

# Trace Element Distributions in Aquatic Insects: Variations among Genera, Elements, and Lakes

Landis Hare, André Tessier, and Peter G. C. Campbell

I.N.R.S.-Eau, Université du Québec, C.P. 7500, Sainte-Foy (Québec) G1V 4C7, Canada

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The distribution patterns of trace element contaminants (As, Cd, Cu, Pb, Zn) among the tissues of freshwater insects (*Chironomus*, *Clinotanypus*, *Procladius*, *Hexagenia*, *Sialis*) are both taxon and element specific. In quantity terms (micrograms per animal), the gut is the major organ of Cd accumulation, while most of the As, Cu, Pb, and Zn accumulates in the body (i.e. animal minus gut and other organs). In concentration terms (micrograms per gram), Cd, Cu, and Zn are often highest in the gut, while Pb and As are not. Much of the As, and in some genera the Pb, appears to be bound externally. Respiratory and excretory structures generally contain small proportions of the total trace element burdens, except for Cu. Trace elements are not uniformly distributed along the gut but tend to accumulate either in the mesenteron (midgut) or in the proctodeum (hindgut), depending on the element and insect concerned. The distribution patterns of most of the trace elements vary little among populations of the mayfly *Hexagenia* from different lakes with the exception of Cd, for which the proportion of the total Cd burden localized in the gut increases markedly with increasing Cd concentrations in the whole nymph.

Les distributions inter-tissus des éléments traces (As, Cd, Cu, Pb, Zn) chez les insectes d'eau douce (*Chironomus*, *Clinotanypus*, *Procladius*, *Hexagenia*, *Sialis*) sont spécifiques au taxon ainsi qu'à l'élément trace. En termes de quantité (microgrammes par animal), l'intestin est l'organe majeur d'accumulation du Cd, alors que les éléments As, Cu, Pb et Zn sont accumulés en plus grande quantités dans le corps (c'est-à-dire l'animal moins l'intestin et d'autres organes). En termes de concentration (microgrammes par gramme), les éléments Cd, Cu et Zn sont normalement les éléments traces les plus abondants dans l'intestin, tandis que l'As et le Pb y sont peu élevés. La plus grande proportion de l'As, ainsi que le Pb chez certains genres, semble être liée à la surface extérieure de l'insecte. Les structures excrétrices et respiratoires contiennent généralement faibles proportions des quantités totales des éléments traces cités, à l'exception du Cu. Les éléments traces ne sont pas distribués de façon uniforme le long de l'intestin mais ont tendance à s'accumuler soit dans le mésentéron (partie médiane), soit dans le proctodéum (partie postérieure); la localisation précise varie selon l'élément et l'insecte étudiés. Les distributions des éléments traces chez *Hexagenia* varient peu entre les individus provenant de différents lacs pour tous les éléments traces, à l'exception du Cd pour lequel la proportion de métal localisé dans l'intestin croît avec une augmentation des concentrations de Cd dans la larve entière.

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Animals in most freshwater systems are contaminated to some extent and thus can potentially be used as pollution biomonitors. Knowledge of the routes by which a species takes up contaminants is essential to its effective use as a biomonitor (NRCC 1988). Routes of contaminant uptake can be estimated by determining the distributions of contaminants within animals, e.g. the presence of a large proportion of a contaminant in the gills or the gut could suggest that it was taken up primarily from water or food, respectively (Luoma 1983; King and Davies 1987; Hare et al. 1991). The study of internal contaminant distributions can also be useful in identifying the tissues in which toxic effects are most likely to occur and in the planning of related histological and physiological studies. Within animals, contaminant concentrations can vary widely among respiratory, digestive, and muscle tissues of individual freshwater animals, including fish (e.g. Bendell-Young and Harvey 1989), molluscs (e.g. Tessier et al. 1984), and crayfish (e.g. Anderson and Brower 1978). Such patterns have been little studied in insects.

Freshwater insects have been little used as biomonitors, yet they are both common and are known to accumulate organic and inorganic pollutants (Ciborowski and Corkum 1988; Timmermans et al. 1989). The internal distributions of contaminants in insects have until recently been measured only in individuals exposed over short times to high concentrations of single pollutants in the laboratory (Sumi et al. 1984; Meyer et al. 1986; Seidman et al. 1986a, 1986b). Results of such studies are difficult to extrapolate to animals in nature, which often are subjected to long-term exposure to low concentrations of several contaminants, in both food and water. In addition, many of the microtechniques used to study internal contaminant distributions produce artifacts (i.e. many fixation and embedding procedures; Brunk and Sköld 1967; George et al. 1976) or cannot discriminate among contaminants and/or permit accurate measurement of their concentrations (e.g. using autoradiography, silver sulphide staining, and X-ray microprobe analysis). In contrast, the direct dissection of organisms allows the accurate quantification of individual contaminants in body parts, but

is limited by the extent to which organisms can be physically subdivided.

In the present study, aquatic insects from several taxonomic groups (Diptera, Ephemeroptera, Megaloptera) and having a variety of feeding habits (predators, detritivores, generalists) were dissected and the concentrations of several trace elements (As, Cd, Cu, Pb, Zn) were measured in various tissues. Comparisons were made among individuals collected from different lakes contaminated by trace elements.

Measurements of trace elements in shed nymphal skins (exuviae) and in adult insects were compared with those in whole nymphs to estimate the partitioning of trace elements between the surface and the interior of animals. Such information is used to assess the potential for trace element loss during nymphal moulting and the transfer of trace elements from aquatic to terrestrial systems through adult emergence. Animals used for this purpose had been exposed to trace elements in nature. In laboratory studies, animals are often exposed to high concentrations of dissolved trace metals in the absence of food over short time periods. Exposure by this latter means could result in a bias towards the surface binding of trace elements (e.g. Kormondy 1965; Frick and Herrmann 1990).

## Study Areas

Insects were collected from 17 lakes located in southern Ontario and Quebec, Canada (Table 1). All of the lakes are contaminated to a greater or lesser degree by trace metals, either through direct input and fallout from nearby mines and smelters (Chibougamau, Rouyn-Noranda, Sudbury) or by long-range atmospheric fallout (Dorset, Eastern Townships, Quebec City). The ranges of total surficial sediment metal concentrations among these lakes are wide: Cd 0.3 – 12; Cu 2 – 2300, Pb 10 – 240, and Zn 40–400  $\mu\text{g}\cdot\text{g}$  dry weight<sup>-1</sup>. All of the study lakes are located on Precambrian Shield bedrock with the exception of Lake Brompton which is located on sedimentary and volcanic rocks of Ordovician age in the Quebec Appalachians.

## Methods

### Sampling and Depuration

Multiple Ekman grab samples were collected in June (of several years; Table 1) at one shallow site (3–6 m) in each lake and sieved in the field using a 0.5-mm-mesh aperture net. Insects and sediment retained in the net were transferred to 4-L plastic bags with lake water and were transported to the laboratory in coolers maintained close to field temperatures. In the laboratory, insects were sorted from the remaining sediment. Nymphs of *Hexagenia* were dissected immediately, while the other taxa were left to depurate in lake water for 2 d, in the dark, at field temperatures. The water was changed twice daily to minimize coprophagy.

Five insect taxa were chosen for study: the mayfly *Hexagenia limbata* (Serville) (Ephemeroptera), the alderfly *Sialis* sp. (Megaloptera), and the midges (Diptera, Chironomidae) *Chironomus* sp. (Chironominae), *Clinotanytus* sp. (Tanypodinae), and *Procladius* sp. (Tanypodinae). *Sialis* and the chironomid genera were not identified to species. Nymphs/larvae of each genus were dissected from two moderately to highly contaminated lakes located in the mining areas of either Sudbury and/or Rouyn-Noranda. Lake pairs were chosen according to the abundance of each genus in the lakes sampled.

Insects from contaminated lakes were used so as to minimize problems of trace element detection in the body parts of these small animals. In order to study the influence of ambient contaminant concentrations on the body part distribution of trace elements in more detail, *H. limbata* nymphs were collected from several additional lakes (Table 1) showing a wide range of sediment trace element concentrations.

To quantify the proportion of trace elements bound externally on nymphs, nymphal exuviae of recently emerged (<12 h) *H. limbata* adults were collected on the surface of Lake St. Joseph at the same time as nymphs were collected from this lake. In addition, adults of *H. limbata* (hereafter referred to as *Hexagenia*) were reared from Lake Joannès nymphs that had been held in the laboratory in sediments collected from that lake.

### Dissection

Individuals were dissected with stainless steel instruments in small amounts (2 mL) of deionized water under a microscope at magnifications of 120 and 250 $\times$ . As each part of an animal was removed, it was placed on a preweighed piece of acid-washed Teflon which was held in a longitudinally cut piece of Tygon or Teflon tubing cemented or sewn onto a plastic petri dish (Hare et al. 1989). The parts of several animals were pooled (Table 1) in order to obtain sufficient quantities of tissue for weighing and analysis.

*Hexagenia* and depurated *Sialis* nymphs were dissected in the following manner. An animal was immobilized by a ventral incision between the head and thorax (which severed the gut and nerve cord) and the abdominal gills were removed. A mid-ventral incision was made along several posterior abdominal segments to extract the gut from the posterior end of the animal. The Malpighian tubules were separated from the gut and, in the case of the nondepurated *Hexagenia*, the gut was opened to remove its contents (Hare et al. 1989). The gut was cut into three portions: the anterior and posterior halves of the mesenteron (midgut) and the proctodeum (hindgut).

In the case of the three chironomid genera, the head and the terminal portion of the last abdominal segment were cut off to allow the withdrawal of the gut from the body. There was little fluid loss, since the elasticity of the body wall sealed the body cavity after withdrawal of the gut. The gut was separated into four regions: the esophagus (or stomodeum), the anterior dilated portion of the mesenteron, the posterior mesenteron, and the proctodeum (Fig. 1). The Malpighian tubules remained attached to the proctodeum.

### Analytical Procedures

To minimize trace element contamination, all labware was soaked in 15% nitric acid for 24 h and rinsed repeatedly in deionized water prior to use. Deionized water for all purposes was obtained from a Milli-Q3RO/Milli-Q2 system (Millipore Corp.). Manipulations of samples were performed when required under a filtered air hood.

The petri dish containing the animal parts was placed in a freezer at  $-40^{\circ}\text{C}$  for at least 1 h and then in a lyophiliser to dry the tissues for at least 12 h. Each pooled animal part was weighed on its piece of Teflon with a Mettler ME30 electronic microbalance (balance pan covered with Teflon). Digestion procedures varied somewhat among samples, a consequence both of the refinement of methods with time and the competing needs of parallel studies. Dried insects were digested either in

TABLE 1. Locations of the study lakes, times of sampling (June of each year), and the number of individuals of each genus dissected from each lake.

Lake	Genus (no. of samples × no. of individuals)	Year
Sudbury, Ontario		
Clearwater (46°22'N, 81°03'W)	<i>Procladius</i> (1 × 5)	1988
	<i>Sialis</i> (2 × 4)	1988
Fairbank (46°28'N, 81°25'W)	<i>Hexagenia</i> (1 × 5)	1988
McFarlane (46°25'N, 80°57'W)	<i>Chironomus</i> (1 × 6)	1988
Wavy (46°17'N, 81°06'W)	<i>Procladius</i> (1 × 7)	1988
Dorset, Ontario		
Bigwind (45°03'N, 79°03'W)	<i>Hexagenia</i> (3 × 5)	1988
Gullfeather (45°06'N, 79°01'W)	<i>Hexagenia</i> (1 × 2)	1988
Rouyn-Noranda, Quebec		
Bousquet (48°14'N, 78°34'W)	<i>Clinotanypus</i> (1 × 14)	1988
	<i>Hexagenia</i> (3 × 5)	1988
La Bruère (48°09'N, 78°56'W)	<i>Hexagenia</i> (3 × 5)	1988
Caron (47°56'N, 78°58'W)	<i>Chironomus</i> (3 × 5)	1988
	<i>Hexagenia</i> (3 × 5)	1988
D'Alembert (48°23'N, 79°01'W)	<i>Hexagenia</i> (3 × 5)	1988
	<i>Sialis</i> (3 × 8)	1990
	<i>Procladius</i> (2 × 14)	1990
Heva (48°11'N, 78°19'W)	<i>Clinotanypus</i> (1 × 8)	1989
	<i>Hexagenia</i> (1 × 8)	1989
Joannès (48°11'N, 78°41'W)	<i>Hexagenia</i> (3 × 5)	1987
Opasatica (48°06'N, 79°17'W)	<i>Hexagenia</i> (3 × 5)	1988
Chibougamua, Quebec		
aux Dorés (49°54'N, 74°18'W)	<i>Hexagenia</i> (1 × 5)	1987
Norhart (49°29'N, 74°26'W)	<i>Hexagenia</i> (3 × 5)	1987
Eastern Townships, Quebec		
Brompton (45°25'N, 72°09'W)	<i>Hexagenia</i> (3 × 5)	1987
Quebec City, Quebec		
St. Joseph (46°55'N, 71°40'W)	<i>Hexagenia</i> (4 × 10)	1989
Tantaré (47°04'N, 71°32'W)	<i>Hexagenia</i> (1 × 4)	1987

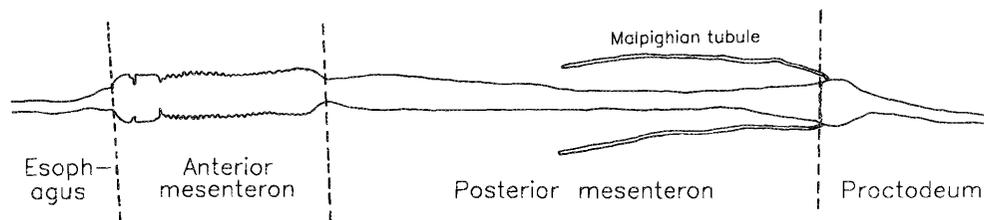


FIG. 1. Gut of a generalized chironomid larva indicating the four regions in which trace element measurements were made. Two of the four Malpighian tubules are shown.

thickwalled, screw-cap, Teflon vials (animals from Lakes Joannès and St. Joseph) or in Teflon bombs (other lakes) in hot concentrated nitric acid (Aristar grade; 100  $\mu\text{L}\cdot\text{mg}$  tissue dry weight<sup>-1</sup>). Digestion was carried out either in a water bath at 70°C for 48 h (Lake Joannès), a pressure cooker at 120°C for 4 h (Lake St. Joseph), or in a microwave oven up to a pressure of 2760 kPa for  $\approx 1$  min (other lakes).

Cooled digested samples were diluted with deionized water and trace element concentrations were measured either by flameless atomic absorption spectrophotometry (Varian GTA-95 graphite tube atomizer; As, Cd, Cu, Pb) or flame atomic absorption spectrophotometry (Varian AA-575; Zn). Repeated measures of similar-sized samples of a certified reference material (lobster hepatopancreas, Tort 1, National Research Council of Canada, Ottawa, Ont.) varied little over time (coefficient of

variation 3–7%;  $n = 10$  for each element) and were close to the expected certified mean values (within 3.5–6.0%;  $n = 10$  for each element).

## Results and Discussion

### General Trends

In all of the genera studied, most of the mass was represented by the body (tissues remaining after dissection) (Fig. 2). Similarly, the majority of the Zn present was located in the body portion (Fig. 2), indicating that concentrations of this element generally do not differ greatly among the various parts. However, substantial quantities of Zn were also present in the guts of two genera, *Hexagenia* and *Sialis* (Fig. 2), which reflects the somewhat higher concentrations of Zn in their guts

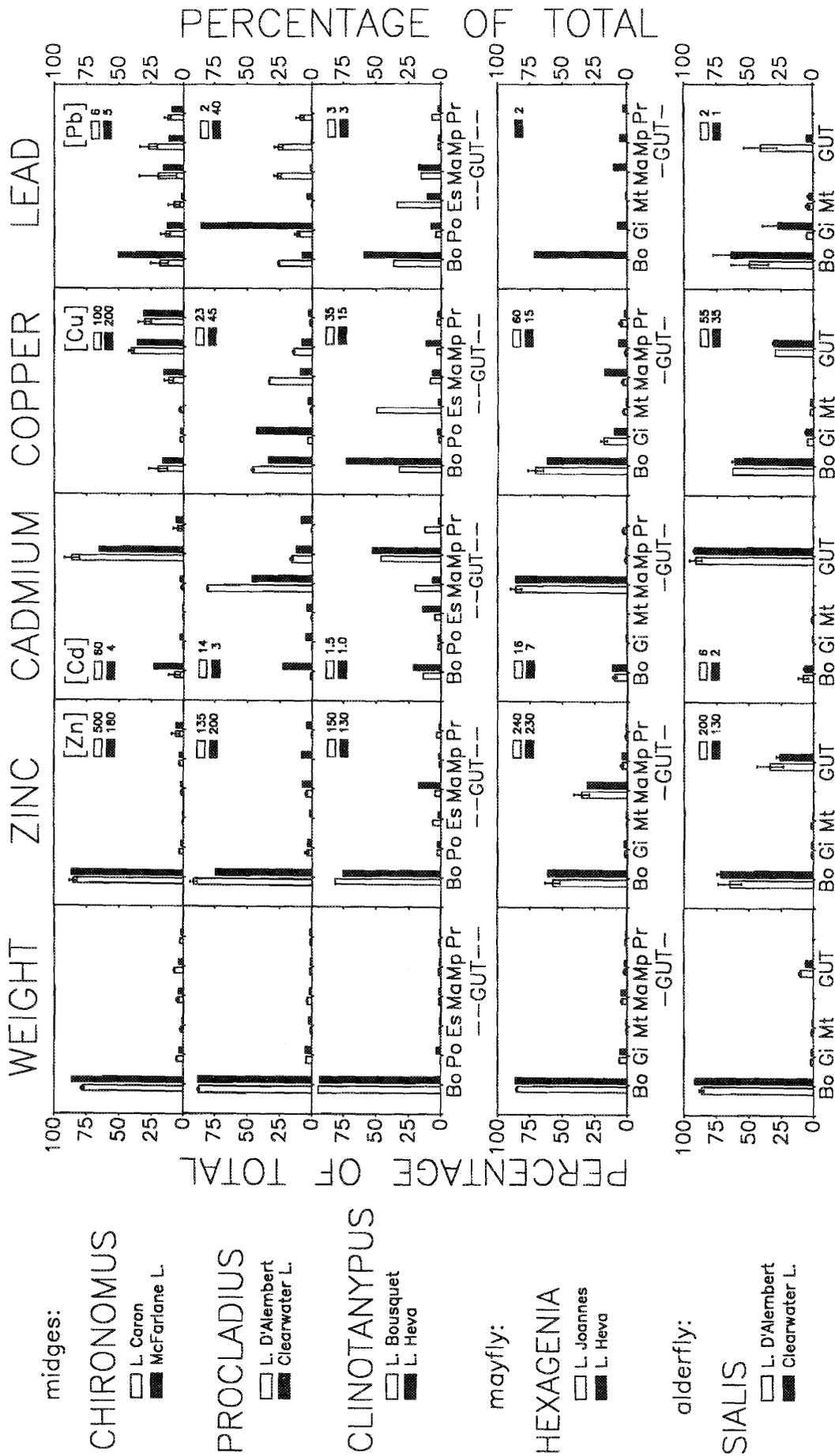


FIG. 2. Percentages of whole-animal dry weight and trace element burdens in the various parts of five aquatic insect genera, each collected from two lakes. Where multiple samples for a given genus from a given lake are available, mean values with their standard deviations are indicated. The number of samples per genus per lake is given in Table 1. Values given in the panels are trace element concentrations ( $\mu\text{g}\cdot\text{g}^{-1}$ ) for whole animals from each lake. No data for Pb are available from Lake Joannès.

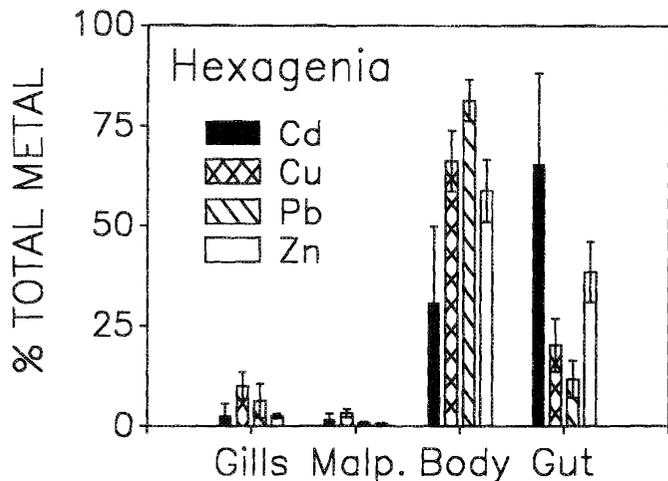


FIG. 3. Mean percentages ( $\pm$ SD,  $n = 12$ ) of whole-animal metal burdens represented by each of the body parts of *Hexagenia* nymphs collected from lakes listed in Table 1 (Malp. = Malpighian tubules).

compared with their other parts. Arsenic (data not shown) was localized to an even greater extent in the body portion of the single genus studied, i.e. the proportions of As in the parts of *Hexagenia* were body  $95 \pm 4\%$ , gills  $2 \pm 2\%$ , and gut  $3 \pm 2\%$  ( $\bar{x} \pm$  SD,  $n = 7$  from Lake Joannès). In marked contrast with Zn and As, the bulk of the Cd in all genera was localized in the gut (Fig. 2), where Cd concentrations often exceeded those in other parts by several orders of magnitude.

Copper tended to be localized in the body portion except in *Chironomus* in which greater quantities were present in the gut. Lead also tended to be associated with the body although the distribution patterns are much more variable among genera and between lakes. For example, in replicate samples of *Sialis* from each of two lakes, either 5 or 45% of the Pb was located in the gut (Fig. 2). Similarly, the proportions of Cu and Pb measured in the esophagus of *Clinotanytus* were very different between the two lakes studied; however, the higher values (Fig. 2, Lake Bousquet) may represent an artifact, as these measurements are based on a single pooled sample and are the only case in which large quantities of metals were measured in the esophagus of any genus.

A striking difference between lakes was also observed in the quantities of Cu and Pb located in the posteriormost abdominal segment (Po) of *Procladius* (Fig. 2). In this case, however, the higher unreplicated values cannot be dismissed, since in a third lake even higher proportions were measured in the posteriormost segment of this genus (Wavy Lake, data not shown; 80% of Cu and 95% of Pb in posteriormost segment). Possible explanations for this difference are either that the species of *Procladius* may be different in the two lakes or that the proportions of Cu and Pb accumulated in the posteriormost segment increase with increasing organism trace metal concentrations (Fig. 2; Wavy Lake whole larvae contained  $85 \mu\text{g Cu}\cdot\text{g}^{-1}$  and  $35 \mu\text{g Pb}\cdot\text{g}^{-1}$ ). This latter possibility should be tested on larvae of a wide range of metal concentrations.

#### Constancy of Patterns among Lakes

For a given trace element the distribution pattern within each genus was on the whole similar between the two lakes studied, in spite of differences in trace element concentrations between individuals from the lakes (Fig. 2). This suggests that the dis-

tribution patterns observed may be independent of trace element concentrations in the whole insect.

This hypothesis was tested on a larger data set comprising nymphs of the mayfly *Hexagenia* collected from many lakes having a wide range in sediment trace element concentrations. Overall, the metal distribution patterns observed for nymphs from the two lakes studied initially (Fig. 2) resembled those for the larger data set (Fig. 3), i.e. the bulk of the Cu, Pb, and Zn was located in the body, while the bulk of the Cd was located in the gut. However, a closer examination of the data for Cd suggests that there was substantial variability in the distribution pattern for Cd among nymphs from different lakes, as evidenced by the large standard deviations around the means for this metal (Fig. 3).

The proportion of a trace metal located in that metal's major organ of accumulation was compared with the concentration of the metal in whole nymphs (Fig. 4). The proportion of Cd contained in the gut was positively correlated with the log of whole-animal Cd concentrations (Fig. 4; Pearson product-moment correlation coefficient ( $r$ ) = 0.75,  $0.002 > P > 0.001$ ). Since animal Cd concentrations tend to be a function of environmental Cd concentrations (Hare et al., in prep.), these results suggest that given higher Cd concentrations in the environment both the quantity and proportion of Cd stored in the gut will increase.

In contrast with Cd, the proportion of Zn in the body shows a significant negative correlation with the log of whole-animal Zn concentrations (Fig. 4;  $r = 0.62$ ,  $0.02 > P > 0.01$ ). This suggests that nymphs contaminated to a greater extent than those measured in the present study could contain the bulk of their Zn in the gut. Comparable measures for Pb were significantly positively correlated (Fig. 4;  $r = 0.79$ ,  $0.02 > P > 0.01$ ); however, the calculated value of the intercept on the vertical axis (65%) suggests that even at low Pb concentrations the bulk of this metal would be found in the body portion. No significant correlation between comparable Cu values was observed (Fig. 4;  $r = 0.35$ ,  $P > 0.2$ ) and thus the bulk of the Cu should always lie in the body portion of nymphs.

Since trace element concentrations in aquatic insects can be correlated with nymph size (Smock 1983; Darlington et al. 1987), the relationships above (Fig. 4), although based on differences among nymphs from different populations, could also apply to the members of a single population. That is, the distribution pattern of a trace element within individuals from the same lake may vary according to their size and age. This possibility remains to be tested.

The ranges in concentration of metals in whole *Hexagenia* nymphs from the various lakes differed greatly among metals and increased in the following order: Zn  $2.0 \times$ , Pb  $3.5 \times$ , Cu  $5.0 \times$ , and Cd  $125 \times$  (Fig. 4, note log scale for [Cd]). The ranges of corresponding concentrations of total metal in surficial sediment were greater for all metals except Cd but increased in the same order: Zn  $10 \times$ , Pb  $15 \times$ , Cu  $20 \times$ , and Cd  $30 \times$  (data not shown). The wide range of Cd concentrations in organisms compared with those in sediments suggests that Cd is stored in nymphs to a greater extent than are the other metals. Furthermore, the similarity in the order of the ranges for metals in organisms and in sediments suggests that organism metal concentrations are a function of environmental metal concentrations.

#### Internal versus External Localization of Trace Elements

The trace elements measured in aquatic insects can be localized both externally on the chitinous body covering and inter-

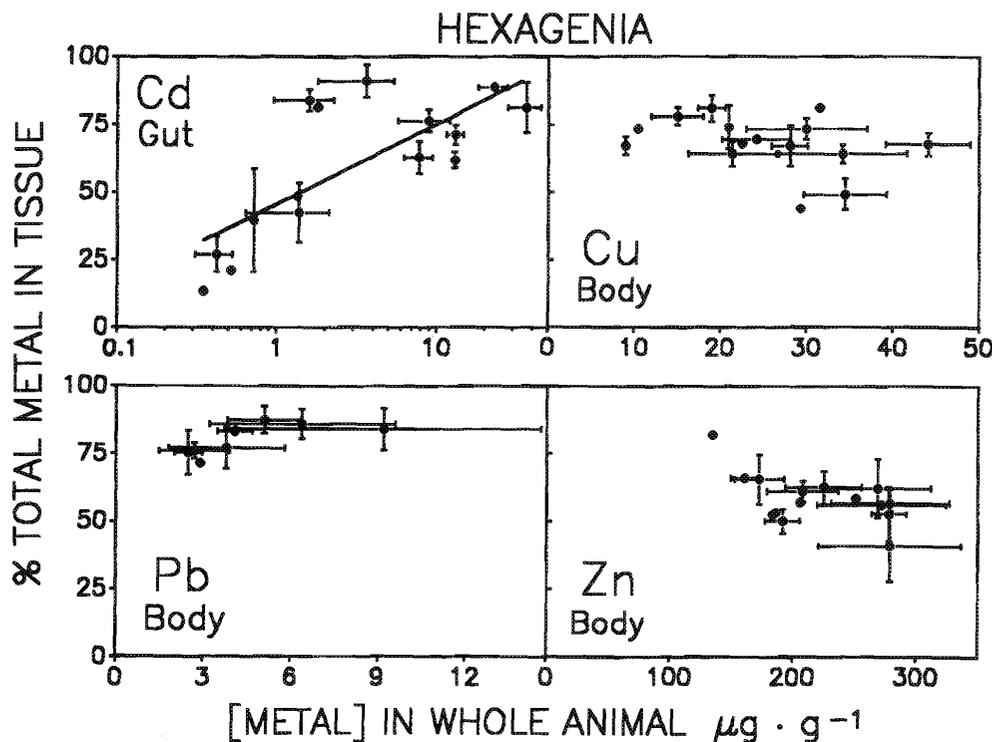


FIG. 4. Comparisons between the mean ( $\pm$ SD) percentage of metal found in the major storage tissue for each metal, with the mean ( $\pm$ SD) metal concentrations in whole *Hexagenia* nymphs collected from various lakes (Table 1). Log-linear regression of Cd:  $r^2 = 0.56$ . Note the logarithmic scale for [Cd].

nally in body tissues. In the mayfly *Hexagenia*, trace element concentrations in exuvial parts were equal to or greater than those in corresponding nymphal parts (Fig. 5). Metal concentrations were highest in the chitinous portion of the gut lining shed with the exuvium; however, given its small weight, it represented only a minor proportion of the total exuvial metal content (Fig. 5). Likewise, given the small proportion of the whole-gut weight represented in the exuvium (1%), trace elements associated with the chitinous gut lining represented small proportions of the whole gut burden: Cd 10%, Cu 5%, and Zn 2%.

The body portions of nymphs and exuviae always represented the bulk of the trace metal content in spite of their low metal concentrations (Fig. 5). The weight of the exuvium body represents  $\approx 10\%$  of that of the whole body and thus the proportions of each metal associated with the nymph body covering were approximately Cd 30%, Cu 15%, and Zn 5%. The exuvial gills, given their high surface area to volume ratio, represent a large proportion,  $\approx 30\%$ , of the weight of the gills of the whole nymph, and correspondingly high proportions of trace metals were associated with the shed exuvial gills: Cd 70%, Cu 30%, and Zn 40%. Based on the above values, the proportions of trace elements in the whole nymph associated with the entire animal surface were estimated at approximately Cd 40%, Cu 20%, and Zn 10%.

Comparisons of trace element concentrations were also made between recently emerged *Hexagenia* adults and their nymphal exuviae. It should be noted that adult/exuvium comparisons may be biased by metal losses during and after moulting, e.g. losses of Cd through postmoulting defecation by the adults of some terrestrial insects can represent 50% of the larval Cd burden whereas losses of Cu or Zn are apparently negligible (Aoki and Suzuki 1984).

Final instar exuviae and emergent adults had comparable concentrations of Cd, Cu, and Zn (Fig. 6); however, the exuvium, given its small weight ( $\approx 25\%$  of adult weight), represented minor proportions of whole-nymph trace element burdens. Arsenic was present in the exuvium but was not detectable in adults (Fig. 6) and thus a high proportion of the As associated with *Hexagenia* nymphs appears to be bound on/in the exoskeleton. A similar pattern has been observed for As in several stream-dwelling insects (Cain and Luoma 1990). Aluminum also appears to be largely associated with the surfaces of aquatic insects (Frick and Herrmann 1990).

Much of the Pb associated with aquatic insects from several taxonomic groups appears to be bound externally, e.g. Ephemeroptera (*Hexagenia*; Hare et al. 1991), Diptera (*Chironomus*; Krantzberg and Stokes 1988), and Odonata (*Libellula* and *Aeshna*; Meyer et al. 1986). In the present study, the positive correlation observed between the proportion of Pb associated with the body of *Hexagenia* and whole-nymph Pb concentrations (Fig. 4) is consistent with increasing quantities of Pb bound to the surface of organisms living along a gradient of increasing environmental Pb concentrations.

Both the nymph/exuvium and the adult/exuvium comparisons were consistent in suggesting that approximately 20–25% of the Cu and 10% of the Zn associated with nymphs of *Hexagenia* was bound to the exoskeleton. There was, however, a substantial difference between the estimated values for Cd; nymph/exuvium and adult/exuvium comparisons suggest that either  $\approx 40$  or  $\approx 7\%$ , respectively, of the whole-animal Cd burden was externally bound. This difference was consistent with, and can be explained by, the previously observed positive relationship between Cd concentrations in the nymph and the pro-

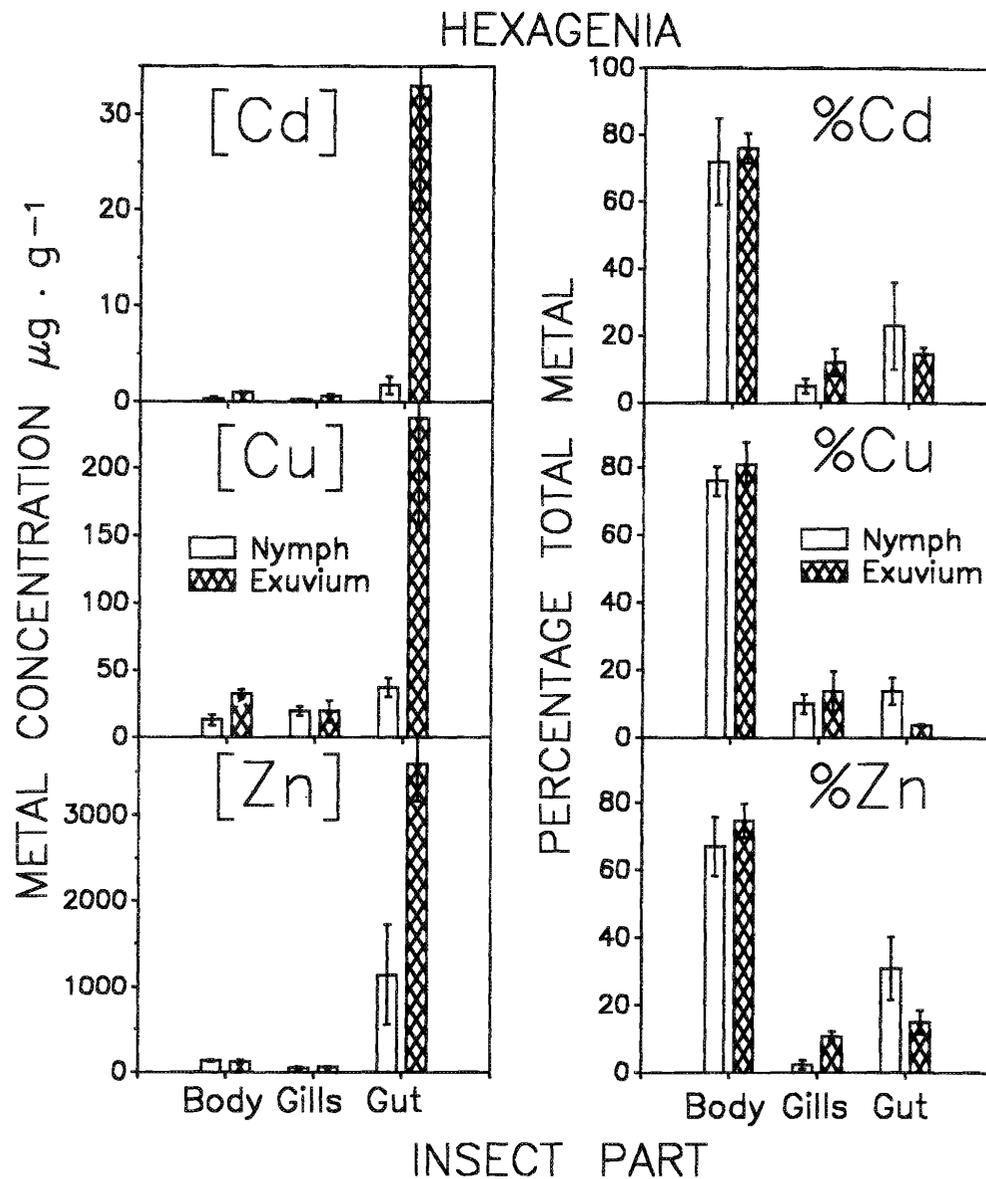


FIG. 5. Comparisons of both the concentrations and the proportional distributions of trace metals among the body parts of final instar nymphs and final instar exuviae of *Hexagenia* collected in Lake St. Joseph, Quebec.

portion of Cd that was associated with the gut (i.e. is internal; Fig. 4). Nymphs on which the adult/exuvium comparisons were made (from Lake Joannès) had much higher Cd concentrations (50×) and a higher proportion of Cd localized in the gut (80%) than did nymphs on which the nymph/exuvium comparisons were based (from Lake St. Joseph; 30% of Cd in the gut).

In addition to the possible influence of trace element concentrations in nymphs, environmental variables such as ambient pH will also be important in determining the degree of surface binding of some trace metals (Krantzberg and Stokes 1988). Furthermore, the proportion of trace elements bound externally will tend to vary negatively with body size, since the ratio of the surface area of an animal to its volume decreases with increasing body size. Such a tendency will be reinforced if there is an increase over time in the quantities of trace elements stored internally, e.g. in granules. Nymphs used in the above comparisons were in or near the final instar and thus the estimated proportions of externally bound metal should represent minima.

The proportion of trace elements bound externally can also vary according to the age of the exoskeleton (Hare et al. 1991), i.e. for a given instar, recently moulted individuals should have lower concentrations of trace elements bound externally than those about to moult.

Trace elements associated with the chitinous exoskeleton are unlikely to have toxic effects on the insect, but could be taken up by predators that consume these insects whole. Trace elements on the exoskeleton will remain in the aquatic system (the exoskeleton is shed periodically), while those absorbed internally can be transferred to the terrestrial system, on emergence of the adult insect, to be consumed there by terrestrial predators such as birds (Nyholm 1981).

#### Gut

Although concentrations of Zn, Cu, and Pb were often highest in the gut, the bulk of these metals was generally found in the body portion (Fig. 2). A similar pattern has been observed

## HEXAGENIA

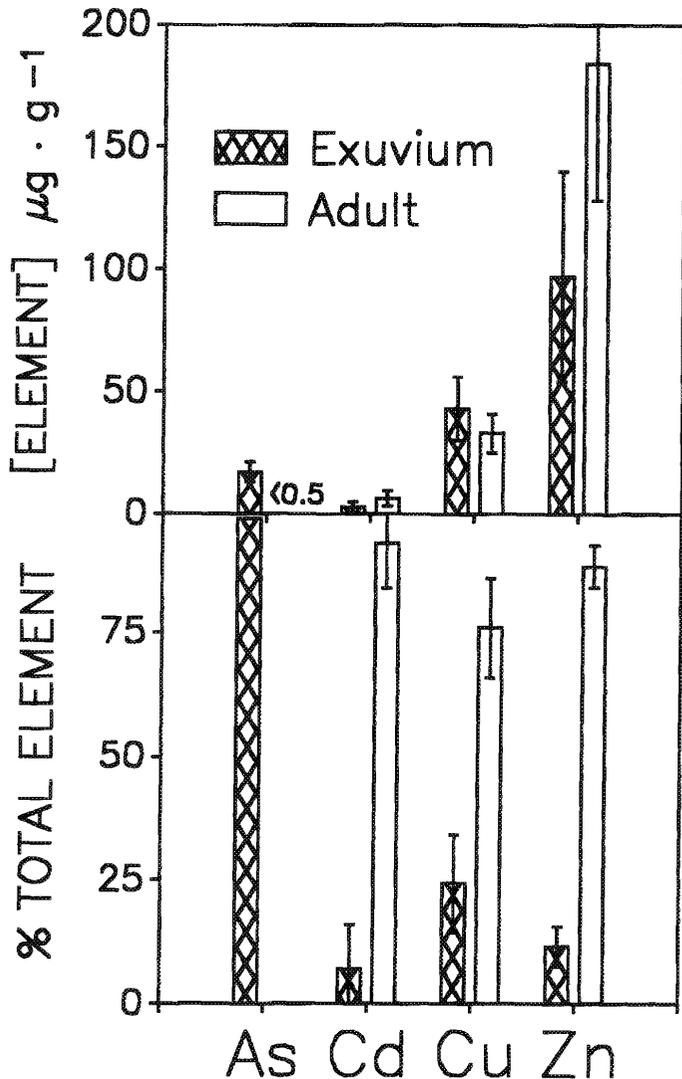


FIG. 6. Comparisons of both the concentrations and the proportional distributions of trace elements among the body parts of *Hexagenia* adults (subimago stage) and their final instar exuviae. Adults were reared in the laboratory from nymphs collected in Lake Joannès, Quebec.

for Pb in dragonflies (Odonata; Meyer et al. 1986). In contrast, Cd is largely associated with the insect gut in both aquatic (Seidman et al. 1986a; Hare et al. 1991; this study) and terrestrial (Aoki et al. 1984; Suzuki et al. 1984) taxa. The potentially large quantities of trace metals present in the gut could be stored in granules and/or lysosomes which have been observed in both aquatic (Seidman et al. 1986b) and terrestrial (Jeantet et al. 1977; Tapp and Hockaday 1977) insects. The rate at which such storage structures are excreted could determine the capacity of a given species to resist the toxic effects of metals (Humbert 1977). The depuration of metals from the gut could also be favored by their binding to and excretion with the peritrophic membrane, a protective chitinous sheet that is produced continuously by the mesenteron and excreted from the anus at rates of up to several millimetres per hour (Waterhouse 1954).

The digestive tract and accessory organs (the hepatopancreas, midgut gland, or digestive glands) are the major storage

sites for Cd, Cu, and Zn in both decapod crustaceans and in marine deposit-feeding molluscs, e.g. Zn and Cu (Bryan 1968), Cd and Zn (Coughtrey and Martin 1976), Cd and Zn (Bryan and Hummerstone 1978), Cd (Ray et al. 1980), Zn (White and Rainbow 1984), Cd (Engel et al. 1985), Cd (Uthe and Chou 1987), and Cu (Bagatto and Alikhan 1987). The functions of these accessory organs are no doubt carried out by the gut in insects, suggesting that there is some similarity in the patterns of metal storage among these disparate groups of invertebrates. In contrast, burdens and concentrations of these trace metals in some suspension-feeding freshwater molluscs are much higher in the gills and mantle than in the digestive gland (Tessier et al. 1984).

The gut of aquatic insects can be subdivided into regions based on differences in gross anatomy (Fig. 1), the presence or absence of a cuticular lining (Chapman 1984), and the proportions of various cell types present (Pierson 1956). These structural differences imply corresponding functional differences that could lead to differences in trace metal accumulation along the gut. In addition, the concentrations of trace elements available for uptake from gut contents will also vary among gut regions depending on the conditions in the gut lumen. The presence and type of digestive enzymes (Terra 1990) and the pH (Phillips et al. 1986) have all been shown to vary along the length of the insect gut.

In the genera studied herein, the cuticle-lined foregut (or stomodeum) consists simply of an esophagus (crop and gizzard absent) and thus probably functions only in the transport of food from the mouth to the midgut and not in its digestion or absorption. Low concentrations of trace elements were usually measured in this region (Fig. 2 and 7).

The midgut, or mesenteron, functions in the production of digestive enzymes and in nutrient absorption, while the chitin-lined hindgut, or proctodeum, is implicated in the reabsorption of salts, water, and other substances from the urine and faeces and in defecation (Chapman 1984; Dow 1986). In the aquatic genera studied herein the region of greatest metal concentration varied according to the genus and metal under consideration (Fig. 2 and 7). Concentrations of Cd and Zn were generally highest in the mesenteron region with the exception of *Chironomus*, in which concentrations of Zn were highest in the proctodeum (Fig. 7). Copper concentrations were highest in the proctodeum or, in the case of *Procladius*, in the mesenteron. Pb concentrations tended to be similar among gut regions (Fig. 7). In the gut tissues of terrestrial insects, trace metals tend to be concentrated in the mesenteron, where they are stored either in the form of granules (Jeantet et al. 1977; Humbert 1978) or in cytolysosomes (Tapp and Hockaday 1977).

Even within a gut region, trace element concentrations differed substantially. For example, in members of the family Chironomidae, Cd was found largely in the anterior region of the mesenteron in *Procladius* (and in the mayfly *Hexagenia*) but in the posterior region of the mesenteron in *Clinotanypus* and *Chironomus* (Fig. 2 and 7), possibly in membrane-enclosed crystals (Seidman et al. 1986a, 1986b). These patterns do not correspond to differences in feeding habits among the genera; *Chironomus* is a deposit/suspension feeder (Johnson 1987), while *Clinotanypus* and *Procladius* tend to have more predatory feeding habits (Roback 1976 and Sephton 1987, respectively). Thus they are probably a consequence of differences in the functional and/or structural organization of the mesenteron among these genera. A gradient of functions along the mesenteron has been reported previously for both chironomid (Pier-

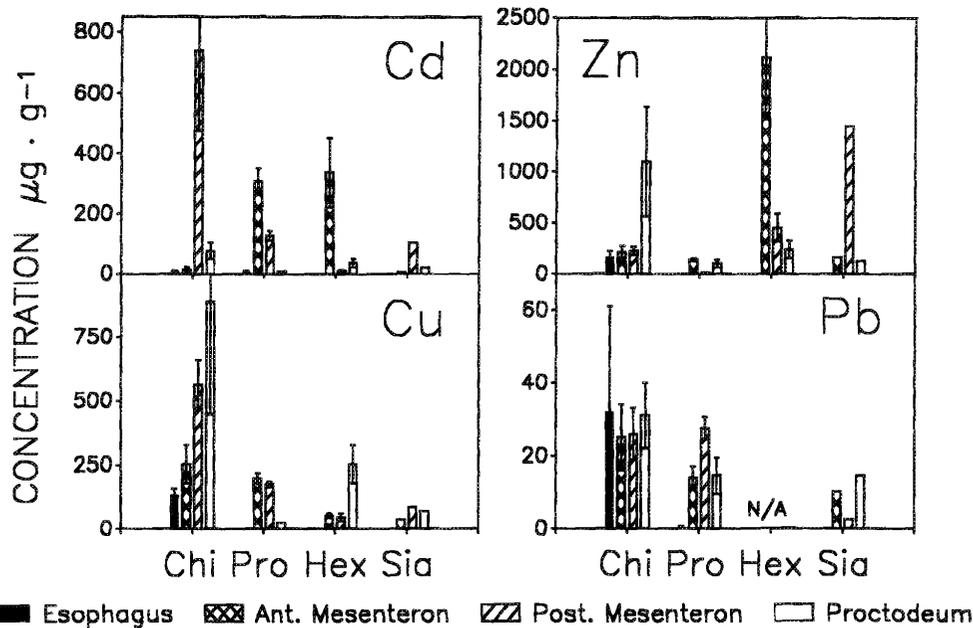


FIG. 7. Variations in trace metal concentrations along the guts of four insect genera. Values are means ( $\pm$  SD), with the exception of those for *Sialis* (a single pooled sample). Organisms were collected from either Lake Caron (Chi = *Chironomus*), Lake D'Alembert (Pro = *Procladius* and Sia = *Sialis*), or Lake Joannès (Hex = *Hexagenia*). N/A = measurements not available.

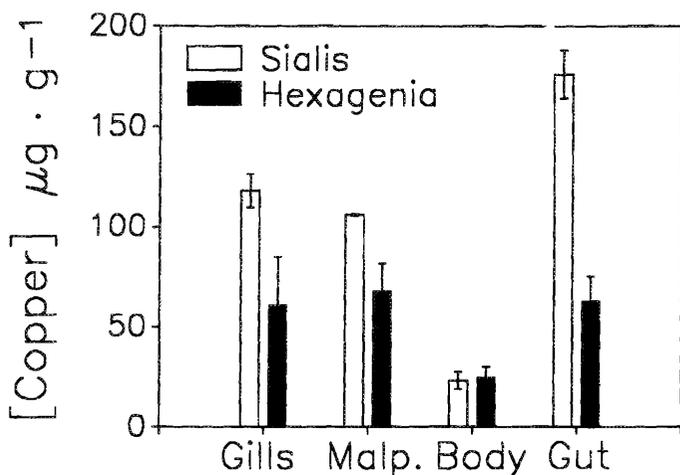


FIG. 8. Comparisons of mean ( $\pm$  SD) Cu concentrations among the body parts of *Sialis* and *Hexagenia* collected from Clearwater Lake or Lake D'Alembert, respectively (Malp. = Malpighian tubules).

son 1956) and mosquito larvae (Clements 1963). These differences suggest that generalizations about internal trace metal distributions based on taxonomic or nutritional similarities among insects should be made with caution.

#### Respiratory and Regulatory Organs

Trace elements were measured in respiratory (gills) and excretory structures (Malpighian tubules) of *Hexagenia* and *Sialis*. These structures never contain large quantities of any trace element compared with the quantities present in the body (Cu, Pb, Zn) or in the gut (Cd) (Fig. 2). Of the elements studied, Cu is distinctive in being present in fairly high concentrations in the gills and Malpighian tubules (Fig. 8) and in higher concentrations in the proctodeum than in other gut regions (of

*Hexagenia* at least; Fig. 7). A tentative explanation for this pattern could be the following.

Copper-based blood pigments are not present in insects. Copper extracted from body fluids by the Malpighian tubules could be in part stored in granules (Sohal et al. 1976) and in part excreted into the gut at the juncture of the mesenteron and the proctodeum (Fig. 1). Copper-containing granules have been observed in the Malpighian tubules of Trichoptera (Darlington and Gower 1990). In the proctodeum, Cu could be reabsorbed, perhaps by cells resembling the so-called chloride cells (Kornick 1977; Chapman 1984), and stored in granules (Balland-Dufrançais 1974). The high proportions of Cu measured in the gills suggest that the initial source of Cu to the body fluids may be from water via the gills.

Copper and Pb distributions in *Procladius* from two Sudbury area lakes were unique in that both the quantities and concentrations of these metals were higher in the terminal abdominal segment than in any other animal part (Clearwater Lake, Fig. 2; Wavy Lake, data not shown). Possible sites of trace metal accumulation in this segment could be either the claws of the posterior prolegs or the anal tubuli. The anal tubuli are thought to function in ionic regulation, at least in mosquito larvae (Stobart and Shaw 1974), and thus could also play a role in the storage and exchange of metals such as Cu and Pb. Trace metals have been detected previously in the anal tubuli of *Chironomus* (Krantzberg and Stokes 1990). Why this pattern should be observed for only one of the three chironomid genera studied bears further examination.

#### Conclusions

The study of contaminant distributions within aquatic insects is a useful extension of whole-animal studies, since, for a minimum of extra effort and expense, information can be obtained on the potential sites and mechanisms of contaminant exchange and toxicity within these animals. In addition, potential routes

of contaminant uptake can be determined, knowledge of which will facilitate the use of these animals as biomonitors of contamination. Such information is also useful in the design of complementary histological and biochemical studies aimed at characterizing processes at the tissue and cell levels. Measurements of the internal versus external localization of trace elements can be used to quantify moult-related losses of elements during insect development and the transfer of trace elements from the aquatic to the terrestrial system.

The results of the present study suggest that for a given insect taxon the internal distribution pattern of each trace element is fairly distinctive. Among taxa, the distribution patterns of both Cd, which is largely localized in the gut, and Zn, which is found in the body, are fairly constant, while those of Cu and Pb are more variable among both taxa and lakes.

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