodeo Park (Redding). A large proportion (>50 percent) of the Cd was accumulated in the cytosol. This finding may have important implications for the trophic cycling of Cd in the upper Sacramento River. Metals associated with the cytosol of algal and invertebrate food items are taken up across the gut wall during digestion (Reinfelder and Fisher, 1991; 1994; Wallace and Lopez, 1996). Other studies have shown metal accumulation and toxic effects in trout fed benthic invertebrates contaminated with metals (Woodward and others 1995), although the form(s) of bioavailable metal in the meal was not identified. However, it is possible that the cytosol represents a dietary source of Cd to the fish.

Summary

The results of this study reaffirm the concern for the biological fate of metals in the Sacramento River. Caddisfly larvae cytosol data show that elevated concentrations of bioavailable Al, Cd, Cu, Pb, and Zn are present in the Sacramento River. The cytosol was a major accumulation site for Cd, Cu, and Zn, but represented only a small fraction of the total body burden of Al and Pb. The downstream concentration patterns indicated a primary upstream source of Al, Cd, Cu, and Pb near or upstream from Redding (river km 479), consistent with documented sources of metals from the East Shasta and West Shasta mining districts. Exposures to Cd, Cu, and Zn appeared to increase moderately in the reach between Balls Ferry (river km 444) and Bend Bridge (river km 415). The data did not delineate the downstream extent of general contamination or of bioavailable metals; however, at the time of sampling, elevated exposures to bioavailable metals were evident at Tehama (river km 368), 120 km downstream of the Keswick Dam.

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Appendices

Appendix 1. The names, numerical designations, and locations of stations sampled for *Hydropsyche californica* in October 1996. [R, River; A, at; NR, near; CA, California; AB, above; C, Creek]

Station Name	Station number	Station ID	Latitude	Longitude	River Mile (km)
SACRAMENTO R A RODEO PARK NR REDDING CA	1	403528122224301	403528	1222243	298 (479)
SACRAMENTO R AB CHURN C NR ANDERSON CA	2	402827122185801	402827	1221858	285 (459)
SACRAMENTO R A BALLS FERRY CA	3	402507122113201	402507	1221132	276 (444)
SACRAMENTO R AB BEND BRIDGE NR RED BLUFF CA	4	11377100	401719	1221108	258 (415)
SACRAMENTO R A TEHAMA CA	5	400139122070301	400139	1220703	229 (368)
COTTONWOOD C NR COTTONWOOD CA	6	11376000	402314	1221415	273 (439)

Appendix 2. Operating conditions for inductively coupled plasma-mass spectrometer. [L/min, liter per minute; MHz, Megahertz; w, watt; msec, millisecond]

Argon flow rates (L/min)	
Plasma coolant	15
Auxilary	0.8
Nebulizer	0.87
Generator	
Frequency	40 MHz
Power	1065 w
Torch/Interface	
Injector	Al_2O_3
Sampler cone	Ni
Skimmer cone	Pt
Spectrometer	
Reading	1
Sweeps/reading	3
Replicates	5
Dwell time	175 msec
Measured isotopes	
Al	27
Cd	111
Cu	65
Pb	208
Zn	66

Appendix 3. Comparison of metal concentrations determined in the study with certified values reported by the National Institute of Standards and Technology for SRM 1566a (oyster tissue) and SRM 50 (albacore tuna), and the National Research Council Canada for Tort – 2 (lobster hepatopancreas), Dorm – 2 (dogfish muscle). Reported concentrations are the mean ± 95 percent confidence limits. Observed concentrations are the mean ± standard deviation for n=2-6. Units are micrograms per gram (µg/g) dry weight.

Metal	NIST 1566a		NIST 50		Tort –2		Dorm - 2	
	Reported	Observed	Reported	Observed	Reported	Observed	Reported	Observed
Aluminum	202.5 ± 13	136 ±7		6.26 ± 1.4				
Cadmium	4.15 ± 0.4	4.35 ±0.10		0.061 ± 0.02				
Copper	66.3 ± 4.3	61.7 ±2.6		3.02 ± 0.2				
Iron	539 ± 15	518 ± 19		52 ± 1				
Lead	0.371 ± 0.01	0.37 ± 0.06	0.46	0.53 ±0.1				
Mercury	0.06 ± 0.01	0.06			5.6 ± 0.7	5.6	4.26 ± 0.26	4.0
Zinc	830 ± 57	824 ± 21	13.6±1	13.8 ± 0.7				