

Metal exposure in a benthic macroinvertebrate, *Hydropsyche californica*, related to mine drainage in the Sacramento River

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Abstract: A biomonitoring technique was employed to complement studies of metal transport in the upper Sacramento River affected by acid mine drainage. Metals (Al, Cd, Cu, Fe, Hg, Pb, and Zn) were determined in a resident invertebrate, *Hydropsyche californica* (Insecta: Trichoptera), and streambed sediments (<62 µm) to assess metal contamination within a 111-km section of the river downstream of the mining area. Metals in *H. californica* also were interpreted to be broadly indicative of metal exposure in fish. Total Hg was determined in the whole body of the insect, whereas Al, Cd, Cu, Fe, Pb, and Zn were additionally separated into operationally defined cytosolic (used as an indicator of exposure to bioavailable metal) and particulate fractions. Total concentrations of Cd, Cu, Hg, Pb, and Zn in sediments were consistent with documented upstream sources of acid mine drainage. Metal distribution patterns in *H. californica* and sediments were generally consistent for Cd, Cu, and Pb but inconsistent for Hg and Zn. Concentrations in *H. californica* indicated that bioavailable Cd, Cu, Pb, and Zn was transported at least 120 km downstream of the mine sources. Zinc in *H. californica* was elevated, but unlike sediments, did not decrease downstream. Mercury in *H. californica* was not elevated.

Résumé : Une technique de biosurveillance a été utilisée pour compléter des études sur le transport de métaux dans le cours supérieur de la rivière Sacramento contaminée par l'eau d'exhaure acide. Les métaux (Al, Cd, Cu, Fe, Hg, Pb et Zn) ont été mesurés chez un invertébré résident, *Hydropsyche californica* (Insecta : Trichoptera), et dans les sédiments du lit (<62 µm) afin d'évaluer la contamination par les métaux dans un tronçon de 111 km de la rivière en aval de la région minière. La présence de métaux chez *H. californica* a aussi été interprétée comme une indication générale d'exposition des poissons à ces métaux. La concentration totale de Hg a été mesurée dans tout le corps des insectes, tandis que Al, Cd, Cu, Fe, Pb et Zn ont en outre été séparés en fractions cytosolique (utilisée comme indicateur de l'exposition au métal biodisponible) et particulaire définies opérationnellement. Les concentrations totales de Cd, Cu, Hg, Pb et Zn dans les sédiments concordaient avec l'information connue sur les sources d'eau d'exhaure acide en amont. Les profils de répartition des métaux chez *H. californica* et dans les sédiments étaient en général cohérents dans les cas du Cd, du Cu et du Pb, mais irréguliers dans le cas de Hg et de Zn. Les concentrations chez *H. californica* ont montré que le Cd, le Cu, le Pb et le Zn biodisponibles étaient transportés à au moins 120 km en aval des sources minières. La concentration de Zn chez *H. californica* était élevée, mais à la différence de ce qu'on observait dans les sédiments, elle ne diminuait pas en aval. La concentration de Hg chez *H. californica* n'était pas élevée.

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Introduction

Metal contamination of freshwaters by mining is widespread (Moore and Luoma 1990). While total metal concentrations in environmental media (e.g., water and sediment) indicate the relative degree and extent of contamination, they

do not necessarily reflect exposures in resident fauna. Benthic insects are one group of organisms used to monitor metal exposures and assess biological effects in freshwaters (Cain et al. 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 and 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.

Metals analysis of the cytosol and (or) other intracellular components provides an unambiguous indicator of metal bioavailability. The cytosol appears to be an important accumu-

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lation site for essential metals such as Cu and Zn and certain nonessential elements, including Cd (Seidman et al. 1986; Cain and Luoma 1998; Suzuki et al. 1988). Furthermore, sublethal effects have been shown to coincide with a redistribution of Cd among cytosolic ligands that occurs with increasing Cd accumulation (Jenkins and Mason 1988). Therefore, concentrations of cytosolic metals reflect intracellular dose and may be a better diagnostic of toxicity than either whole-body or whole-tissue concentrations (Thorpe and Costlow 1989; Roesijadi 1994).

Cytosolic metal also 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 Ca 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.

The upper Sacramento River is affected by acid mine drainage. Metal contamination of the aquatic food web has been documented (Wilson et al. 1981; Saiki et al. 1995), but the downstream extent of metal contamination has not been fully resolved, and little is specifically known about the biological availability of metals within the river. The upper Sacramento River is of special concern because it includes spawning ground for several salmonid fish species, including four distinct runs of chinook salmon (*Oncorhynchus tshawytscha*), steelhead trout (*Oncorhynchus mykiss*), and resident rainbow trout (*O. mykiss*) (U.S. Environmental Protection Agency 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 have recently been listed as threatened species (National Oceanic and Atmospheric Administration 1994, 1997).

This study was one component of a multidisciplinary study of the distribution, transport, and fate of metals in the Sacramento River. Detailed studies specific to metal geochemistry and transport are reported elsewhere (Alpers et al. 1999). Here, the principal objective was to assess the occurrence and distribution of biologically available metals in the upper Sacramento River, relative to sediment metal contamination, downstream of documented sources of acid mine drainage. Also, because of the concern for resident fish, there was a need for data that were at least broadly indicative of dietary metal exposure in fish.

To satisfy our objectives, metal concentrations were determined in larvae of the hydropsychid caddisfly *Hydropsyche californica*. The genus *Hydropsyche* is widely distributed and abundant in many rivers, including the Sacramento River. The larva lives for about 1 year as a sedentary, omnivorous filter-feeder. Therefore, metal concentrations in the larva are site specific, and the period of exposure is on the

order of 1 year or less. Larvae of *Hydropsyche* are relatively metal tolerant (Spehar et al. 1978; Clements et al. 1992), thereby making them a good organism for monitoring metal contamination (Cain et al. 1992). Consumption of metal-contaminated benthic macroinvertebrates, such as *Hydropsyche*, can be a significant cause of chronic metal contamination in resident trout (Farag et al. 1995; Woodward et al. 1995), although studies to identify the form(s) of metal absorbed during digestion have not been conducted. We suggest that an analysis of the metal partitioning in prey species of fish may help identify metals most likely to be accumulated from food.

Methods

Site description

Drainage from base-metal mines at Iron Mountain has been a principal source of metals to the Sacramento River in northern California that has threatened resident fauna for many years (Finlayson and Verrue 1980; Wilson et al. 1981; National Oceanic and Atmospheric Administration 1989). Recurring fish kills instigated the construction of the Spring Creek Debris Dam (SCDD) in 1963 to reduce 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 3–4 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% and Zn (and probably Cd also) by about 90%. Prior to lime-neutralization treatment, about 90% 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, Calif., unpublished data). Since 1994, this component of the overall Cu loading has been reduced to about 50%. 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).

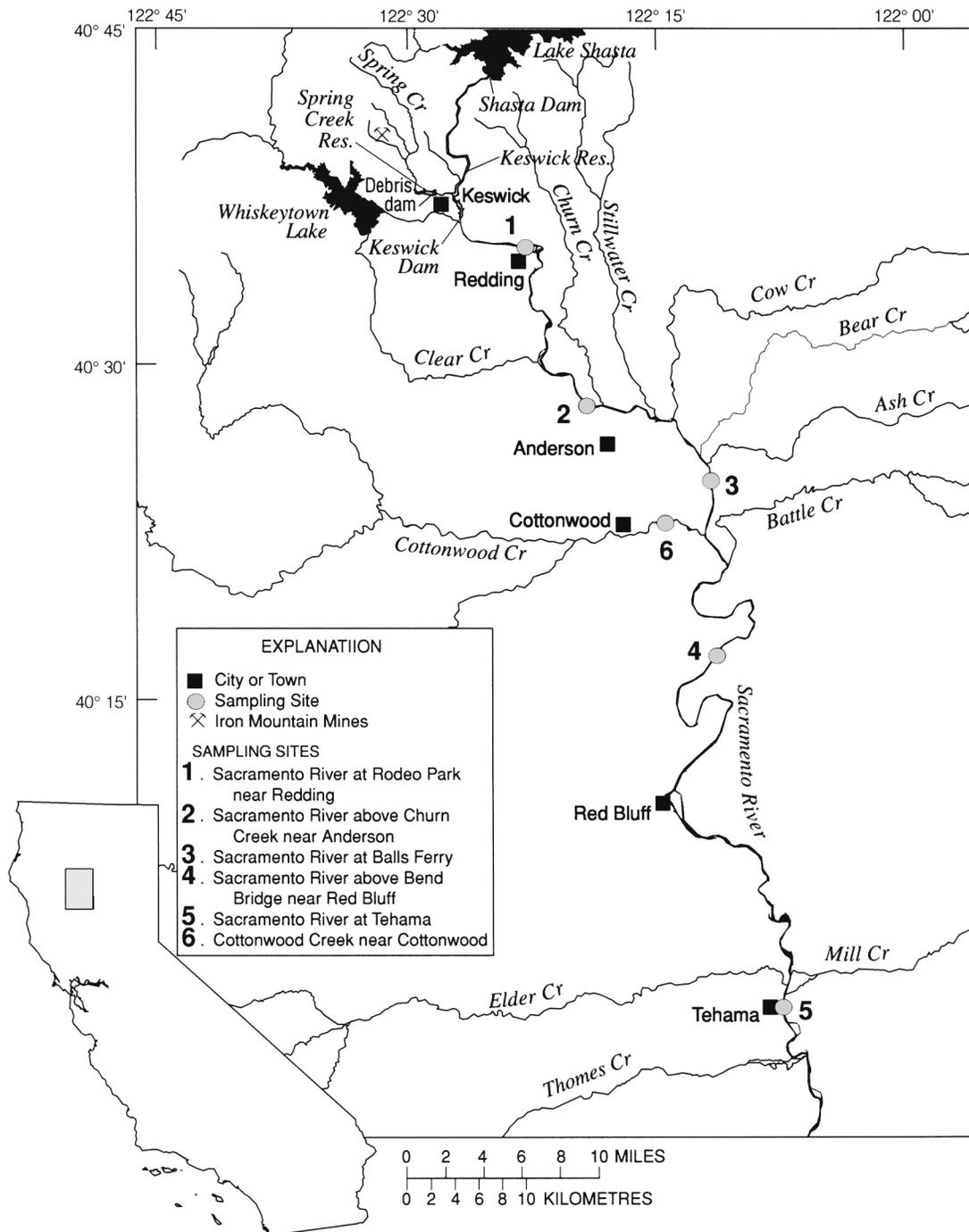
Flows from the SCDD are regulated by the Bureau of Reclamation, together with dilution flows from Shasta Lake and Whiskeytown Lake (via the Spring Creek Power Plant), to meet water quality criteria for the protection of resident fish at a compliance point below Keswick Dam (Fig. 1). Nevertheless, these water quality criteria are periodically exceeded during periods of heavy rainfall when water in the Spring Creek Reservoir overtops the SCDD and insufficient dilution flows are available.

Sample collection

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). Station locations were determined by the availability of suitable habitat and proximity to stations where water quality data were collected for related studies of metal transport. High seasonal flows and managed releases of water from Shasta Dam prohibited sampling during much of the year. Samples for this study were collected during October 21–23, 1996, coincident with seasonal lows in discharge, sediment transport, and metal loading. In addition, a sample was collected from Cottonwood Creek, near Cottonwood (river km 439) (Fig. 1), and used as a local reference to evaluate metal levels in samples from the Sacramento River.

At each station, *Hydropsyche* larvae were collected from a single, shallow (<0.5 m deep) riffle using large kick nets constructed of cotton mesh (approximately 1-mm mesh size) and plastic (PVC) handles. Specimens were picked from the net with nylon forceps

Fig. 1. Names, numerical designations, and locations of stations in the upper Sacramento River and Cottonwood Creek where *H. californica* and sediments were collected in October 1996.



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 h of collection. The field collections were moved to the laboratory where they were stored at -70°C until sample preparation. Specimens for taxonomic identification were preserved in 10% formalin in the field and transferred to 75% ethanol in the laboratory. Collections contained a single species, *H. californica*.

Sample preparation

Samples for the determination of Al, Cd, Cu, Fe, Pb, and Zn

were prepared following the method described by Cain and Luoma (1998). Specimens collected from a station 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. *Hydropsyche californica* were collected for metals analysis. Instars 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 (1–2 h), the animals were blotted dry with tissue paper, pooled into replicate, composite

samples ($n = 4-6$) of approximately the same wet weight, and then temporarily refrigerated.

Cold 0.05 M Tris-HCl buffer (pH 7.4, previously degassed and bubbled with N_2) was added to each sample at a ratio of 8:1 (millilitres of Tris per gram wet weight of subsample). Samples were homogenized with a stainless steel high-speed tissue homogenizer under a nitrogen atmosphere for 1 min. The homogenate was subsampled for 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 $100\,000 \times g$ for 1 h at $5^\circ C$. 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 $-20^\circ C$ as they were prepared. Later, they were freeze-dried, weighed, and digested by reflux in hot, isopiestically distilled 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 mL of 1% high-purity HNO_3 . Five millilitres of this solution was diluted to 50 mL for trace metal analysis.

All plastic and glassware used for sample preparation was cleaned by soaking overnight in a Micro⁶² solution, rinsed with deionized water, and then washed in either 10% HCl or 10% HNO_3 and rinsed with deionized water. The tissue homogenizer was cleaned by soaking overnight in a solution of RBS[®] and rinsed in deionized water.

Total Hg concentrations were determined in the whole body only to assess the occurrence of Hg contamination. Additional samples from all stations were sorted and cleaned as described above. Station samples were composed of single or duplicate composites, which were immediately frozen at $-20^\circ C$. The composites 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).

Streambed sediments

Streambed sediment was collected from three to six depositional areas within 100 m at each station following procedures developed for the U.S. Geological Survey's National Water Quality Assessment Program (Shelton and Capel 1994). The sediment was scooped from the surface (<1 cm depth) with an acid-washed plastic spatula and composited into an acid-washed 8-L container. The sediment and associated river water were homogenized and then sieved through a $62\text{-}\mu m$ nylon mesh. The sediment passing through the mesh was collected in acid-washed, plastic screw-cap jars and then chilled on ice for transport to the laboratory. Sediments were transferred to a freezer ($-5^\circ C$) until further processing. The sediment was freeze-dried for 24 h, weighed, and then digested in a microwave with a mixture of HCl, HNO_3 , and HF. Prior to analysis, H_3BO_3 was added to the solutions to complex excess fluoride.

Metals analysis

Metals were determined using several different techniques. Aluminum, Cd, Cu, Pb, and Zn were determined on the digested samples by inductively coupled plasma - mass spectrometry using a modification of a direct analysis procedure (Taylor and Garbarino 1991). Iron was determined by a modified inductively coupled plasma - atomic emission spectrometric technique (Taylor and Garbarino 1985) at a wavelength of 259.94 nm. Mercury in the caddisflies was determined by cold-vapor atomic absorption spectrophotometry using conditions described by Elrick and Horowitz (1986). Mercury in streambed sediments was determined by automated cold-vapor atomic fluorescence as described by Roth (1994).

Quality assurance

Laboratory determinations of Al, Cd, Cu, Fe, Pb, and Zn were

performed in triplicate on each composite sample. Single or duplicate determinations of Hg were performed. Standard deviations reported for concentrations of each station represent the combined precisions associated with sample collection, processing, and analysis of the composite samples.

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 of Canada (NRC). Four materials were selected to simulate invertebrate tissue: NIST SRM 1566a (oyster tissue), NIST SRM50 (albacore tuna), NRC Tort-2 (lobster hepatopancreas), and NRC Dorm-2 (dogfish muscle). NIST 2704 (Buffalo River sediment) was used for streambed sediments. Standards were processed in a manner identical to the procedure used for the samples. The medians of the observed concentrations for the analysis of the biological reference materials ranged from 92 to 105% of the reported concentrations except for Al (68%) and Pb in SRM 50 (120%). In addition, selected representative caddisfly 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% (95-102) for Cd, 94% (82-100) for Cu, 93% (87-98) for Pb, and 96% (93-108) for Zn. Median recoveries of total digests of NIST 2704 ranged from 93 to 107% of the certified concentrations.

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

Metal concentrations in streambed sediments are reported as the mean and standard deviation ($n = 3$). The mean, standard deviation, and standard error of composite caddisfly samples ($n = 4-6$ for all samples except those analyzed for Hg where $n = 1$ or 2) from each station are reported. The number of samples generally reflected the abundance of *H. californica* at each station. Tissue 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 percentage 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 Kruskal-Wallis ANOVA. Pearson product-moment correlations were determined between whole-body, particulate, 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 than among-station variance). Correlation analysis also was done between metal concentrations in *Hydropsyche* and streambed sediments. Interelement correlations were performed separately on tissue and sediment metal concentrations among stations to assess similarities in the spatial distributions of metals within each type of sample. Results of statistical tests were considered significant if $\alpha < 0.05$.

Results

Metal enrichment in *Hydropsyche*

Mean concentrations of Cd, Cu, Pb, and Zn in the whole body, pellet, and cytosol of *Hydropsyche* from all stations in

² Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Table 1. Metal concentrations ($\mu\text{g}\cdot\text{g}^{-1}$ dry weight⁻¹) in *H. californica* collected from the Sacramento River and Cottonwood Creek during October 21–23, 1996.

Element	Body fraction	Station						EF
		1 (Rodeo Park)	2 (Churn Creek)	3 (Balls Ferry)	4 (Bend Bridge)	5 (Tehama)	6 (Cottonwood Creek)	
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±0.01	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±0.01	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

Note: Values are the mean \pm 1 SD ($n = 1-6$). Metal enrichment factors (EF) in the Sacramento River (metal concentration of Sacramento River station/metal concentration in Cottonwood Creek) are shown. NE, not enriched (i.e., EF < 1).

the Sacramento River were significantly greater than in those from Cottonwood Creek, the reference site (Table 1). Aluminum concentrations in the cytosol also were consistently higher in samples from the Sacramento River; however, concentrations in the whole body (and pellet) were not significantly different from those in the Cottonwood Creek sample. Iron concentrations in all body fractions in Sacramento River samples were variable but were not either uniformly higher or lower than concentrations in the Cottonwood Creek sample. Mercury concentrations in samples from the Sacramento River were $\leq 0.06 \mu\text{g}\cdot\text{g}^{-1}$ and lower than the concentration in the sample from Cottonwood Creek. Among the metals analyzed, Cd showed the greatest degree of enrichment, regardless of body fraction. Enrichment factors for other metals in the whole body and pellet followed the order $\text{Cu} > \text{Pb} > \text{Zn} > \text{Al}$. Relative to Cottonwood Creek, Al and Pb were more enriched in the cytosol than in the whole body, and therefore, the order of enrichment was $\text{Cd} > \text{Pb} > \text{Al} \geq \text{Cu} > \text{Zn}$.

Metal partitioning in the insect

Whole-body concentrations represent the accumulation of both cytosolic and noncytosolic, particulate metal forms. (Particulate metal forms are operationally defined as metal retained in the pellet after ultracentrifugation.) Partitioning between cytosolic and particulate forms differed greatly among different groups of elements. The cytosol was an important accumulation site for Cd, Cu, and Zn, accounting for approximately 50–100% of the total body burden of these elements (Table 2). Much lower percentages ($\leq 20\%$) of the body burdens of Al, Fe, and Pb occurred in the cytosol (Table 2). Particulate form(s) accounted for more than 99% of

the total Al body burden and at least 85% of the total Fe body burden. The proportion of particulate Pb ranged between 80 and 92%.

As expected, the results of correlations between the whole-body and cytosolic and particulate metal concentrations were affected by partitioning of metal in the insect. 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), and therefore, whole-body Cd, Cu, and Zn concentrations were indicative of exposures to biologically available metal. Concentrations in the particulate fraction, which represented up to 53% of the body burden, also correlated with the whole-body concentrations (results not shown). Aluminum and Fe concentrations in the whole body did not correlate with cytosolic Al and Fe concentrations. Whole-body and cytosolic concentrations of Al were negatively correlated among stations in the Sacramento River ($p = 0.04$) (Fig. 2). Cytosolic Fe concentrations were independent of whole-body concentrations. A weak, positive relationship between whole-body and cytosolic Pb was influenced by the low concentrations in the Cottonwood Creek sample (Fig. 2). Among samples from the Sacramento River, whole-body Pb concentrations were not predictive of cytosolic Pb concentrations (Fig. 2). For Al, Fe, and Pb, concentrations in the whole body and particulate fraction were highly correlated ($p \leq 0.02$), reflecting the dominant accumulation of these elements in noncytosolic forms.

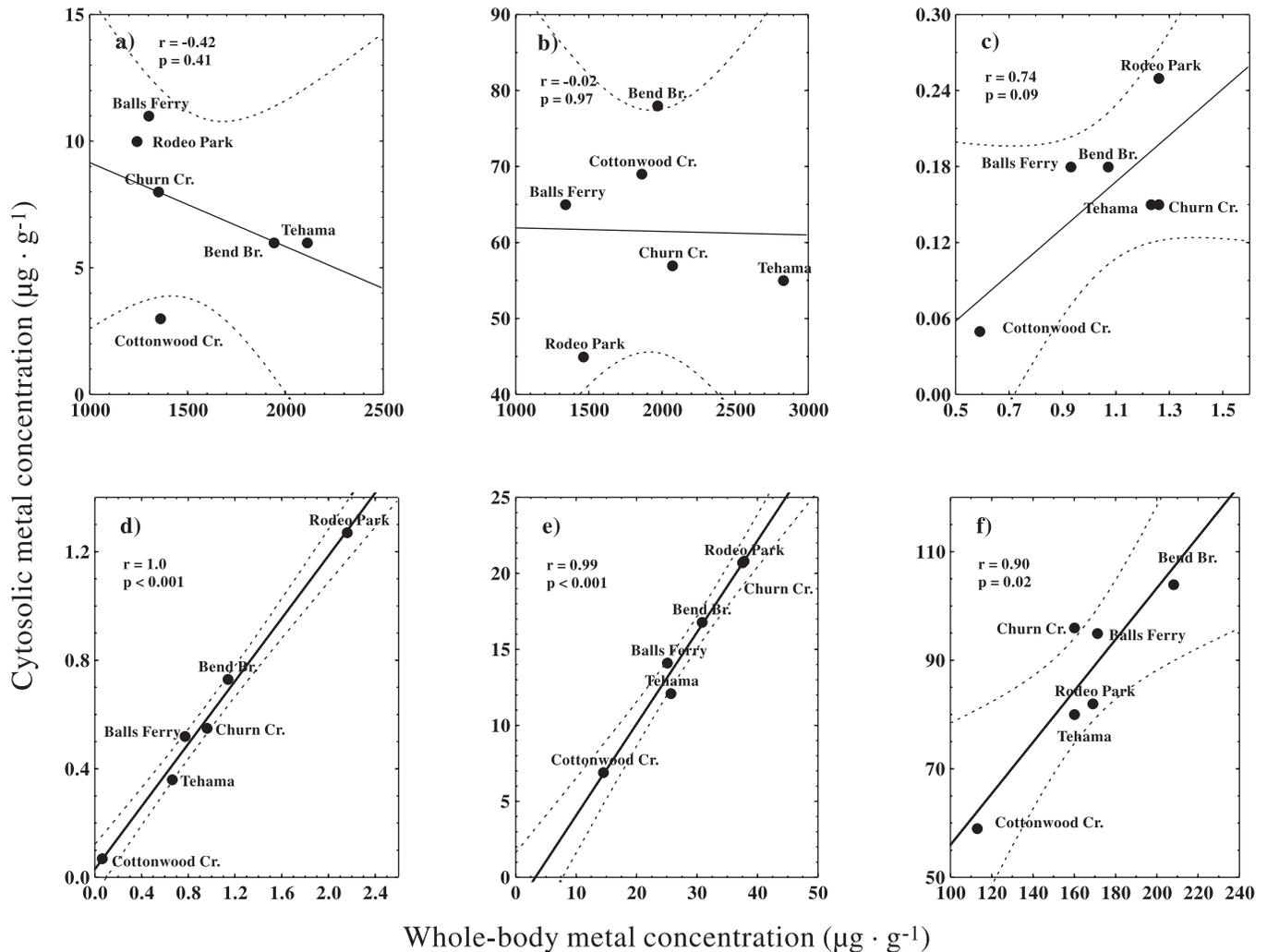
Spatial patterns in whole-body concentrations of Al, Fe, Pb, and Hg

As suggested above, spatial patterns in the concentrations of Al, Fe, and Pb in the whole body (and the particulate

Table 2. Mean percentage of total metal body burden recovered in the cytosol (calculated as cytosolic metal concentration/whole body metal concentration $\times 100$) of *H. californica* collected from the Sacramento River and Cottonwood Creek.

Element	Station					
	1 (Rodeo Park)	2 (Churn Creek)	3 (Balls Ferry)	4 (Bend Bridge)	5 (Tehama)	6 (Cottonwood Creek)
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

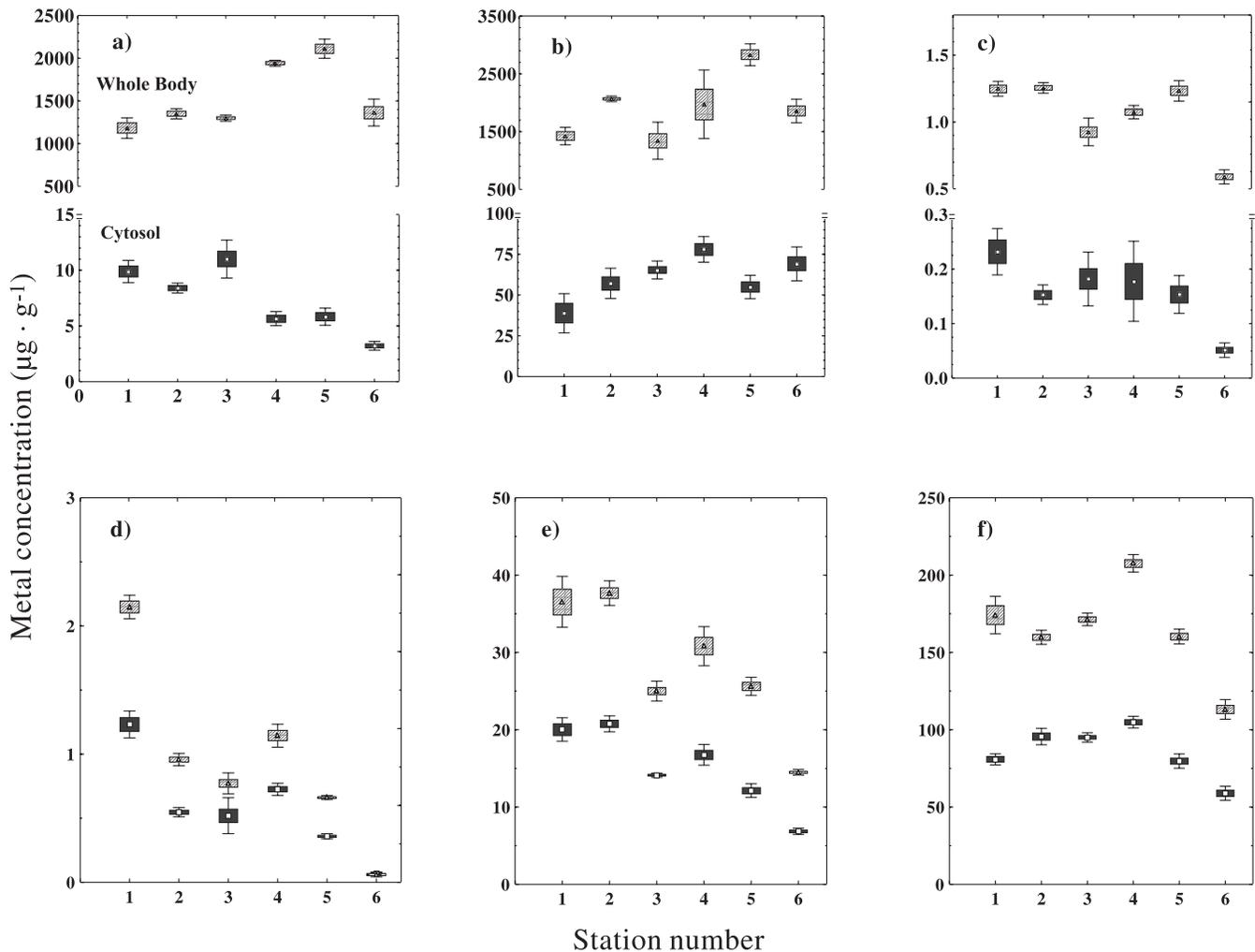
Fig. 2. Correlation between the mean metal concentrations in the whole body and cytosol of *H. californica*. Data are identified by station. (a) Al; (b) Fe; (c) Pb; (d) Cd; (e) Cu; (f) Zn. The correlation coefficient r and probability level p are given for each element. Data are fitted using a linear regression. Dotted lines are the 95% confidence intervals.



fraction) were distinctly different from those displayed in the cytosol. Whole-body concentrations of Al and Fe exhibited similarities in their longitudinal distribution within the Sacramento River that differed from the distributions of other elements. Concentrations between Rodeo Park and Balls Ferry (stations 1–3) were generally lower than concentrations at Bend Bridge (station 4) and Tehama (station 5) (Fig. 3). Between Balls Ferry and Tehama, concentrations of

Al and Fe in the whole body increased significantly. Lead concentrations were highest at Rodeo Park, Churn Creek, 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 and then progressively increased downstream to Tehama. The concentration pattern of Pb between Balls Ferry and Tehama closely resembled

Fig. 3. Metal concentrations in the whole body and cytosol of *H. californica* from stations in the upper Sacramento River and Cottonwood Creek (metal reference site). Stations in the Sacramento River are numbered sequentially (1–5) from upstream to downstream, as described in Fig. 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 \pm 1 SE (shaded box) and the mean \pm 1 SD (whiskers).



that of Fe. Mercury concentrations in samples collected from the Sacramento River were slightly less than in the Cottonwood Creek sample and did not exhibit any organized spatial pattern (Table 1).

Spatial patterns in enriched, cytosolic metals

Cytosolic metals differed in their distribution in the Sacramento River. Maximum concentrations of cytosolic Cd, Cu, Pb, and Al occurred in the samples from the three most upstream stations (Rodeo Park, Churn Creek, and Balls Ferry). From these stations, cytosolic metal concentrations decreased downstream to Tehama, although the attenuation patterns differed in some respects. Cadmium displayed the greatest attenuation in concentration, decreasing about 70% from Rodeo Park to Tehama. The majority (80%) of this decrease occurred between Rodeo Park and Churn Creek (Fig. 3). Copper concentrations decreased by roughly 40% between Rodeo Park and Tehama. Concentrations were similar at Rodeo Park and Churn Creek and then decreased significantly at Balls Ferry. Between Balls Ferry and Tehama, Cd and Cu concentrations followed one another closely. A

small, but significant increase in concentrations occurred at Bend Bridge; then, concentrations decreased at Tehama. Lead concentrations were not significantly different among stations in the Sacramento River, although concentrations decreased by 40% between Rodeo Park and Churn Creek. Cytosolic Al concentrations ranged between 8 and 11 $\mu\text{g}\cdot\text{g}^{-1}$ in the reach between Rodeo Park and Balls Ferry and then declined significantly to 6 $\mu\text{g}\cdot\text{g}^{-1}$ at Bend Bridge and Tehama. The distribution pattern of Zn contrasted with 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.

Metal concentrations in sediments and relationships with *Hydropsyche*

Concentrations of Cd, Cu, Hg, Pb, and Zn in streambed sediments (<62 μm) were typically greater in the Sacramento River than in Cottonwood Creek (Table 3). The maximum concentrations of Cd, Cu, Hg, and Zn at Churn Creek were three to nine times greater than concentrations in Cottonwood Creek, and enrichment of these metals was still evi-

Table 3. Metal concentrations ($\mu\text{g}\cdot\text{g}^{-1}$ dry weight⁻¹ except for Al and Fe, which are $\text{mg}\cdot\text{g}^{-1}$) in streambed sediments collected from the Sacramento River and Cottonwood Creek during October 21–23, 1996.

Element	Station					
	1 (Rodeo Park)	2 (Churn Creek)	3 (Balls Ferry)	4 (Bend Bridge)	5 (Tehama)	6 (Cottonwood Creek)
Al	76±0	67±1	84±2	75±2	58±2	70±0
Cd	2.7±0	3.6±0.2	1.5±0.1	0.9±0.2	1.3±0.1	0.4±0
Cu	240±10	250±0	100±0	75±2	78±2	61±2
Fe	56±1	56±2	61±2	49±2	48±2	50±2
Hg	0.40±0.01	0.39±0	0.15±0.01	0.08±0	0.25±0.02	0.06±0.01
Pb	36±2	36±1	24±0	14±1	14±1	14±0
Zn	410±10	550±20	310±10	220±10	230±10	110±0

Note: Values are the mean \pm 1 SD ($n = 3$).

Table 4. Pearson product-moment correlation coefficients r shown for correlations between sediments and *H. californica* (whole body and cytosol) collected from the Sacramento River and Cottonwood Creek during October 21–23, 1996.

Element	Correlation coefficient r	
	Sediment \times whole body	Sediment \times cytosol
Al	-0.57	0.57
Cd	0.51	0.46
Cu	0.83*	0.84*
Fe	-0.75	-0.31
Hg	-0.31	—
Pb	0.54	0.56
Zn	0.26	0.50

Note: The mean metal concentrations from all stations were used in the analysis. Significant correlations ($p < 0.05$) are indicated with an asterisk.

dent in sediment at Tehama. Compared with those metals, Pb was less enriched in Sacramento River sediment (maximum concentrations were about twice as high as in Cottonwood Creek sediment), and enrichment did not appear to extend further downstream than Balls Ferry. The mean concentrations of Cd, Cu, Hg, Pb, and Zn among all stations correlated significantly (results not shown), reflecting the overall decrease in concentrations from upstream to downstream (Table 3).

Aluminum and Fe in streambed sediments of the Sacramento River were not clearly enriched relative to those in Cottonwood Creek sediment (Table 3). For both elements, the lowest concentrations occurred at Tehama and the highest concentrations occurred at Balls Ferry; however, the mean concentrations among all stations were not significantly correlated.

With the exception of Cu, correlations between metal concentrations in streambed sediments and *Hydropsyche* were insignificant (Table 4). Most metals that were enriched in sediments were also enriched in *Hydropsyche*, and downstream attenuation in the concentrations of Cd, Cu, and Pb occurred in both sediments and the insect. However, the spatial distributions of Cd and Pb differed in some respects between the sample types, affecting the correlations. Spatial patterns for Cd in sediments and *Hydropsyche* were fairly similar, except that maximum Cd concentrations in sediments and the insect occurred at adjacent stations: the former at Churn Creek and the latter at Rodeo Park (Tables 1 and 3). Lead contamination of sediments was only evident

between Rodeo Park and Bend Bridge, a distance of about 60 km, whereas cytosolic concentrations in *Hydropsyche* at Tehama indicated that contamination of bioavailable Pb extended at least 111 km downriver. Concentrations of Hg and Zn in sediments and the insect were generally inconsistent. Mercury contamination, evident in sediments, was not observed in *Hydropsyche*. Unlike the sediments, cytosolic (and whole-body) Zn did not decrease downstream, although concentrations in samples from all five Sacramento River stations were significantly higher than in the sample from Cottonwood Creek.

Discussion

Metal concentrations in *Hydropsyche* and streambed sediment samples taken in October 1996 from the Sacramento River indicate that metal contamination occurs between Redding (Rodeo Park) and Tehama (368 km from river mouth), which is approximately 120 km downstream of the Keswick Dam. Furthermore, analysis of metal accumulation in the cytosol of *Hydropsyche* verified that this contamination includes biologically available Cd, Cu, Pb, and Zn. Mercury contamination was evident in streambed sediments but not in *Hydropsyche*.

Metal partitioning patterns were indicative of the location and form of metal within the animal and thus provided some insight into accumulation processes. Differences in the partitioning of different metals between the cytosol and particulate (pellet) fractions in our study were consistent with results reported by Cain and Luoma (1998) for the same genus. Most of the Al, Fe, and Pb in *Hydropsyche* was present in a particulate form(s). 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 suggested that substantial amounts of Al, Fe, and Pb can be sorbed to external body surfaces (Krantzberg and Stokes 1988; Hare et al. 1991; Cain et al. 1992). In un-depurated animals, variable amounts of metals are also associated with undigested gut content (Smock 1983; Hare et al. 1989; Cain et al. 1995). Thus, it is likely that much of the Al, Fe, and Pb could be characterized as extracellular. Similarities in the spatial patterns of noncytosolic, particulate Al, Fe, and Pb, particularly downstream of Balls Ferry, suggest that accumulation occurred by similar processes, possibly by sorption to external body parts and (or) inadvertent ingestion of sediment. There was no correspondence between concen-

trations of Al and Fe in the particulate fraction (and whole body) and in the cytosol. A small proportion (8–20%) of the Pb body burden was recovered 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 that the relatively narrow range of low concentrations in the Sacramento River influenced the 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). In contrast with Al, Fe, and Pb, a large portion of the total Cd, Cu, and Zn occurred in the cytosol, which explains the high degree of correspondence between cytosolic and whole-body concentrations for these elements.

The sampling design was not able to either isolate the source of metal or completely delineate the downstream extent of contamination. However, the spatial patterns of Cd, Cu, and Pb in *Hydropsyche* are consistent with documented inputs of dissolved and particulate-bound metals from mineralized areas upstream of Keswick Reservoir (Nordstrom et al. 1977). Inputs of metals from Keswick Reservoir increase during winter high river flow, creating a seasonal gradient in dissolved and suspended particulate concentrations (Alpers et al. 1999). Metal inputs appear to be preserved in streambed sediments because concentration gradients were evident in sediments downstream of Keswick Reservoir in October. Thus, streambed sediments and *Hydropsyche* were basically consistent in characterizing Cd, Cu, and Pb contamination in the Sacramento River.

Inconsistencies between environmental (e.g., sediments) and biological indicators can arise from both abiotic and biotic causes. In this study, metal concentrations in sediments were determined for total sediment digestions, which may not provide reliable estimates of bioavailable metal. Furthermore, exposure pathways for *Hydropsyche*, a filter-feeder, are probably more closely related to metal concentrations in water and food in suspended material than to those in bed sediments. Physiological processes also influence metal concentrations and distributions in the organism. Relationships between metal concentrations in streambed sediments and *Hydropsyche* were generally not statistically significant, Cu being the only exception. As discussed above, both sediments and *Hydropsyche* exhibited concentration gradients for Cd and Pb in the Sacramento River. However, the features of those gradients differed between the sample types, affecting the correlations. For Al, Hg, and Zn, the differences were more basic. While neither sedimentary nor whole-body Al concentrations exhibited any obvious contamination, cytosolic Al suggested increased exposure to bioavailable Al between Rodeo Park and Balls Ferry. However, Al concentrations in the cytosol were very low relative to the whole body, and it is possible that the cytosolic concentrations reflect some low-level contamination of the cytosol by particulate Al during the preparation of the sample. Mercury concentrations in streambed sediments were elevated in the upper Sacramento River relative to Cottonwood Creek. However, concentrations in *Hydropsyche* were $<0.1 \mu\text{g}\cdot\text{g}^{-1}$ at all stations, which appears to be indicative of background concentrations for the Sacramento River watershed (Slotton

et al. 1997). Mercury contributions from the upstream acid mine drainage site (Iron Mountain via Spring Creek) are apparently not a major source to the river. Dissolved Hg concentrations in the Sacramento River are low ($<0.4\text{--}2.2 \text{ ng}\cdot\text{L}^{-1}$). The majority of Hg in suspension occurs with colloids in oxidizable and residual (mineral) phases (Alpers et al. 1999). Evidently, these sources are not sufficient to cause Hg enrichment in *Hydropsyche*. Inconsistencies between Zn concentrations in bed sediments ($<62 \mu\text{m}$) and in the cytosol of *Hydropsyche* observed in the Sacramento River have been observed in the Clark Fork River, also (Cain and Luoma 1998). Zinc accumulation in the cytosol reflected gross differences in contamination (e.g., between uncontaminated and contaminated sites) but was inconsistent with some environmental indicators of metal gradients (e.g., bed sediment concentrations). One explanation is that Zn uptake is relatively slow (Hare et al. 1991) and (or) that efflux of excess Zn from the cytosol is relatively rapid.

Metal exposures in *Hydropsyche* in the Sacramento River can be placed into context by comparison with 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 $3 \mu\text{g}\cdot\text{g}^{-1}$ (Hornberger et al. 1997). Cytosolic Cd concentrations in this same area ranged from approximately 0.25 to $1.5 \mu\text{g}\cdot\text{g}^{-1}$ (Cain and Luoma 1998). Some of the highest concentrations in the Clark Fork are similar to Cd concentrations at Rodeo Park near Redding ($2.16 \mu\text{g}\cdot\text{g}^{-1}$ in the whole body and $1.27 \mu\text{g}\cdot\text{g}^{-1}$ in the cytosol). Concentrations of Cu, Pb, and Zn in the Sacramento River appear to be indicative of moderate contamination, relative to other studies (Cain et al. 1992; Hornberger et al. 1997). Metal concentrations in samples from Cottonwood Creek are characteristic of uncontaminated rivers (Cain et al. 1992; Fuhrer et al. 1994; Hornberger et al. 1997; Slotton et al. 1997).

High tissue concentrations can be symptomatic of toxic effects (Jarvinen and Ankley 1999). However, dose–response relationships are complex, and application of threshold concentrations observed in laboratory studies for a few test species to a natural population of a different species or to a whole community is prone to inherent uncertainty. Also, the effects of simultaneous, multiple-metal exposures that occur in nature are poorly understood. Nonetheless, the results for Cd seem pertinent to any future consideration of ecological risk in the upper Sacramento River. It is worth noting that tissue concentrations of Cd were comparable with concentrations in *Hydropsyche* in the upper Clark Fork River where metal exposure is considered a factor affecting changes in the composition and abundance of benthic macroinvertebrates (McGuire 1995). While *Hydropsyche*, a relatively metal-tolerant organism (Spehar et al. 1978; Clements et al. 1992), might exhibit no obvious effect, more metal sensitive taxa could be affected. As in the Clark Fork (Cain and Luoma 1998), a large proportion ($>50\%$) of the Cd accumulated by *Hydropsyche* was associated with the cytosol. Because predators can efficiently assimilate cytosolic metals from their prey (Reinfelder and Fisher 1991, 1994; Wallace and Lopez 1997), food could be an important source of Cd to higher trophic animals such as fish.

Conclusions

Comparison of metals accumulated in the whole body and the cytosol of caddisfly larvae facilitated interpretations of the metal exposures, relative to metal concentrations in streambed sediments, in the upper Sacramento River, downstream of Keswick Reservoir. Metal concentrations in the cytosol indicated exposure to elevated concentrations of bioavailable Cd, Cu, Pb, and Zn. Exposures, compared with a regional reference sample, were greatest for Cd. The downstream concentration patterns indicated a primary upstream source of Cd, Cu, and Pb near or upstream from Redding (river km 479), consistent with concentration gradients in streambed sediments and documented inputs from Iron Mountain mine and Shasta Lake, which receives drainage from abandoned mining areas. The data did not delineate the downstream extent of general contamination and bioavailable metals; however, it was evident that bioavailable forms of these metals occurred downstream as far as Tehama (river km 368), 120 km downstream of the Keswick Dam.

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