

Influence of gut content in immature aquatic insects on assessments of environmental metal contamination

D.J. Cain, S.N. Luoma, and E.V. Axtmann

Abstract: We evaluated the effect of metal associated with the gut content in immature aquatic insects (larvae and nymphs) on spatial and interspecific comparisons of whole-body metal concentrations. Four species, common to cobble-bottom rivers and streams, were collected along an established contamination gradient in the Clark Fork River, and from tributaries of the Clark Fork. Metal concentrations were determined in the gut and its content and in the insect body. Whole-body metal concentrations were higher and more variable as a result of gut content. The positive bias produced by the gut content did not alter interpretations of site contamination in most cases. Interspecific comparisons of metal bioaccumulation also were not greatly affected by the presence of gut content. The influence of gut content was specific for metal, species, and site. Feeding habit, gut size, and metal bioaccumulation in the body affected the relative contribution of the gut and its content to metal concentrations in the whole insect.

Résumé : Nous avons évalué l'effet des métaux associés au contenu du tube digestif d'insectes aquatiques immatures (larves et nymphes) au cours de comparaisons spatiales et interspécifiques de concentrations de métaux dans tout l'organisme. En suivant un gradient de concentrations déterminé au préalable, on a recueilli dans la rivière Clark Fork et ses tributaires des spécimens de quatre espèces communes dans les rivières et cours d'eau à fond recouvert de cailloux. On a dosé les métaux du tube digestif et de son contenu, ainsi que de tout l'organisme des insectes. Les concentrations de métaux pour tout l'organisme étaient plus élevées et plus variables à cause du contenu du tube digestif. Dans la plupart des cas, l'erreur systématique positive due au contenu du tube digestif n'a pas modifié les interprétations de la contamination des lieux. De plus, les comparaisons interspécifique de la bio-accumulation des métaux n'ont pas été fortement influencées par la présence de contenus de tubes digestifs. L'influence de ces valeurs était caractéristique pour un métal, une espèce et un lieu. De plus, les habitudes alimentaires, les dimensions des tubes digestifs et la bio-accumulation des métaux dans l'organisme avaient une influence sur la contribution relative du tube digestif et de son contenu aux concentrations de métaux mesurées dans tout l'organisme.

[Traduit par la Rédaction]

Introduction

The measurement of contaminants in the tissues of aquatic organisms is widely used to assess the environmental distribution of biologically available pollutants (Phillips 1980; Hare 1992; Phillips and Rainbow 1993). Immature life stages of aquatic insects meet most requirements of bioindicator species (Hare 1992; Cain et al. 1992), and are used to monitor contaminants in freshwater (Nehring 1976; Lynch et al. 1988; Dukerschein et al. 1992). Recent studies demonstrated that whole-animal metal concentrations in river-dwelling insect species correlated significantly with metal

concentrations in unfiltered water or sediments, at least along contamination gradients (Moore et al. 1991; Cain et al. 1992; Kiffney and Clements 1993). In some instances differences in the tissue concentrations of metals among taxa also were related to differences in feeding habit (Cain et al. 1992; Kiffney and Clements 1993). However, it was not determined whether metals associated with the content of the digestive tract affected either the relationship to feeding habit or the correlation with environmental contamination.

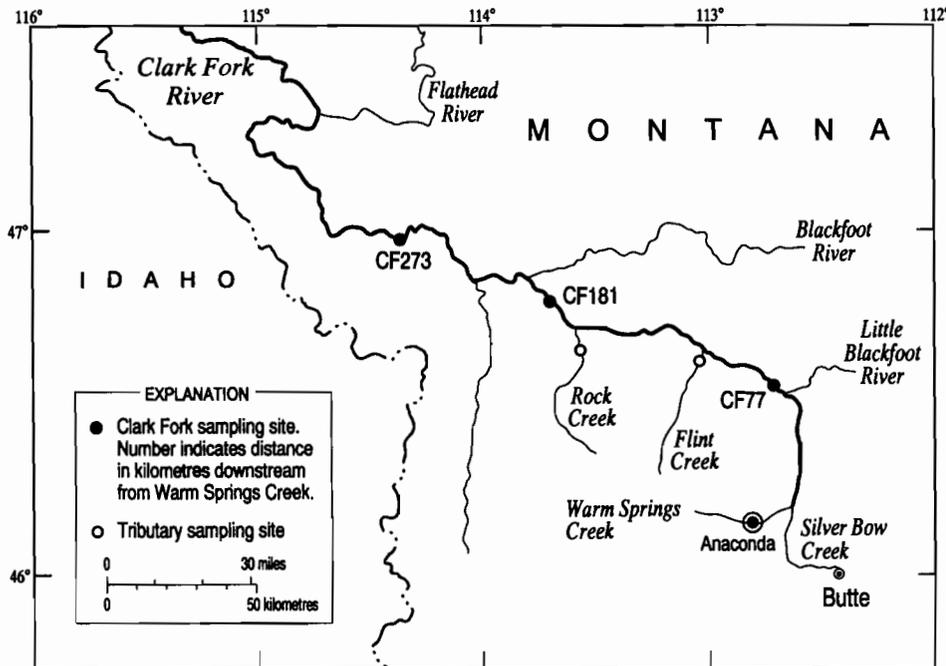
The gut content can represent a significant fraction of the contaminant body burden (metal mass) in some aquatic insects (Elwood et al. 1976; Hare et al. 1989), particularly species that ingest sediment or detritus (Smock 1983). Predators also might incidentally ingest sediment that is present in the gut of their prey. Gut content could introduce a substantial error to studies that analyze whole organisms to assess the assimilation of metals into tissues (i.e., bio-availability) (Elwood et al. 1976; Phillips 1980; Chapman 1985; Hare et al. 1989). Lobel et al. (1991) suggested that

Received February 13, 1995. Accepted June 22, 1995.
J12776

D.J. Cain and S.N. Luoma. U.S. Geological Survey,
MS 465, 345 Middlefield Road, Menlo Park, CA 94025,
U.S.A.

E.V. Axtmann. U.S. Geological Survey, 3215 Marine
Street, Boulder, CO 80303, U.S.A.

Fig. 1. Mainstem and tributary stations of the Clark Fork River basin sampled in August 1992.



such effects should be greatest at more contaminated sites. Although the potential problems with gut content on spatial assessments of metal contamination are generally recognized, studies have not quantitatively determined the severity of biases in aquatic insects.

The principal objective of this study is to determine how interpretations of spatial metal contamination patterns are biased by the gut content in whole-animal analyses. Secondly, we examine the effect of gut content on comparisons of metal bioaccumulation patterns among insect species. Cadmium, copper, iron, and lead were quantified in either the gut content alone or the gut content including the gut in four insect species employed in previous studies (Luoma et al. 1989; Cain et al. 1992). Metal concentrations also were determined in the gut-free body. Samples were collected at five sites that differed widely in degree of metal contamination. Results are discussed relative to using whole-insect metal concentrations to assess the distribution of biologically available trace metals.

Methods

Study site

The Clark Fork River is located in western Montana (Fig. 1), flowing northwest into Idaho from its headwaters near Butte, Mont. The river is contaminated over much of its length (>500 km) with trace metals, including Cd, Cu, and Pb, from the past operation of a large Cu mining and smelting complex in and around Butte (Moore and Luoma 1990; Axtmann and Luoma 1991).

Insects and bed sediments were collected for the study of gut content effects from three stations in the Clark Fork and two of its tributaries in 1992 (Fig. 1). Rock Creek was included in the study because it is one of the most

pristine tributaries in the watershed (Axtmann and Luoma 1991). Flint Creek, the second tributary, is moderately contaminated from mining in its watershed. Clark Fork stations are designated by river kilometre, downstream of the confluence of Warm Springs Creek. Locations are the same as reported in earlier studies (Axtmann and Luoma 1991; Cain et al. 1992).

Sample collection

Species collected for this study included two caddisflies (Trichoptera), *Hydropsyche occidentalis* and *Arctopsyche grandis*, and two stoneflies (Plecoptera), *Pteronarcys californica* and *Claassenia sabulosa*. These taxa were chosen because they represent different functional feeding groups, and they have been used as bioindicators of metal contamination in the Clark Fork River (Luoma et al. 1989; Cain et al. 1992; Lambing et al. 1994). The caddisflies are net-spinning, omnivorous, filter feeders (Merrit and Cummins 1984). *Pteronarcys californica* feeds on detritus and *C. sabulosa* is an active predator (Merrit and Cummins 1984).

Immature insects (larvae and nymphs) were collected with kick nets and by hand-picking rocks from riffle areas at all stations. Specimens were separated to genus on location and immediately frozen on dry ice. Most individuals were late instars (e.g., instars 3–5 for the caddisflies). Because of differences in species distributions, not all taxa were collected at every station. Sample sizes also varied depending on species abundance.

Oxic surface sediments were collected from depositional areas of shallow, slow-moving water near the water's edge as described by Axtmann and Luoma (1991). These areas were immediately adjacent to riffle areas where insects were sampled. Several areas were sampled and then combined into one composite sample. Composite

samples were taken in triplicate at each station. Where access was possible, both sides of the river were sampled. Sediment samples were immediately sieved with native river water through 60- μm nylon mesh, transported to the laboratory on ice, and then dried at 60°C.

Sample preparation

Sediment samples were homogenized by grinding in a ceramic mortar and pestle. Duplicate subsamples were digested by subboiling 16 M HNO_3 reflux (Luoma and Bryan 1981), evaporated to dryness, reconstituted in 0.6 M HCl, and then filtered (0.4 μm) for analysis.

Insects were partially thawed, rinsed with deionized water to remove particles, and separated by species. Identifications were verified with preserved specimens using descriptions in Claassen (1931), Baumann et al. (1977), Alstad (1980), Merritt and Cummins (1984), and Scheffter and Wiggins (1986).

Individual insects were divided by dissection into the gut, gut content (or gut including gut content), and the eviscerated body. Insects were dissected on a piece of Teflon[®] coated vinyl sheeting under a stereomicroscope using stainless steel tools that had been previously washed in a detergent (Micro[®] or RBS[®]) and rinsed in deionized water.¹ To expose the gut, the head and the last abdominal segment were removed, and an incision was made ventrally along the length of the body. For the stoneflies, a second lateral incision was made along the abdomen and a section of the abdominal integument was removed. The gut was lifted from the body and placed on the Teflon[®] surface. For *P. californica* the gut was separated from its content. The gut content included the peritrophic membrane. In the smaller caddisflies and the stonefly *C. sabulosa*, and in *P. californica* at site CF273, the gut and its content were analyzed together because they could not be cleanly separated. For these samples, metal concentrations are reported for the gut and gut content together and are hereafter collectively referred to as the gut plus content. The bodies of the stoneflies and the gut content of *P. californica* were placed in separate, acid-washed, tared 20-mL glass vials, and then capped. Other smaller animal parts were first placed on tared, acid-washed, microscope cover glasses to facilitate handling and weighing. The cover glasses were in turn placed inside an acid-washed 20-mL glass vial. Samples were freeze dried to constant weight. Approximately 2–5 mL (depending on the tissue weight) of 16 M HNO_3 was added to each vial. The vials were covered with glass bulbs, and the organic material was digested by hot acid reflux. When the solution turned clear, the glass bulbs were removed, the samples were evaporated to dryness, and the sample residue was reconstituted in 0.2 M HNO_3 for metals analyses.

Metal concentrations (micrograms per gram dry weight) and content (micrograms) were separately determined in the body, the gut, and the gut content (or gut plus content) of individual insects. These data and the weights of the respective body parts were used to reconstruct the metal concentration and content for the whole insect.

¹ Use of trade or product names is for descriptive purposes only and does not imply endorsement by the U.S. government.

Table 1. Metal concentrations ($\mu\text{g/g}$ dry weight; mean \pm 1 SD) in bed sediments ($<60 \mu\text{m}$) from stations in the Clark Fork River (CF77–CF273) and two tributaries sampled in 1992.

Station	Cd	Cu	Fe	Pb
CF77	5.83 \pm 0.03	729 \pm 34	20 500 \pm 1300	107 \pm 5
CF181	3.40 \pm 0.40	322 \pm 23	17 300 \pm 600	74 \pm 4
CF273	1.14 \pm 0.12	106 \pm 7	19 500 \pm 300	35 \pm 3
Rock Creek	\leq 0.5	7 \pm 0	17 600 \pm 1200	11 \pm 1
Flint Creek	2.30 \pm 0.34	55 \pm 1	21 100 \pm 400	204 \pm 5

Analytical methods

Samples were analyzed for metals by inductively coupled argon plasma emission spectrophotometry (ICAPES). Cd, Cu, and Pb in insect samples were determined by graphite furnace atomic absorption spectrophotometry (AAS) when sample concentrations were below the analytical detection limits of ICAPES.

Quality assurance included analysis of procedural blanks, standard reference material (National Bureau of Standards SRM 1566a, oyster tissue, and NBS SRM 1645, river sediment), and metal-spiked samples. Analysis of SRM 1566a by ICAPES yielded recoveries of Cd and Cu within the reported certified limits. Iron recovery in this material averaged 90% of the certified mean, most samples falling slightly outside the 95% confidence limits for the mean. Pb in samples of SRM 1566a was generally below the limit of quantification for ICAPES. The percent recoveries for postdigestion metal spikes in samples analyzed by AAS were 102 \pm 6 (mean \pm SD), 98 \pm 5, and 113 \pm 17 for Cd, Cu, and Pb, respectively. Concentrations of Cu and Fe measured in SRM 1645 were the same as the certified values. Recoveries of Cd and Pb in this material averaged 80 and 92% of the certified mean concentrations, respectively, but were slightly outside the reported 95% confidence limits.

Statistical analysis

The effect of gut content on comparisons of spatial and species-specific patterns in metal bioaccumulation was tested by analysis of variance (ANOVA). Differences between whole-animal metal concentrations and the body only were tested for each station and for each metal. Metal concentrations were log transformed for the analysis. Tukey's honestly significant difference test for unequal n was used to analyze differences among taxa within stations. If data were heteroscedastic, then either the Mann–Whitney or Kruskal–Wallis test was used in lieu of ANOVA. Differences in the percent contribution of the gut content (or gut plus content) to the whole-insect metal burden were tested by ANOVA after the data were transformed to arc sines (Sokal and Rohlf 1969). Differences were considered significant if $\alpha \leq 0.05$.

Results

Comparison of sediments and the gut content

Metal concentrations in bed sediments were greatest at stations in the upper Clark Fork River, nearest the original source of metal input (CF77 > CF181 > CF273) (Table 1).

Table 2. Metal concentration ($\mu\text{g/g}$ dry weight) and dry weight (mg) for the gut and gut content (either separately or combined), the body, and the whole insect in taxa from stations in the Clark Fork River and tributaries.

	Station	Body part	Weight	Metal concentration				
				Cd	Cu	Fe	Pb	
<i>P. californica</i>	CF181	Gut	4 \pm 1	1.0 \pm 0.1	89 \pm 20	525 \pm 144	4.1 \pm 0.8	
		Gut content	18 \pm 3	1.4 \pm 0.3	131 \pm 15	3600 \pm 740	14.9 \pm 4.3	
		Body	122 \pm 24	0.1 \pm 0.02	48 \pm 9	162 \pm 18	1.0 \pm 0.2	
		Whole	140 \pm 26	0.3 \pm 0.04*	63 \pm 9	633 \pm 130*	3.3 \pm 0.6*	
	CF273	Gut plus content	14 \pm 6	1.0 \pm 0.3	65 \pm 13	5020 \pm 1460	5.7 \pm 0.1	
		Body	97 \pm 65	0.06 \pm 0.03	38 \pm 2	292 \pm 53	0.4 \pm 0.1	
		Whole	112 \pm 71	0.3 \pm 0.1	43 \pm 4	1210 \pm 490*	1.4 \pm 0.3*	
	RC	Gut	7 \pm 1	0.4 \pm 0.05	18 \pm 4	854 \pm 108	0.4 \pm 0.2	
		Gut content	26 \pm 5	0.5 \pm 0.03	9 \pm 1	4050 \pm 260	2.2 \pm 0.3	
		Body	122 \pm 15	0.08 \pm 0.01	25 \pm 2	180 \pm 17	0.05 \pm 0.02	
	FC	Whole	149 \pm 53	0.2 \pm 0.02*	22 \pm 2	764 \pm 103*	0.4 \pm 0.1*	
		Gut	6 \pm 2	0.2 \pm 0.02	31 \pm 4	770 \pm 625	5.7 \pm 2.3	
Gut content		23 \pm 5	1.0 \pm 0.4	26 \pm 2	1920 \pm 510	14.5 \pm 4.7		
Body		139 \pm 18	0.05 \pm 0.01	25 \pm 2	288 \pm 25	2.1 \pm 0.1		
<i>H. occidentalis</i>	CF77	Whole	179 \pm 21	0.2 \pm 0.1*	26 \pm 2	567 \pm 80*	3.7 \pm 0.4*	
		Gut plus content	0.5 \pm 0.03	2.9 \pm 1.0	94 \pm 16	1610 \pm 240	17.3 \pm 5.7	
		Body	4.1 \pm 0.4	0.6 \pm 0.1	26 \pm 2	409 \pm 63	4.2 \pm 0.8	
	CF181	Whole	4.6 \pm 0.4	0.8 \pm 0.2	34 \pm 4	546 \pm 52*	5.9 \pm 1.0	
		Gut plus content	0.5 \pm 0.1	3.7 \pm 1.2	105 \pm 26	2630 \pm 270	33.5 \pm 10.5	
		Body	3.0 \pm 0.3	0.5 \pm 0.1	27 \pm 2	500 \pm 91	6.4 \pm 0.8	
	CF273	Whole	3.4 \pm 0.3	0.9 \pm 0.2*	36 \pm 3*	791 \pm 96*	9.3 \pm 1.4	
		Gut plus content	0.7 \pm 0.1	1.9 \pm 0.4	71 \pm 17	1850 \pm 300	16.1 \pm 6.0	
		Body	4.7 \pm 0.6	0.4 \pm 0.04	23 \pm 3	526 \pm 136	2.2 \pm 0.6	
	<i>A. grandis</i>	CF181	Whole	4.9 \pm 0.8	0.6 \pm 0.1*	30 \pm 4	730 \pm 118	3.8 \pm 1.1
			Gut plus content	0.9 \pm 0.1	4.7 \pm 1.3	64 \pm 6	2220 \pm 351	19.5 \pm 2.5
			Body	7.6 \pm 1.6	0.6 \pm 0.1	16 \pm 2	195 \pm 31	2.9 \pm 0.6
RC		Whole	8.5 \pm 1.5	1.1 \pm 0.3	21 \pm 2	452 \pm 66*	4.8 \pm 0.8	
		Gut plus content	0.5 \pm 0.1	0.8 \pm 0.4	39 \pm 20	882 \pm 208	5.7 \pm 3.8	
		Body	4.0 \pm 0.7	0.1 \pm 0.05	20 \pm 10	91 \pm 18	0.2 \pm 0.2	
CF273		Whole	4.4 \pm 0.7	0.2 \pm 0.1	20 \pm 8	166 \pm 21*	0.8 \pm 0.6	
		Gut plus content	3.2 \pm 0.3	3.0 \pm 0.4	31 \pm 5	612 \pm 145	7.4 \pm 6.5	
		Body	64 \pm 13	0.2 \pm 0.03	45 \pm 4	58 \pm 12	0.1 \pm 0.01	
<i>C. sabulosa</i>		CF273	Whole	68 \pm 13	0.3 \pm 0.02	45 \pm 4	95 \pm 8	0.3 \pm 0.2

Note: Data are given as the means \pm 1 SE ($n = 5-10$, except the following: *P. californica*, $n = 4$ for the gut at Flint Creek and $n = 2$ or 3 at CF273; *C. sabulosa*, $n = 2$ for Pb). Asterisk indicates that the whole-insect metal concentration is significantly different from the metal concentration in the body.

The differences among stations were consistent with the downstream gradient in metal concentrations observed in previous studies (Axtmann and Luoma 1991). Concentrations of Cd, Cu, and Pb were lowest in Rock Creek. Flint Creek sediments were somewhat enriched in Cd and Cu and had the highest Pb concentration of any station sampled. Fe concentrations were not significantly different among stations ($p = 0.251$, Kruskal-Wallis test).

Although none of the insects collected in this study feed directly on sediments, all could ingest sediment incidentally with detritus, filamentous algae, suspended organic matter, or the gut content of prey. Visual inspection showed that the gut content of *P. californica* contained mainly coarse pieces of leaf litter and *Cladophora* sp., but also numerous, apparently inorganic, particles. Some smaller particles also were observed in the filter feeders. These

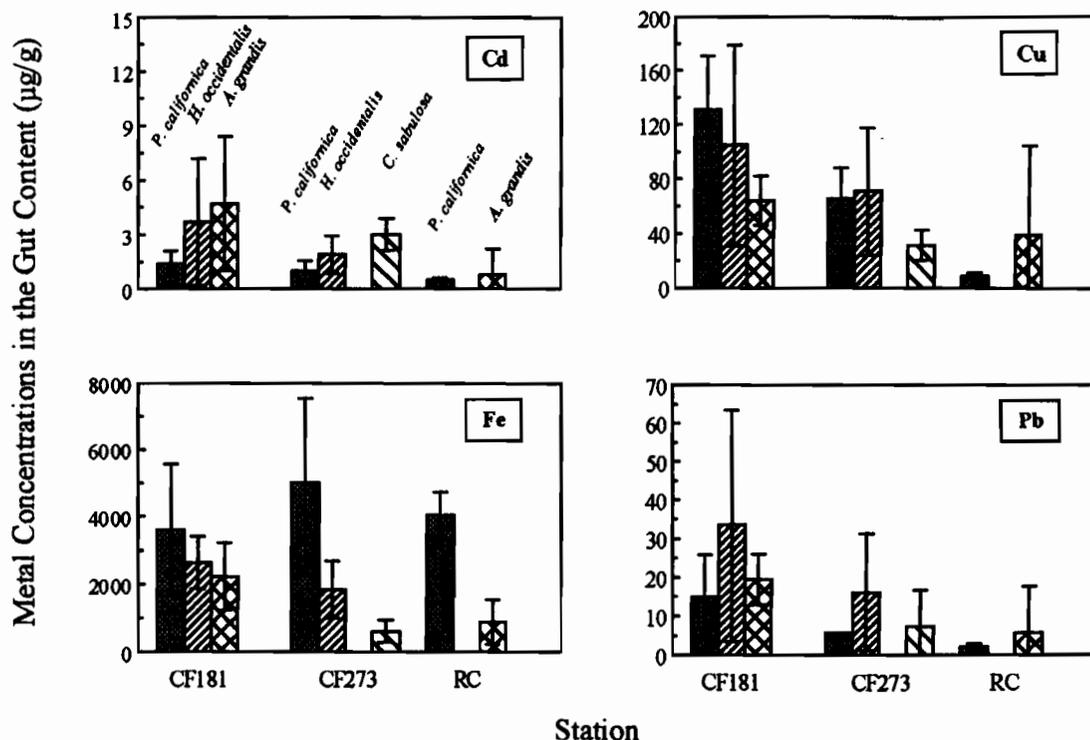
observations tended to be supported by the presence of inorganic residue remaining after acid digestion of the samples. Little residue remained in samples of *C. sabulosa*.

Metal concentrations in the gut content were not the same as in fine-grained sediments. The ratios of gut content to sediment metal concentrations ranged from 0.03 to 5.6 depending on taxon, metal, and station. Metal concentrations in the gut content were usually less than in the sediments, especially at contaminated stations (Tables 1 and 2). Only Cd concentrations in the gut content of samples from the less contaminated stations (CF181, CF273, and RC) and Cu in samples from tributaries were as high as those in sediment.

Metal concentrations in the insects

Metal concentrations in *P. californica* were highest in the gut content, intermediate in the gut, and lowest in the body

Fig. 2. Comparison of the mean metal concentrations (± 1 SD) in the gut content (or gut plus content) among taxa (identified in the panel for Cd) at stations in the Clark Fork River basin.



in most instances (Table 2). Cu concentrations varied least among the three parts of the insect, with differences never exceeding a factor of three. Cu was not enriched in the gut content compared with the gut and the body in *P. californica* from either tributary. For other metals, concentration differences between the gut content and the body varied between 7- and 44-fold, depending on station. The gut had relatively high Cd concentrations at CF181 and Rock Creek.

In other species, metal concentrations in the gut plus content were at least 3 times greater than in the body, except for Cu in *A. grandis* at Rock Creek (2 \times) and *C. sabulosa* at CF273 (<1 \times) (Table 2). Cu was the metal least enriched in the gut plus content relative to the body, as in *P. californica*. Metal-specific differences were least distinct in *H. occidentalis*. Cu concentrations in the gut plus content did not exceed 4 times the concentrations in the body. The difference for other metals ranged from 4- to 7-fold.

Metal concentrations in the gut content or gut plus content exhibited few significant differences among species, when comparisons were made where the species co-occurred (Fig. 2). Cd concentrations in the detritivore, *P. californica*, were significantly lower than in the predator, *C. sabulosa* (at CF273). In contrast, Fe concentrations were generally highest in *P. californica*, and were significantly higher than in *C. sabulosa* (CF273) and in *A. grandis* (Rock Creek). Cu concentrations were higher in *P. californica* than in *A. grandis* at CF181, but at Rock Creek the concentrations were not significantly different.

Weight of insects and their gut content

The total body weight of each species was similar among stations except for *A. grandis*. In that species, specimens collected at CF181 were larger than those collected at Rock Creek ($p < 0.05$; Table 2).

The average weight of the gut content and the gut plus content varied among species from less than 1 mg in the caddisflies *H. occidentalis* and *A. grandis* to about 30 mg in *P. californica*, the large detritivorous stonefly (Table 2). The gut content or the gut plus content never represented more than 22% of the total body weight in any sample. Considerable variation occurred among individuals within samples (the coefficient of variation typically exceeded 30%), but differences in means among stations within species were small (Fig. 3). The gut plus content accounted for a slightly lower proportion of the total weight in *A. grandis* ($12 \pm 1\%$, mean ± 1 SE) and *H. occidentalis* ($13 \pm 1\%$) than in *P. californica* ($19 \pm 1\%$). In the latter species, the gut content and the gut represented 15 and 4% of the total dry weight, respectively (Fig. 3). The gut plus content of the predator, *C. sabulosa*, was proportionately smaller than that of the other species, accounting for $5 \pm 1\%$ of the total weight of the insect.

Contribution of the gut content to the total metal burden

For a given metal, the proportion contributed by the gut content was surprisingly similar among species. Cu exhibited little difference among taxa (27–34%) at CF181 and was relatively consistent for most samples except *P. californica*

Table 3. Percentage (mean \pm 1 SE, $n = 2$ –10) of the total body burden contributed by the gut plus content or gut content (*P. californica*) in insects from stations in the Clark Fork River and tributaries.

Taxon	Station	Cd	Cu	Fe	Pb
<i>H. occidentalis</i>	CF77	34 \pm 6a	38 \pm 8a	34 \pm 5a	34 \pm 7a
	CF181	46 \pm 4a,u	34 \pm 4a,u	45 \pm 6a,u	40 \pm 3a,u
	CF273	40 \pm 7a,w	32 \pm 8a,x	39 \pm 7a,w	40 \pm 11a,w
<i>A. grandis</i>	CF181	47 \pm 7b,u	33 \pm 3b,u	56 \pm 8b,uv	47 \pm 7b,u
	RC	37 \pm 15b,y	37 \pm 11b,y	50 \pm 7b,y	87 \pm 13c,y
<i>P. californica</i>	CF181	56 \pm 5d,u	27 \pm 5d,u	71 \pm 5d,v	57 \pm 6d,u
	CF273	52 \pm 4d,w	26 \pm 6de,wx	74 \pm 8d,x	71 \pm 4def,w
	RC	54 \pm 4d,y	7 \pm 1f,z	78 \pm 3d,y	88 \pm 5f,y
	FC	66 \pm 10d	14 \pm 4ef	45 \pm 24d	44 \pm 8de
<i>C. sabulosa</i>	CF273	20 \pm 9w	4 \pm 1w	38 \pm 13w	62 \pm 38

Note: Within columns, values with the same letter are not significantly different (*a*–*f* compare among stations within taxa; *u*–*z* compare among taxa within stations).

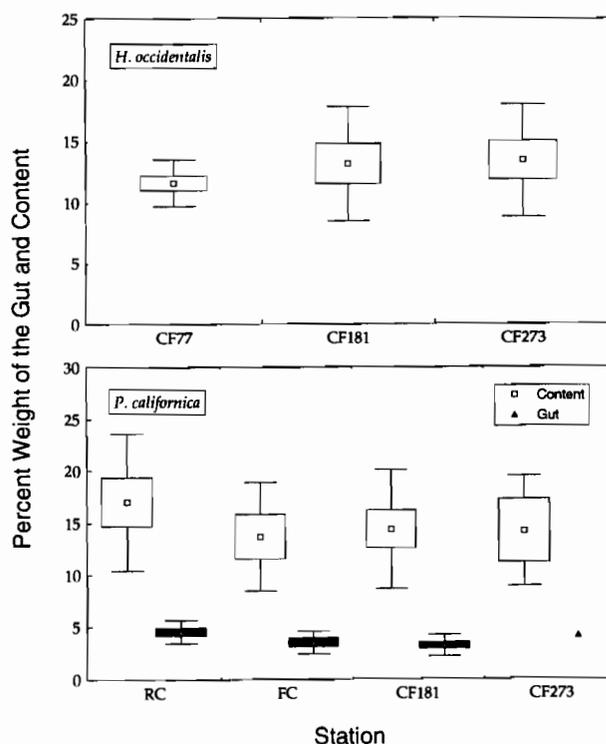
in Rock Creek and *C. sabulosa* at CF273 (Table 3). *Pteronarcys californica* tended to have proportionally more Cd, Fe, and Pb in the gut content than did the caddisflies and *C. sabulosa*, but only Fe was significantly less in *H. occidentalis* than in *P. californica*. (Note that an evaluation for Pb in *C. sabulosa* based on two individuals was not warranted.) Because of its low weight, the gut accounted for only 3–6 % of the total metal burden in *P. californica*, except in Rock Creek and at CF181 where the contribution was 11% for Cd.

The percent contribution of the gut content to metal burdens within taxa varied little among stations in most cases, although levels of environmental contamination differed greatly. For example, in *H. occidentalis* and *P. californica*, the proportional contribution from the gut content for every metal was the same at different stations within the Clark Fork (Table 3). For Cd, the contribution from the gut content within species was the same at all stations. However, less Cu was contributed by the gut content in *P. californica* in uncontaminated Rock Creek, than at contaminated stations in the Clark Fork. In contrast, the gut content contributed significantly more Pb to *P. californica* in Rock Creek (88%) than in Flint Creek (44%) or at CF181 (57%). Iron exhibited a pattern similar to that of Pb, but the differences among stations were insignificant. Pb in *A. grandis* from Rock Creek was detected only in trace amounts (approximately 3 ng/individual) in several specimens. In these animals, Pb was almost exclusively (87%) associated with the gut plus content.

Influence of gut content on whole-insect concentrations

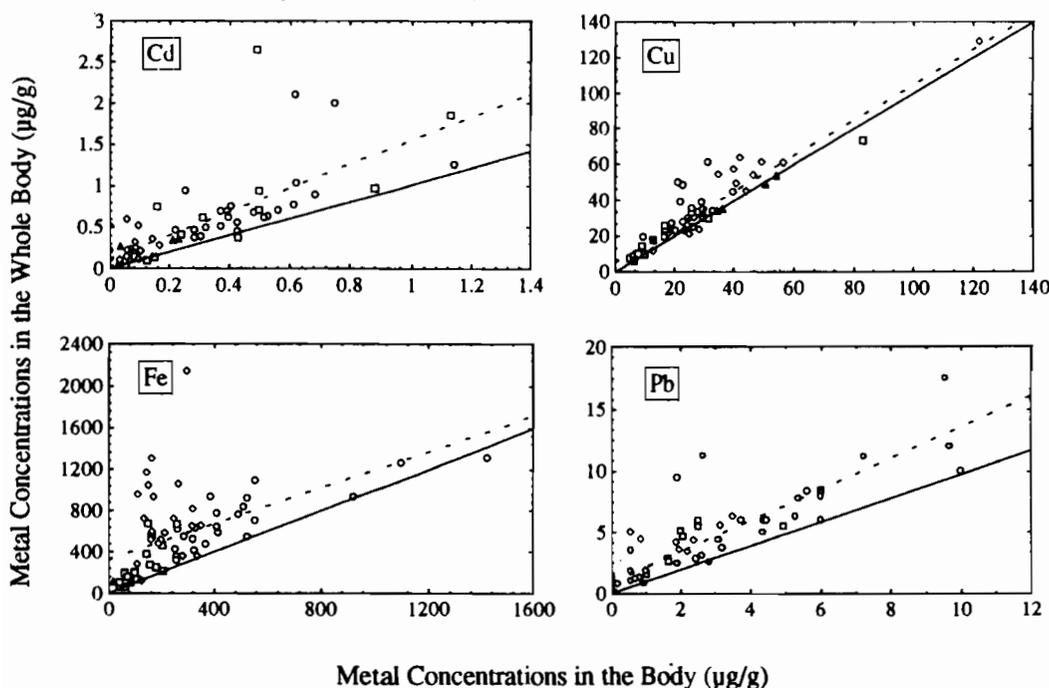
Metal concentrations in the body were consistently lower than in the whole insect. The greatest differences were observed in *P. californica*. Gut content significantly increased Cd, Fe, and Pb concentrations in whole *P. californica* (Table 2). Differences were insignificant for Cu. Differences in metal concentration between the body and the whole insect were significant in fewer cases in other species than in *P. californica* (Table 2).

Fig. 3. Weight of the gut and gut content in *P. californica*, and the gut plus content in *H. occidentalis* as a percentage of the total dry weight of the insect. Data are given as station means, \pm 1 SE, and \pm 1 SD.



The gut content usually did not affect comparisons of metal bioaccumulation among species. In several instances, metal concentrations in the body were more sensitive than whole-insect metal concentrations in detecting differences among species. At CF181, Fe concentrations in whole *H. occidentalis*, *A. grandis*, and *P. californica* were not significantly different. However, concentrations in the body were significantly greater in *H. occidentalis* than in the

Fig. 4. Metal concentrations in the body (gut and content removed) compared with whole-insect metal concentrations in samples from all stations. Data are fitted with a simple linear regression (broken line). A solid line representing the 1:1 relationship between the body and the whole insect is drawn in each panel to illustrate biases resulting from the presence of the gut content. ○, *H. occidentalis*; □, *A. grandis*; ◇, *P. californica*; and △, *C. sabulosa*.



other two species. Similarly, Cu concentrations in whole *H. occidentalis*, *P. californica*, and *C. sabulosa* were not significantly different at CF273, but body concentrations of Cu were significantly higher in *C. sabulosa* than in *H. occidentalis*. In contrast, at CF181, high Cu concentrations in the gut plus content of *H. occidentalis* resulted in a significant difference in Cu between whole *H. occidentalis* and *A. grandis*. Cu concentrations in the bodies of these species were not significantly different.

Metal concentrations in the whole insect and the insect body were significantly correlated among all samples (Fig. 4). The correlation was strongest for Cu ($r = 0.92$) and had a slope of 1. This reflects the relatively high and consistent proportion of Cu associated with the body. Therefore, influences of gut content on interpretation of Cu in whole insects would be small. The correlation was significant for other elements, although weaker than for Cu (Fig. 4). The correlation was weakest for Fe ($r = 0.54$). The largest, positive biases in whole-insect metal concentrations from the 1:1 trend line were indicative of individuals in which the gut content contributed unusually high proportions of metals to the whole insect.

Most trends in metal concentrations were similar for the whole insect and the insect body, relative to environmental contamination, as illustrated by the examples in Fig. 5. As suggested above, the greatest inaccuracies in trend evaluations occurred where the gut content was a high (>70%) and (or) variable proportion of the whole-animal metal burden. Station comparisons were most affected for Fe in *P. californica*. Differences in Fe concentrations

among stations were not significant in whole insects, but were significant in the body (Fig. 5).

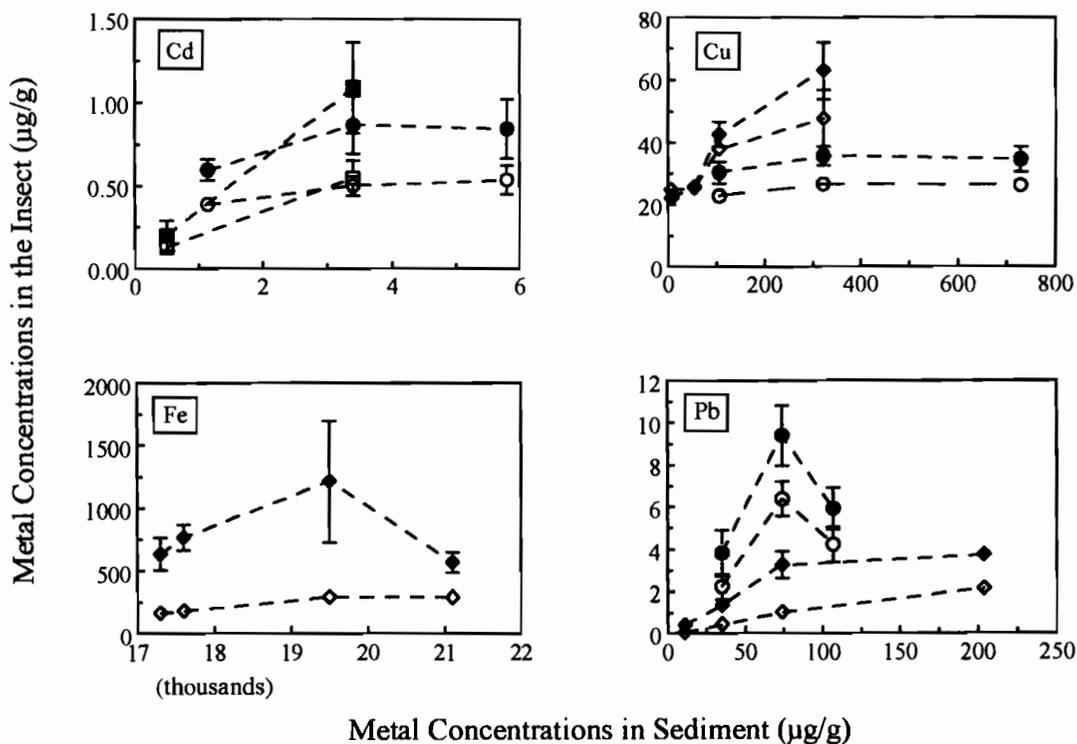
Discussion

The effects of gut content

Metal concentrations in biological indicators should be indicative of environmental metal exposure. The presence of metals in the gut content complicates precise measurements of the tissue metal burdens in whole animals (Phillips 1980). In our study, there were two effects of gut content on site assessments. First, inclusion of gut content increased whole-insect metal concentrations, producing a positive bias in assessments of site contamination. Biases were greatest for elements that occurred in the highest concentrations in the gut content. Second, contaminants in the gut content increased the variability in whole-insect metal concentrations. The greater variability hampered statistical determination of site differences. Surprisingly, however, in most cases the effect of gut content did not alter basic interpretations of relative differences in site contamination. Misinterpretation would only have occurred for Fe in whole *P. californica*.

Species comparisons also were not greatly affected by metal associated with the gut content. Generally, greater variation in metal concentrations in the whole insect, affected by gut content, made species-specific differences less distinct. Thus, some small species-specific differences in metal concentrations in the body were not evident in the whole insect. In one case only, a species-specific difference

Fig. 5. Comparison of the mean metal concentrations (± 1 SE) in the body (gut and content removed) and the whole insect relative to the mean sediment metal concentrations at different stations. Open and solid symbols are the body and the whole insect, respectively, for *H. occidentalis* (circles), *A. grandis* (squares), and *P. californica* (diamonds).



detected in whole-insect concentrations was an artifact of gut content (Cu in *H. occidentalis* and *A. grandis*). Thus, the primary effect of gut content was to mask rather than to impose differences in species bioaccumulation patterns.

A number of factors appear important in influencing the relative contribution of the gut content to whole-animal metal burdens. These factors include feeding habit, size of the gut, metal accumulation in the body, and the degree of environmental contamination.

Feeding habit basically determines metal concentrations in the gut content. Smock (1983) studied seven metals in 40 taxa grouped into five functional feeding groups. He concluded that for sediment-bound metals the gut content contributes higher concentrations of metal in species that directly or indirectly ingest sediments than in predators. However, our results are not entirely consistent with this conclusion. Fe, Pb, and Cu concentrations in the gut content of detritivores and filter feeders in the Clark Fork were typically greater than in a predator, as observed by Smock (1983). However, Cd was most concentrated in the gut content of the predator, *C. sabulosa*.

Metal concentrations in the different foods selected by insects vary greatly. These differences, which are reflected in the gut content, might be affected to some extent by metal-specific biogeochemical processes. For example, the lower Cd concentrations in the gut content of species that ingest detritus, such as *P. californica*, could reflect the loss of cytosolic Cd that occurs during the degradation of plant and animal material (Lyngby and Brix 1989; Lee

and Fisher 1993, 1994; Reinfelder et al. 1993; Reinfelder and Fisher 1994). Particle-reactive metals that are primarily surface-bound (e.g., Pb) are retained longer in detritus (Lee and Fisher 1993, 1994; Reinfelder et al. 1993). Thus, the relatively high Pb concentrations in the gut content of detritivores and filter feeders are as expected. These feeding groups would also be expected to have high concentrations of Fe in their gut content because of incidental ingestion of sediment or sediment-contaminated detritus.

The concentrations of metals in the gut content do not adequately explain all differences in the contribution of the gut content to whole-animal metal burdens, as implied by Smock (1983). For example, metal concentrations alone do not explain why the gut content contributed similar percentages of Fe in the predator, *C. sabulosa*, and in the filter feeder, *H. occidentalis*. This result and others were affected by the weight of the gut content, relative to the body. The higher Cd concentration in the food of *C. sabulosa* was offset by the species' relatively small gut (5% of the total body weight). As a result, the fraction of the total Cd contributed by the gut content was comparatively low. In contrast, the larger gut of *P. californica* (18% of the total body weight) accommodated a greater quantity of food and contributed higher proportions of Cd (and other metals), even though Cd concentrations in food were relatively low.

The gut content has less influence on whole-animal metal concentrations for elements that accumulate in the body. Body concentrations of Cu were more similar to

concentrations in the gut content than for other metals, implying efficient uptake of this metal. Because the body accounts for most of the tissue mass of the insect, most of the Cu was associated with the body (63–96%). Consequently, among the metals analyzed, Cu was least affected by gut content. Efficient mechanisms of uptake and regulation of this essential metal by insects and relatively high biological availability of Cu in contaminated ecosystems have been reported previously (Krantzberg 1989; Darlington and Gower 1990). It also is possible that Cu associated with the body of Clark Fork insects was bound externally (e.g., Timmermans et al. 1989; Gower and Darlington 1990), although several studies suggest that sorption of Cu to the insect exoskeleton is relatively minor compared with accumulation in tissues (Timmermans and Walker 1989; Hare et al. 1991a; Timmermans et al. 1992).

Sorption to external body parts could have affected the proportions of Pb and Fe contributed by the gut content. Insects appear to accumulate little intracellular Fe and Pb. Unpublished data (D.J. Cain and S.N. Luoma) show that cytosolic Pb in *H. occidentalis* and *A. grandis* at CF181 accounted for only 3 and 14%, respectively, of the total Pb body burden. The proportions of Fe were $\leq 2\%$. Other studies indicate that Pb occurs principally as a surface contaminant in insects (Timmermans et al. 1989; Hare et al. 1991a), possibly associated with iron oxide deposits (Hare et al. 1991b). In *P. californica* and *A. grandis* proportionately more Pb (and Fe in *P. californica*) was associated with the gut content at Rock Creek than at contaminated stations. As expected, body concentrations in samples from Rock Creek were low. In these clean streams, the biological availability of Pb is low. Pb might occur primarily in discrete mineral phases that insects might occasionally ingest with food. In the contaminated reaches of the Clark Fork and in Flint Creek, sorption to external body parts could increase body concentrations of Pb and Fe, thereby reducing the relative proportions of Pb and Fe contributed by the gut content. Iron and manganese oxides, which often occur as precipitates, have been found on the external surfaces of insects in the Clark Fork River (Boggs 1994). In insect samples collected from the Clark Fork for this study, Pb concentrations correlated with Fe in the eviscerated bodies ($r^2 = 0.42$, $p < 0.001$), suggesting that Pb is associated with these precipitates.

The relative distribution of metals between the gut content and the body can vary with contamination levels. Depending on whether metals accumulate primarily in the gut content or the body, the proportional contribution of the gut content could either increase or decrease. However, because of the interaction of the processes discussed above, the influence of contamination on the relative contribution by the gut content and the body is not necessarily direct, and might only become evident where large differences in contamination levels exist. For example, the proportion of total Cd contributed by the gut in the mayfly, *Hexagenia limbata*, increased with higher contamination (Hare et al. 1991a). Such a relationship was not evident in this study, but the Cd concentrations in the Clark Fork were much lower than those observed by Hare et al. (1991a). The proportion of Cu in the gut of the detritivore, *P. californica*, was higher at contaminated stations

than in an uncontaminated tributary. However, the site-specific effect was relatively small because the bulk of the Cu was contained in the body.

Inclusion of the gut with the gut content in *H. occidentalis*, *A. grandis*, and *C. sabulosa* could have confounded some results. The influence of the gut content on comparisons among stations or taxa would be less than we observed if a high portion of the metal within the animal is in the gut (e.g., Cd; Seidman et al. 1986; Hare et al. 1991a). The gut represented a minor portion of the total metal burden in *P. californica*, however. If the gut accounts for a similarly small portion of metal in the other species, then our results should mainly represent the influences of the gut content.

Correction for gut content

Three approaches have been proposed to minimize the effect of gut content on whole-animal metal analysis: depuration, dissection, and correction. The limitations of each to studies of aquatic insects have been discussed by Hare et al. (1989). Passive depuration of the gut content before metal analysis is widely employed for invertebrates (Phillips 1980). However, depuration experiments indicate that complete evacuation of the gut of the stream-dwelling species examined in this study is difficult to achieve (D.J. Cain and S.N. Luoma, unpublished data), and the effects of partial depuration have not been quantified. Dissection of gut content is unambiguous, but it is labor intensive and impractical for large numbers of samples. Correction for gut content in whole-animal concentrations can be accomplished by subtracting the metal content and weight of the gut material from those of the whole animal. As practiced (e.g., Chapman 1985), this procedure substitutes measured metal concentrations of sediments for the gut content. This concentration is then multiplied by the weight of the gut content, which is estimated from the inorganic residue remaining after acid digestion of the sample. Hare et al. (1989) assessed the applicability of this method to *Hexagenia limbata*, a species that specifically ingests sediments. Reasonable estimates ($\pm 20\%$) were obtained compared with animals in which the gut content was removed by dissection. We applied the procedure to the species studied here by substituting sediment metal concentrations for the concentrations in the gut content (weights used are those reported in Table 2). Not surprisingly, the method worked poorly (high sediment concentrations often resulted in negative insect metal concentrations) because the diets of these species include materials other than sediments.

Conclusions

The difficulties of effectively compensating for gut content complicate, but do not nullify, the use of aquatic insects as bioindicators of trace metal contamination. Interpretations for most site and species comparisons in the Clark Fork River basin were not qualitatively affected by the presence of gut content.

The effects of gut content on metal concentrations in whole insects were site, metal, and species specific. This variation is affected by biotic and abiotic processes, which should be considered for assessments of metal bioavailability. The portion of metal contributed by the gut content

can be influenced by feeding habit (Smock 1983), gut size, and bioaccumulation. In this study, a detritivore was most prone to biases from gut content. Biases appear to be greatest for metals that are highly concentrated in sediments (e.g., Fe), bind strongly to iron and manganese oxides, or have low biological availability (e.g., Pb). The upward bias and variability caused by gut content tend to be greatest in contaminated conditions, as suggested by Lobel et al. (1991), but are not necessarily limited to these types of sites. In a pristine stream, nearly all the measurable Pb was associated with gut content because the body burden of Pb was low. The essential metal Cu appeared to be efficiently bioaccumulated and was least biased by gut content.

Acknowledgments

Landis Hare, Barbara Scudder, Terry Short, and two anonymous reviewers provided valuable suggestions on earlier drafts of the manuscript. The authors thank Stacey Andrews and Michelle Hornberger for their help in collecting samples for this study.

References

- Alstad, D.N. 1980. Comparative biology of the common Utah Hydropsychidae (Trichoptera). *Am. Midl. Nat.* 103: 167–174.
- Axtmann, E.V., and S.N. Luoma. 1991. Large-scale distribution of metal contamination in the fine-grained sediments of the Clark Fork river, Montana, U.S.A. *Appl. Geochem.* 6: 75–88.
- Baumann, R.W., A.R. Gaufin, and R.F. Surdick. 1977. The stoneflies (Plecoptera) of the Rocky Mountains. American Entomological Society, Philadelphia, Pa.
- Boggs, S.J. 1994. Temporal and spatial variability of metal concentrations in fine-grained bed sediments and benthic insect larvae of the Clark Fork River, Montana, U.S.A. M.Sc. thesis, University of Montana, Missoula, Mont.
- Cain, D.J., S.N. Luoma, J.L. Carter, and S.V. Fend. 1992. Aquatic insects as bioindicators of trace element contamination in cobble-bottom rivers and streams. *Can. J. Fish. Aquat. Sci.* 49: 2141–2154.
- Chapman, P.M. 1985. Effects of gut sediment contents on measurements of metal levels in benthic invertebrates: a cautionary note. *Bull. Environ. Contam. Toxicol.* 35: 345–347.
- Claassen, P.W. 1931. Plecoptera nymphs of America (north of Mexico). Charles C. Thomas, Baltimore, Md.
- Darlington, S.T., and Gower, A.M. 1990. Location of copper in larvae of *Plectrocnemia conspersa* (Curtis) (Trichoptera) exposed to elevated metal concentrations in a mine drainage stream. *Hydrobiologia*, 196: 91–100.
- Dukerschien, J.T., J.G. Weiner, R.G. Rada, and M.T. Steingraeber. 1992. Cadmium and mercury in emergent mayflies (*Hexagenia bilineata*) from the upper Mississippi river. *Arch. Environ. Contam. Toxicol.* 23: 109–116.
- Elwood, J.W., S.G. Hildebrand, and J.J. Beauchamp. 1976. Contribution of gut contents to the concentrations and body burden of elements in *Tipula* spp. from a spring-fed stream. *J. Fish. Res. Board Can.* 33: 1930–1938.
- Gower, A.M., and S.T. Darlington. 1990. Relationships between copper concentrations in larvae of *Plectrocnemia conspersa* (Curtis) (Trichoptera) and in mine drainage streams. *Environ. Pollut.* 65: 155–168.
- Hare, L. 1992. Aquatic insects and trace metals: bioavailability, bioaccumulation, and toxicity. *Crit. Rev. Toxicol.* 22: 327–369.
- Hare, L., P.G.C. Campbell, A. Tessier, and N. Belzile. 1989. Gut sediments in a burrowing mayfly (Ephemeroptera, *Hexagenia limbata*): their contribution to animal trace element burdens, their removal, and the efficacy of a correction for their presence. *Can. J. Fish. Aquat. Sci.* 46: 451–456.
- Hare, L.A., A. Tessier, and P.G.C. Campbell. 1991a. Trace element distributions in aquatic insects: variations among genera, elements, and lakes. *Can. J. Fish. Aquat. Sci.* 48: 1481–1491.
- Hare, L., E. Saouter, P.G.C. Campbell, A. Tessier, F. Ribeyre, and A. Boudou. 1991b. Dynamics of cadmium, lead, and zinc exchange between nymphs of the burrowing mayfly *Hexagenia rigida* (Ephemeroptera) and the environment. *Can. J. Fish. Aquat. Sci.* 48: 39–47.
- Kiffney, P.M., and W.H. Clements. 1993. Bioaccumulation of heavy metals by benthic invertebrates at the Arkansas river, Colorado. *Environ. Toxicol. Chem.* 12: 1507–1517.
- Krantzberg, G. 1989. Accumulation of essential and nonessential metals by chironomid larvae in relation to physical and chemical properties of the elements. *Can. J. Fish. Aquat. Sci.* 46: 1755–1761.
- Lambing, J.H., M.I. Hornberger, E.V. Axtmann, and D.A. Pope. 1994. Water-quality, bed-sediment, and biological data (October 1992 through September 1993) and statistical summaries of water-quality data (March 1985 through September 1993) for streams in the upper Clark Fork basin, Montana. Open File Rep. U.S. Geol. Surv. No. 94-375.
- Lee, B.-G., and N.S. Fisher. 1993. Release rates of trace elements and protein from decomposing planktonic debris. 1. Phytoplanktonic debris. *J. Mar. Res.* 51: 391–421.
- Lee, B.-G., and N.S. Fisher. 1994. Effects of sinking and zooplankton grazing in the release of elements from planktonic debris. *Mar. Ecol. Prog. Ser.* 110: 271–281.
- Lobel, P.B., S.P. Belkhole, S.E. Jackson, and H.P. Longrich. 1991. Sediment in the intestinal tract: a potentially serious source of error in aquatic biological monitoring programs. *Mar. Environ. Res.* 31: 163–174.
- Luoma, S.N., and G.W. Bryan. 1981. A statistical assessment of the form of trace metals in oxidized estuarine sediments employing chemical extractants. *Sci. Total Environ.* 17: 165–196.
- Luoma, S.N., E.V. Axtmann, and D.J. Cain. 1989. Fate of mine wastes in the Clark Fork river, Montana, USA. *In* Metals and metalloids in the hydrosphere; impact through mining and industry, and prevention technology in tropical environments. Proceedings of an IHP Workshop, Phuket, Thailand, 6–10 March 1989. A contribution to the International Hydrological Programme of the United Nations Educational Scientific and Cultural Organization (UNESCO). Asian Institute of Technology, Bangkok, Thailand. pp. 63–75.
- Lynch, T.R., C.J. Popp, and G.Z. Jacobi. 1988. Aquatic insects as environmental monitors of trace metal contamination. Red River, New Mexico. *Water Air Soil Pollut.* 42: 19–31.
- Lynby, J.E., and H. Brix. 1989. Heavy metals in eelgrass (*Zostera marina* L.) during growth and decomposition. *Hydrobiologia*, 176–177: 189–196.
- Merritt, R.W., and K.W. Cummins (Editors). 1984. An introduction to the aquatic insects of North America. Kendall/Hunt, Dubuque, Iowa.
- Moore, J.N., and S.N. Luoma. 1990. Hazardous wastes from large-scale metal extraction. *Environ. Sci. Technol.* 24: 1279–1285.
- Moore, J.N., S.N. Luoma, and D. Peters. 1991. Downstream effects of mine effluent on an intermontane riparian system. *Can. J. Fish. Aquat. Sci.* 48: 222–232.
- Nehring, B.J. 1976. Aquatic insects as biological monitors of heavy metal pollution. *Bull. Environ. Contam. Toxicol.* 15: 147–154.

- Phillips, D.J.H. 1980. Quantitative aquatic biological indicators. Applied Science Publishers Ltd., Barking, Essex, England.
- Phillips, D.J.H., and P.S. Rainbow. 1993. Biomonitoring of trace aquatic contaminants. Elsevier Science Publishers Ltd., Barking, Essex, England.
- Reinfelder, J.R., and N.S. Fisher. 1994. Retention of elements absorbed by juvenile fish (*Menidia menidia*, *M. beryllina*) from zooplankton prey. *Limnol. Oceanogr.* 38: 1783-1789.
- Reinfelder, J.R., N.S. Fisher, S.W. Fowler, and J.-L. Teysse. 1993. Release rates of trace elements and protein from decomposing planktonic debris. II. Copepod carcasses and sediment trap particulate matter. *J. Mar. Res.* 51: 423-442.
- Scheffer, P.W., and G.B. Wiggins. 1986. A systematic study of the Nearctic larvae of the *Hydropsyche morosa* group (Trichoptera: Hydropsychidae). Royal Ontario Museum, Toronto, Ont.
- Seidman, L.A., G. Bergtrom, D.J. Gingrich, and C.C. Remsen. 1986. Accumulation of cadmium by the fourth instar larva of the fly *Chironomus thummi*. *Tissue Cell*, 18: 395-405.
- Smock, L.A. 1983. The influence of feeding habits on whole-body metal concentrations in aquatic insects. *Freshwater Biol.* 13: 301-311.
- Sokal, R.R., and F.J. Rohlf. 1969. *Biometry*. W.H. Freeman and Company, San Francisco, Calif.
- Timmermans, K.R., and P.A. Walker. 1989. The fate of trace metals during the metamorphosis of chironomids (Diptera, Chironomidae). *Environ. Pollut.* 62: 73-85.
- Timmermans, K.R., B. van Hattum, M.H.S. Kraak, and C. Davids. 1989. Trace metals in a littoral foodweb: concentrations in organisms, sediment and water. *Sci. Total Environ.* 87-88: 477-484.
- Timmermans, K.R., W. Peeters, and M. Tonkes. 1992. Cadmium, zinc, lead, and copper in *Chironomus riparius* (Meigen) larvae (Diptera, Chironomidae): uptake and effects. *Hydrobiologia*, 241: 119-134.