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

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Nymphs of the burrowing mayfly *Hexagenia limbata* were collected in the field, returned to the laboratory, and dissected to remove the gut contents. Concentrations of four trace elements (As, Cd, Cu, Zn) were determined both for the gut contents and for the body. Trace elements in gut contents represented up to 22% of whole animal trace element burdens. Studies of depuration of *H. limbata* nymphs held in water for up to 48 h indicate that individuals vary substantially in the rate at which they egest gut contents and that 48 h is not sufficient to ensure a complete emptying of the gut. A model developed to compensate for the presence of gut contents in the determination of body trace element burdens was applied to *H. limbata*. Comparisons of model estimates with actual body burdens (without gut contents) show that the model accurately predicts As and Cu concentrations, but overestimates concentrations of Cd and Zn by as much as 20%. We suggest that the biases in the model result from assuming that: (a) trace element concentrations in gut sediments are equivalent to those in sediments sampled from the animal's surroundings, and (b) there is no weight loss of gut contents during sample digestion (a two-thirds weight loss of gut contents was in fact observed). These biases may be minimized by, respectively: (a) measuring trace element burdens of gut contents and (b) compensating for weight loss of gut contents during digestion.

Des nymphes de l'éphémère fouisseur *Hexagenia limbata* ont été recueillies, rapportées au laboratoire et disséquées de façon à retirer le contenu intestinal. Quatre éléments traces (As, Cd, Cu, Zn) ont été dosés dans le contenu du tractus intestinal et dans le corps de l'organisme. Les quantités retrouvées dans le contenu du tractus représentaient jusqu'à 22% de celles présentes dans l'organisme. Les études de dépuration chez les nymphes de *H. limbata* maintenues dans l'eau pendant 48 h montrent que le taux d'excrétion du contenu du tractus intestinal varie substantiellement d'un sujet à l'autre et que 48 h ne suffisent pas à assurer l'évacuation complète du tractus. Un modèle mis au point pour compenser pour la présence du contenu du tractus intestinal lors de l'évaluation des quantités d'éléments traces dans l'organisme a été appliqué à *H. limbata*. La comparaison des estimations fournies par le modèle avec les quantités mesurées dans le corps (sans le contenu du tractus intestinal) montre que le modèle prédit correctement la concentration en As et en Cu mais conduit à une surestimation de la concentration en Cd et Zn, jusqu'à 20% parfois. Nous supposons que les erreurs systématiques du modèle sont le résultat du fait que les hypothèses suivantes sont admises: (a) les concentrations en éléments traces dans les contenus du tractus intestinal sont équivalentes à celles observées dans les sédiments prélevés dans le milieu où vit l'animal, et (b) le contenu du tractus intestinal ne perd pas de poids lors de la digestion de l'échantillon (une perte de poids correspondant aux deux tiers a été observée). Ces erreurs systématiques peuvent être minimisées de la façon suivante: (a) en mesurant les quantités d'éléments traces dans le contenu du tractus intestinal et (b) en compensant pour la perte de poids du contenu du tractus durant la digestion.

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Compared with the ephemeral nature of chemical conditions in lake water, the relatively long life span and reasonably sessile nature of benthic organisms make them attractive as continuous biological monitors of pollutants. Benthic animals are known to accumulate pollutants and can effect their transfer from sediments. A common source of error in determining pollutant levels in benthic organisms is the presence in the digestive tract of pollutant-containing material which may or may not be ultimately incorporated into the organism. Thus a variety of techniques have been developed for compensating for gut contents when body burdens of pollutants are measured. Gut contents may be removed directly by dissection or by placing the live animal in water or in uncontaminated sediments for a length of time sufficient to eliminate its gut contents (depuration). Alternatively, the contaminant level in the whole undepurated animal may be measured and an estimate of the proportion contributed by the gut contents subtracted. Chapman et al. (1980) used such a correction technique for estimating the concentrations of trace metals in Oligochaeta (Annelida). The weight of residue remaining after chemical digestion of the organisms was multiplied by the concentration of the trace metal in the sediments from which the oligochaetes

had been collected. This quantity of trace element was then subtracted from the whole animal trace element burden.

Each of these methods has drawbacks. Dissection may not be possible on very small organisms, or where very large numbers of specimens are required. In addition, pollutant losses and cross-contamination of tissues can occur. Depuration in water may be very slow or incomplete for animals requiring incoming food to push the gut contents through. Depuration in sediments, though probably more rapid than in water, may be difficult to verify unless contaminated and uncontaminated sediments can be visually distinguished. An additional difficulty with depuration is the extreme change in the contaminant concentration gradient between the organism and its surroundings, with the concomitant potential for contaminant loss from the organisms. Laboratory results suggest that losses of some metals by animals held in water without nourishment may be rapid, e.g. larvae of *Chironomus* can lose 50% of their body burden of Cd over 3 d (Yamamura et al. 1983). Losses could be particularly acute in small animals, i.e. those with high surface to volume ratios.

Despite these limitations, very few investigators have attempted to establish the errors associated with either the presence of gut contents or the various methods of their removal. In this paper, we compare the body trace element burdens of *Hexagenia limbata* (Serville) (Ephemeridae, Ephemeroptera, Insecta) determined by dissection with those determined by the compensation model of Chapman et al. (1980). We also describe the pattern of material loss from the gut during depuration in water.

Materials and Methods

Collection of Nymphs

Nymphs of the burrowing mayfly *Hexagenia limbata* were used in the experiments. The species was identified by the examination of nymphs and laboratory-reared imagoes using the descriptions of McCafferty (1975) and Spieth (1941), respectively. Animals were collected on 21 January 1987 from Lake Joannès (surface area 4.5 km^2) situated 25 km to the east of the mining city of Rouyn-Noranda in northwestern Quebec (48°11′N, 78°41′W). The major source of trace element contamination to the lake is by atmospheric fallout from smelter emissions. Nymphs were collected at a mid-lake site, at a depth of 15 m, with a 15 × 15 cm Ekman grab. The population density of nymphs at this site on the sampling date was 310 ± 20 nymphs·m^{-2} ($\bar{X} \pm$ S.E., $n = 4$ grabs). Animals were transported to the laboratory, live, in closed plastic sacs containing lake sediments and water maintained near field temperature (2°C). In the laboratory, nymphs were kept in sediments from the collection site, in the dark, at a temperature of 4°C, for a period of 7 wk prior to dissection. During this time various methods of depuration, dissection, drying, and chemical digestion were tested.

Depuration, Dissection, and Digestion of Nymphs

In order to estimate the contribution of gut contents to animal trace element burdens, 10 nymphs covering a wide range of sizes (0.7–2.0 mm in length, excluding cerci) were sieved individually from sediment and were dissected immediately in small amounts (2 mL) of deionized water under a microscope at magnifications of 120 and 250×. An animal was immobilized by cutting its nerve cord by means of a ventral incision between the head and thorax (which also served to sever the intestine), then the seven pairs of plumose gills were plucked from the abdomen. A mid-ventral incision was made along abdominal segments 7–9 which permitted the extraction of the digestive tract from the posterior end. Once extracted, the anterior end of the gut was torn open, so as to expose the tube of compacted material within. One pair of forceps was used to hold the gut while the contents were withdrawn with another pair. If the cylinder of compacted gut contents broke during removal, an incision was made at the site of the break in order to withdraw

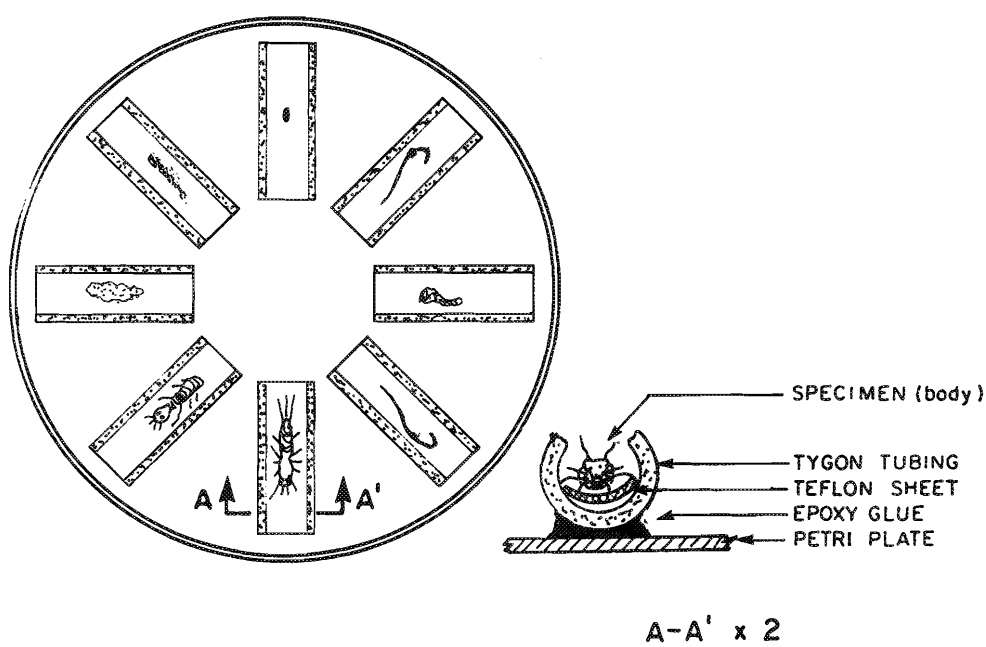

FIG. 1. Apparatus for drying small animal parts; consisting of a plastic petri dish onto which are glued half cylinders of Tygon tubing, into which pieces of Teflon sheet are placed to hold animal parts.

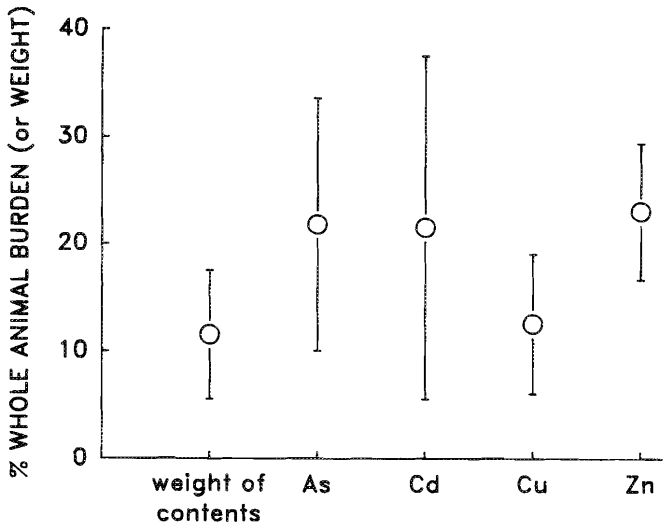

FIG. 2. Contribution (%) of weight and trace element levels of the gut contents of *Hexagenia limbata* to those of the whole animal; $\bar{X} \pm SD$, $n = 10$.

the remainder. A separate incision was made in the region of the gut posterior to the junction of the Malpighian tubules for removal of the contents therein. Animals whose guts broke up during dissection were discarded. As each part of the animal was removed (gills, gut, gut contents, remainder of body) it was placed for drying on a preweighed piece of acid-washed Teflon of appropriate size (thickness 0.013 mm; Warehoused Plastic Sales, Toronto, Ontario, cat. #615) which was held in a longitudinally cut piece of Tygon tubing cemented onto a plastic petri dish (all acid-washed), as illustrated in Fig. 1 (modified from method of N. Yan, Ontario Ministry of the Environment, Dorset, Ontario, pers. comm.). When necessary, a Pasteur pipette was used to transfer scattered material from the gut onto the Teflon piece. This apparatus facilitates the handling and drying of many small samples simultaneously; the pieces of Tygon tubing act to hold samples in place, the placement of samples on Teflon minimizes the possibility of their contamination, and the light weight of the Teflon pieces permits the accurate measurement of sample weights.

The covered petri dish was held in a freezer at −40°C (at least 30 min) and then in a lyophiliser (at least 12 h) in order to dry its contents. Each piece of Teflon holding a body part was weighed with a Mettler ME30 electronic micro-balance (balance pan covered with Teflon) and then placed into a thick-walled, screw-cap, acid-washed Teflon vial (Nalgene; 7.5, 15, or 30 mL capacity, according to specimen weight). Concentrated nitric acid (Aristar purity) was added to each vial (100 μL for each 1 mg tissue dry weight) which was then closed and heated in a water bath at 70°C for 48 h. Small samples (5–15 mg) of a certified standard (lobster hepatopancreas — Tort 1, National Research Council of Canada) were digested at the same time as the *Hexagenia* samples for use in verifying the accuracy of the entire procedure. Measured values of this standard were within the range of certified values for each of the elements studied. After digestion and analysis (see below), gut contents were filtered through acid-washed, preweighed, 0.4-μm Nuclepore filters. These were dried at 60°C to a constant weight and the weight of the filtered material calculated by subtraction. In addition, surface oxic sediments for trace element analysis (four samples of 2–5 mg dry weight) were collected with a pipette from the aquaria containing *H. limbata* and were dried and submitted to the digestion procedure.

TABLE 1. Mean concentrations ($\pm$ SD, $\mu g \cdot g^{-1}$) of various trace elements in the gut contents of nymphs of *Hexagenia limbata* ($n = 10$) and in the surficial lake sediments in which the nymphs live ($n = 4$) and the ratio of the latter to the former.

	As	Cu	Cd	Zn
[TE] in gut contents	39 ± 12	63 ± 9	18 ± 13	530 ± 170
[TE] in sediments	79 ± 5	91 ± 2	4 ± 0.1	290 ± 13
$\frac{\text{[TE] in sediments}}{\text{[TE] in gut contents}}$	2.01	1.44	0.23	0.55

For the measurement of changes with time in the weight of gut contents, nymphs were taken from sediments (at 5°C) and placed in 5°C lake water, which was either maintained at 5°C or allowed to equilibrate to room temperature (25°C), for up to 48 h. Coprophagy was minimized by placing netting (1-mm mesh aperture) in the water-filled containers which allowed faeces to fall to the container bottom out of reach of the nymphs. Dissection, drying, and weighing of nymphs followed the above described procedure except that the body parts of each nymph were dried and weighed together, only the gut contents being weighed separately.

Analytical Procedures

All labware was soaked in 15% nitric acid for 24 h and rinsed in deionized water prior to use. Deionized water for all uses was obtained from a Milli-Q3RO/Milli-Q2 system (Millipore Corp.). Manipulations of samples were performed whenever necessary in a laminar flow hood.

Cooled digested samples were diluted with deionized water to a concentration of 16.6% nitric acid (5:1 v/v). Trace element concentrations of the various solutions were measured with either a Varian AA-1275 series atomic absorption spectrophotometer equipped with a Varian GTA-95 graphite tube atomizer (As, Cd, and Cu) or a Varian AA-575 series flame atomic absorption spectrophotometer (Zn).

Results and Discussion

Contribution of Gut Contents to Weight and Trace Element Burdens of the Whole Animal

Gut contents collected from nymphs immediately after their removal from sediments represented a mean of 12% of the weight of whole animals (including gut contents) (Fig. 2). This proportion varied substantially among individuals (range of 2.5–22.0%) and showed little relation to animal dry weight (linear regression; $r^2 = 0.08$). Gut contents of *Hexagenia limbata* represented means of 12% (Cu) – 22% (As, Cd, and Zn) of whole animal trace element burdens (Fig. 2). These proportions were significantly greater than that for gut contents by weight for both As ($P = 0.025$) and Zn ($P = <0.01$) but not for Cd ($P = 0.10$) and Cu ($P = 0.85$) (Wilcoxon's signed-ranks test). These differences among elements are related to the ratios of the concentrations of these trace elements in the gut contents (Table 1) to those in the animal (Table 2), which were about 2.0 for As and Zn and 1.0 for Cu; although the ratio for Cd was 1.5, great variability among nymphs rendered insignificant the difference between the weight and trace element burden proportions (as compared above and in Fig. 2).

TABLE 2. Mean concentrations ($\pm$ SD, $n = 10$) of various trace elements in the entire body (excluding gut contents) of *Hexagenia limbata* and the percent change in these means if: (a) the sediment concentration of a trace element, $[TE]_s$, is used to estimate that in the gut contents, $[TE]_g$; (b) the weight of residue remaining after digestion of the whole animal in HNO_3 is used to estimate the original weight of the gut contents (W_g); (c) both of these factors are applied. Significance of differences between measured values (row 1) and calculated values (a, b, and c) was assessed using Wilcoxon's signed-ranks test where: **, $P < 0.01$; *, $0.01 < P < 0.05$; *ns*, $P > 0.05$.

	As	Cu	Cd	Zn
[TE] measured in body ($\mu g \cdot g^{-1}$)	22 ± 11	62 ± 13	12 ± 8	244 ± 13
(a) Percent change in mean body [TE] if gut content [TE] taken as sediment [TE]	-28%**	-13%**	$+15\%$ ns	$+3\%$ ns
(b) Percent change in mean body [TE] if weight of gut contents taken as that remaining after digestion	$+10\%$*	$+1\%$ ns	$+13\%$ ns	$+9\%$ **
(c) Percent change in mean body [TE] if both factors (a) and (b) are applied	$+1\%$ ns	-1% ns	$+20\%$ *	$+13\%$ **

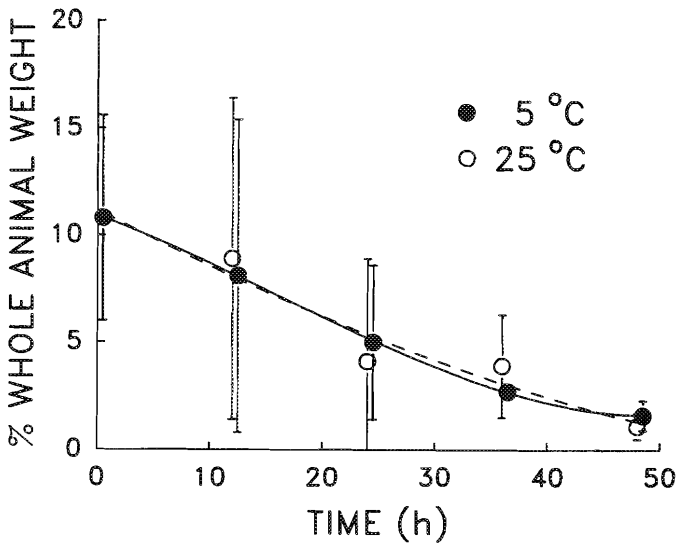

FIG. 3. Proportion of whole animal dry weight represented by gut contents for nymphs of *Hexagenia limbata* which had been removed from sediments (at 5°C) and kept in water (at either 5°C or 25°C) for various lengths of time; $\bar{X} \pm$ SD; $n = 10$ (time 0), 4 (time 24 h), or 3 (others). Second-order curvilinear regression lines are plotted for the two temperatures (5°C solid line, 25°C broken line).

Studies on other benthic animals suggest that gut contents can account for even higher proportions of animal pollutant burdens; e.g. gut contents can represent >60% of Pb and Ni body burdens of oligochaetes and ammocoetes larvae (Chapman 1985). Thus the accurate estimation of animal pollutant burdens usually necessitates the removal of gut contents, or at least a compensation for their contribution to animal burdens. An exception might be in the study of predator–prey relationships where pollutants in prey gut contents contribute to the predator's pollutant intake and thus should be included in prey analyses. Where desirable, contaminated gut contents can be eliminated by dissection, by depuration of the animals in water, or by replacement of gut contents with less contaminated food (e.g. by depuration in clean sediments). Alternatively, compensation can be made for the contribution of gut contents to body pollutant burdens. The merits and drawbacks of each of these approaches for the accurate determination of trace element body burdens are discussed below.

Dissection

Dissection of animals permits the direct and complete removal of gut contents and allows the determination of the distribution of pollutants among body parts. The major drawbacks of dissecting to remove gut contents are: it is time consuming and thus may be impractical when dealing with large numbers of animals; it can not be used for very small animals; cross-contamination among body parts can occur (Uthe and Chou 1987); and there could be a loss of body fluids (including associated pollutants) during dissection. To evaluate this latter point, losses of trace elements during dissection in water were measured by labelling nymphs of *H. limbata* (50-d exposure) in study-lake sediments containing the radio-isotopes ^{109}Cd, ^{210}Pb, and ^{65}Zn (added to sediments one year prior to use). Animals were depurated in nonradioactive sediments prior to dissection. Measures of these radio-isotopes in the water remaining after dissection represented, respectively, $2.8 \pm 3.9\%$, $0.4 \pm 0.1\%$, and $0.7 \pm 1.3\%$ of the mean totals present in the animals ($n = 7$). These results suggest that trace elements are associated in large part with body tissues and thus are not readily lost in body fluids during dissection.

Depuration

The proportion of whole animal dry weight represented by gut contents declined progressively over time during depuration in water (Fig. 3). From an initial mean of 12%, gut contents came to represent less than 2.2% of total body weight after 48 h. However, gut contents represented a highly variable proportion of total animal weight during the depuration period, as evidenced by the large standard deviations about the means, notably at 12 h (Fig. 3).

The times required for complete depuration under various conditions have not been measured for most benthic animals, but periods of 6–48 h have been commonly used, e.g. Pruell et al. 1986 (6 h); Lewis and McIntosh 1986 (24 h); Luoma and Jenne 1977 (48 h). During depuration animals should be inhibited from coprophagy by the placement of netting in the depur-

ation container (as in this study) or by frequent changes of water. As individuals of *H. limbata* can vary greatly in the rate at which they empty their guts (after 12 h of depuration, both individuals with empty and full guts were found), a depuration duration of less than 48 h could actually increase the variability among nymphs in terms of the quantity of gut contents present and thus their apparent trace element burdens. Complete depuration in water could take much longer than 48 h for species that require incoming food to push the gut contents through; e.g. we observed that *Chironomus* spp. larvae and some *H. limbata* nymphs held in water for 2 wk did not clear their guts completely. If trace element concentrations are high in the gut contents compared with those in the body, the persistence of even small quantities of material in the gut (as occurred after 48 h in this study) could bias measures of body trace elements.

Animals taken from the field are usually depurated at either field or room temperatures. Results of this study suggest that animals taken from sediments at 5°C and placed in water at either 5°C or 25°C differ little in the rate at which gut sediments are expelled (cf. similarity of regression lines, Fig. 3). This is not to suggest that in nature gut passage times of invertebrates would be unaffected by temperature. Nymphs of *H. limbata* taken from the field at various times of the year, and depurated at field temperatures in the laboratory, showed a negative relationship between temperature and gut clearance time (Zimmerman and Wissing 1978).

Depuration over a period of several days could lead to the loss of pollutants from an animal. For a given pollutant, rates of such losses could vary among individuals of the same or different species, depending on their sizes, surface area to volume ratios and metabolic rates. Thus, not only might the total pollutant concentration be affected, but the variability among individuals could be increased as well. For sediment feeders, depuration of contaminated gut contents can probably be achieved in a much shorter time by allowing animals to feed on little-contaminated natural or artificial sediments which would act to push through contaminated material. For *H. limbata*, gut clearance times of approximately 5 h in natural sediments (at 20°C) have been reported (Zimmerman and Wissing 1978). Even shorter gut passage times probably occur in nature (Hare, unpubl. data).

Difficulties of depuration in sediments, as opposed to water, can lie in; (a) obtaining sediments or artificial substrates which are consumed readily and which are sufficiently low in pollutant concentrations compared with those of the animal (in the case of lightly contaminated animals), (b) ensuring that an exchange of gut contents has taken place, and (c) measuring gut-content-free body weight (depurated animals will still contain gut contents) for use in calculating body pollutant concentrations.

Correction for the Presence of Gut Contents

An indirect means of compensating for the presence of gut contents in the determination of animal pollutant burdens is to measure pollutant burdens in whole animals (including gut contents) and to correct for the presence of gut contents. As individuals are likely to vary in the fullness of their guts (e.g. SD in Fig. 2), such a correction would be best applied on a sample-by-sample basis and not as a single mean correction factor for all samples of each taxon. The efficacy of a correction factor used by Chapman et al. (1980) for trace metals (equation 1), which corrects for gut contents on a sample-by-sample basis, has been evaluated in the present study. Direct measures of trace elements in the body and in gut contents removed by dissection have been used to assess its accuracy. It can be summarized as:

$$(1)\quad [\mathrm{TE}]_b = \frac{W_t \cdot [Te]_t - W_g \cdot [TE]_s}{W_t - W_g},$$

where [TE] = trace element concentration (micrograms per gram), W = weight (grams), and the subscripts refer to: (b) the entire body (gills + intestine + remainder, but excluding gut contents), (t) the whole animal (total including gut contents), (g) the gut contents, and (s) the sediments in which the animal lived. Direct measures of mean entire body concentrations of various trace elements in *H. limbata* are presented in Table 2 (row 1). The extent and significance of differences between these direct measures, and estimates made according to equation (1) (Table 2, rows *a–c*), will be discussed. Two sources of error that are inherent in the correction method require evaluation.

The first source of error lies in the use of the sediment concentrations of trace elements to approximate those of the gut contents. The assumption that sediment collected by an investigator corresponds in character to that eaten by an invertebrate ignores the substantial heterogeneity of bottom sediments. Trace elements are not usually distributed in lake sediments in a homogeneous manner, concentrations usually differing between sediments in the surface oxic and deeper anoxic layers (Carignan and Nriagu 1985). Furthermore, trace elements may be differentially associated with particles according to their size and/or quality (Warren 1981). Thus species that concentrate their feeding at different depths in the substrate or on different particle sizes or types, or species that show no electivity in feeding, could consume sediments having substantially different trace element concentrations. Even with such knowledge, it will be difficult for an investigator to sample the same quality of sediment as that contained in an animal's gut.

In the present study, the concentrations of trace elements in the neighbouring oxic sediments were higher than those measured in gut contents for two of the elements (As, Cu) and lower for the two others (Cd, Zn) (Table 1). Substituting the concentrations of trace elements in the sediment for those measured directly in the gut contents biases the resultant estimates either in a negative (As, Cu) or in a positive (Cd, Zn) direction (Table 2, a), as would be expected from the ratios of trace element concentrations in sediment to those in gut contents (Table 1). The positive bias for Zn is small and not statistically significant, while that for Cd, although larger, is not statistically significant due to the great variability of Cd concentrations among nymphs. Since the amount and direction of the bias vary among elements, no simple correction factor could be used to take this into account. Where possible, it would be preferable to measure directly the pollutant concentrations of gut contents, either removed from animals by dissection or collected after their depuration in pollutant-free water, rather than use an indirect measure of pollutant concentrations in neighbouring sediments.

A second source of error inherent in corrections with equation (1) lies in equating the weight of undigested gut contents with the weight of residue remaining after digestion of the whole animal. Since a proportion of the gut contents will be dissolved during the digestion, the weight of the residue will be less than that contained initially in the gut, leading to an underestimation of the gut content trace element levels and an error in those for

the body itself. In the present study, gut contents removed from the body by dissection were weighed and digested and their trace element contents measured. The subsequent filtering and reweighing of these samples gave a mean weight after digestion that was only $32 \pm 7\%$ ($\pm$ SD) of initial mean gut content weight. For any one digestion procedure, the extent of such weight loss would depend in large part on the character of the sediments consumed, e.g., organic matter and carbonate contents. For example, the profundal lake sediments studied herein had a total organic carbon content of $5.7 \pm 0.3\%$ ($\bar{X} \pm$ SD, $n = 3$) and were very low in carbonates. Since these components are the ones most likely to be solubilized by the digestion method used, the loss of two-thirds of the weight of gut contents during chemical digestion supports the idea that *H. limbata* sorts sediment so as to increase the quantity of organic matter consumed. Consequently, gut contents and surrounding sediments may differ greatly in composition, as suggested above.

Use of the weight of digested gut contents, as proposed in the correction method, increases the apparent mean body concentrations of the majority of elements studied (Table 2, b). There was little or no change in values of Cu, as the mean concentration of this element in gut contents represented a lower proportion of that for the whole animal than was the case for the other trace elements (Fig. 2). Measures of Cd varied greatly among nymphs and thus, although the approximated mean differed substantially from that measured, this difference was not statistically significant (Table 2). Overall, the effect of this source of error is less than might initially be expected as this factor occurs in both the numerator and denominator of equation (1). A simple and effective means of minimizing this source of error would be to collect gut contents, by either of the above methods, and measure the weight loss during digestion. Sediment from the animal's surroundings could be used if gut contents cannot be obtained. The mean percentage weight loss could then be used to correct the weights of digested gut contents.

These two sources of error may either counteract one another, yielding corrected values that are not significantly different from those measured directly (As, Cu), or be additive, resulting in corrected values that are significantly larger than the actual measures (Cd, Zn) (Table 2, c). However, since these sources of error may be minimized: (a) by measuring trace element burdens of gut contents, and (b) by compensating for weight loss of gut contents during digestion, the correction method could provide good estimates of body pollutant burdens.

Conclusions

The precise measure of pollutant concentrations in the bodies of benthic animals is usually confounded by the presence of contaminated gut contents. Their contribution to body burdens may in some cases be ignored if: (a) gut content pollutant levels are much lower than those of the animal, (b) a crude estimate is required (the cost of analyses may not warrant crude measures), and (c) one is studying the transfer of pollutants from prey to predators that consume their prey in their entirety, i.e. including gut contents.

In order to obtain precise estimates of pollutant body burdens it is usually necessary to remove or somehow compensate for the pollutants present in an animal's gut contents. Three possible approaches have been compared: (i) direct removal of the gut contents by dissection; (ii) induced removal of the gut contents (depuration); (iii) analysis of the whole animal (including gut contents), followed by a correction for the contribution afforded by gut contents. Method (i) provides measures that are precise and unambiguous and, where feasible, is the technique of choice for *H. limbata*. Method (ii) is a possible alternative, but the effects of the necessarily lengthy depuration period in water (48 h or more) on trace element levels of the organism are presently unknown. Depuration in uncontaminated sediments is a better method, provided that suitable sediments can be found (or manufactured). Method (iii) offers simplicity and savings in time; despite the inherent biases regarding the weight and trace element levels of the gut contents (which may be minimized, as discussed above), it yielded reasonably accurate estimates (max. error <25%) of the trace element content of *H. limbata* in the present study. In the final analysis, the choice of method will depend on the goals of and means available to an investigator.

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References

CARIGNAN, R., AND J. O. NRIAGU. 1985. Trace metal deposition and mobility in the sediments of two lakes near Sudbury, Ontario. Geochim. Cosmochim. Acta. 49: 1753–1764.

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.

CHAPMAN, P. M., L. M. CHURCHLAND, P. A. THOMSON, AND E. MICHNOWSKY. 1980. Heavy metal studies with oligochaetes, p. 477–502. *In* R. O. Brinkhurst, and D. G. Cook [ed.] Aquatic oligochaete biology. Plenum Press, NY.

LEWIS, T. E., AND A. W. MCINTOSH. 1986. Uptake of sediment-bound lead and zinc by the freshwater isopod *Asellus communis* at three different pH levels. Arch. Environ. Contam. Toxicol. 15: 495–504.

LUOMA, S. N., AND E. A. JENNE. 1977. The availability of sediment-bound cobalt, silver, and zinc to a deposit-feeding clam, p. 213–230. *In* R. E. Wildung, and H. Drucker [ed.], Biological implications of metals in the environment. Technical Information Center, U.S. Energy Research and Development Administration. (Available as CONF-750929, NTIS Springfield, VA.).

MCCAFFERTY, W. P. 1975. The burrowing mayflies (Ephemeroptera:Ephemeroidea) of the United States. Trans. Am. Entomol. Soc. 101: 447–504.

PRUELL, R. J., J. L. LAKE, W. R. DAVIS, AND J. G. QUINN. 1986. Uptake and depuration of organic contaminants by blue mussels (*Mytilus edulis*) exposed to environmentally contaminated sediment. Mar. Biol. 91: 497–507.

SPIETH, H. T. 1941. Taxonomic studies on the Ephemeroptera II. The genus *Hexagenia*. Am. Midl. Nat. 26: 233–280.

UTHE, J. F., AND C. L. CHOU. 1987. Cadmium in sea scallop (*Placopecten magellanicus*) tissues from clean and contaminated areas. Can. J. Fish. Aquat. Sci. 44: 91–98.

WARREN, L. J. 1981. Contamination of sediments by lead, zinc and cadmium: a review. Environ. Pollut. (Ser. B) 2: 401–436.

YAMAMURA, M., S. H. SUZUKI, AND K. KUBOTA. 1983. Tolerance to cadmium and cadmium-binding proteins induced in the midge larva, *Chironomus yoshimatsui* (Diptera, Chironomidae). Comp. Biochem. Physiol. 75C: 21–24.

ZIMMERMAN, M. C., AND T. E. WISSING. 1978. Effects of temperature on gut-loading and gut-clearing times of the burrowing mayfly, *Hexagenia limbata*. Freshwater Biol. 8: 269–277.