Dynamics of metal adaptation in riverine chironomids.

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CHAPTER IV

Efficient shedding of accumulated metals during metamorphosis in metal-adapted populations of the midge *Chironomus riparius*

ABSTRACT

Metal accumulation and loss during metamorphosis were investigated in *Chironomus riparius* populations in a metal contaminated lowland river. Cadmium and zinc levels were measured in imagoes and larvae at reference and metal-exposed sites. It was hypothesised that the relationship between metal concentrations in biota and environmental compartments would be influenced by the presence of metal-adapted chironomids. In contrast to the large interpopulation differences in larval body burdens of cadmium, body burdens in imagoes vanished to background levels for all midge populations. This indicated that any cadmium accumulated in larval stages was lost during metamorphosis. This nearly 100% efficiency in shedding of cadmium is most likely caused by an increased metal handling capacity present in exposed midges. In agreement with the cadmium measurements, larval body burdens of zinc showed also highly significant interpopulation differences. In contrast with the cadmium values, however, body burdens of zinc in imagoes showed highly significant interpopulation differences and differences were even recorded between the two exposed sites, indicating interpopulation differences in shedding capacity for zinc. It is concluded that the highly efficient shedding of accumulated metals reflected the metal adaptation recorded in earlier studies of metal-exposed *C. riparius* populations from the River Dommel. Based on the differences in metal accumulation and the differences found in shedding of metals between the two exposed midge populations, it was concluded that population differentiation due to metal stress is a gradual process rather than an all-or-nothing situation.

Introduction

Natural selection is the evolutionary force responsible for adaptation to the ever changing environment. Perhaps the best studied examples of natural selection in action are those in which adaptation to an increased anthropogenic metal input is investigated (Brandon 1990). In invertebrates, adaptation to metals has conclusively been demonstrated for a few species (Brown 1976; Klerks & Levinton 1989; Posthuma & van Straalen 1993; Postma & Groenendijk 1999). However, knowledge about the physiological mechanisms to cope with metal pollution is limited. Possible mechanisms include induction of metal binding proteins (Roesijadi 1992) and formation of metal-containing granules (Brown 1982). For example, copper-tolerant *Nereis diversicolor* may contain 100 times more copper compared to non-tolerant worms and absorption of copper is also more rapid in tolerant than in non-tolerant worms (Bryan & Hummerstone 1971). Furthermore, metal-adapted chironomid larvae showed a decreased net accumulation rate as well as higher equilibrium values compared to non-adapted chironomids when exposed to metals (Postma et al 1996). Consequently, the presence of tolerant individuals may disrupt in situ relationships between metals in larval tissues and environmental compartments. Although largely overlooked, the presence of such metal-adapted populations with their specific metal physiology might be significant to an understanding of metal accumulation relationships in the field, which are, however, not always present (Tessier et al 1984; Luoma 1989; van Hattum et al 1991; Pourang 1996; Bervoets et al 1997).

The influence of changed metal physics in adapted specimens was for instance shown in Collembola, which excreted metals very efficiently during moult in adapted populations (Posthuma et al 1992; 1993). In chironomids, metal accumulation is well documented for the larval stage (Bervoets et al 1994; Postma et al 1996; Bervoets et al 1997), but almost no data are available for metal levels in imagoes (Timmermans & Walker 1989). Furthermore, it should be noted that chironomids are holometabolous insects and the effects of an additional moult during pupation on the levels of metals in imagoes

has not yet been investigated. In this study, one of the primary objectives was to determine if a higher net metal accumulation in larvae corresponded with higher metal levels in imagoes.

In a lowland river in northern Belgium, a distinct point source has released a continuous high metal load of zinc and cadmium for more than a century (Postma 1995), creating a strong selective force on the benthic chironomid community. As a consequence, some of the metal-exposed chironomid populations have been shown to be less sensitive to cadmium compared to unexposed midges (Postma et al 1995a; 1995b). Therefore, the possible influence of metal adaptation on metal accumulation relationships in situ have been studied in this river. To this purpose, we determined metal accumulation in larvae and its loss during metamorphosis in reference and metal-exposed midge populations.

Materials and Methods

site description

This study was conducted in the River Dommel, which flows from Belgium to the Netherlands. The Dommel is a second to third order stream fed by rainwater and is one of the numerous lowland rivers in the Meuse River basin. The river is characterised by a sandy substrate and neutral waters with a naturally high iron content. Visibility is often limited to 10-20 cm due to suspended organic material, but seasonal variation does occur. Parts of the Dommel are heavily loaded with cadmium and zinc, which originates from a nearby zinc factory situated on the banks of a small stream, which enters the Dommel close to the Dutch-Belgian border (figure 4.1). The factory started producing zinc and cadmium from ores in 1888. During the 1980s yearly production averaged 120,000 tons of zinc and 600 tons of cadmium. In 1992, production of zinc and cadmium was stopped and the factory switched to recycling and the production of zinc alloys. Since that time, until now no change in metal concentrations in the River Dommel has been measured (Postma 1995).

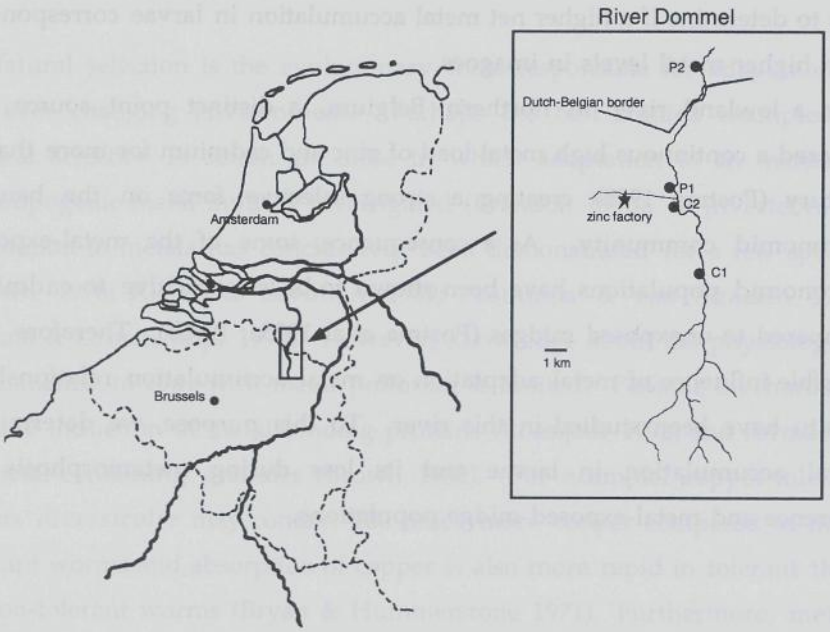

FIGURE 4.1: The location of the River Dommel in the Dutch-Belgian frontier area. The location of the four sampling stations and the zinc factory is indicated in the detailed inset.

Four sampling stations were selected in the Dommel. Two of them are reference locations situated upstream from the zinc factory: Heesakkers, Control 1 (C1), situated circa 7 kilometres upstream from the point source and Eindergatloop upstream (C2), situated only 100 metres upstream from the zinc factory. The other two sampling stations are situated in the metal-polluted downstream area: Neerpelt, Polluted 1 (P1), situated circa 500 metres downstream and Borkel (P2), situated circa 7 kilometres downstream from the zinc factory (figure 4.1).

chironomid sampling

larvae

Fourth instar larvae were collected on August 21, 1997, at all four study sites. Sampling took place in sedimentation areas of the Dommel, which are

a suitable habitat for *C. riparius*. The upper 5 cm of the mud layer was scraped off over several square metres using nylon nets with a mesh size of 300 µm. Sediment was sieved (400 µm) in the field and larvae belonging to the genus *Chironomus* were collected and transported to the laboratory the same day. From every sampling location, 100 chironomid larvae were kept for circa two hours in clean metal-free tap water to remove gut contents. To prevent coprophagy, all faecal pellets were removed by hand every 15-30 minutes. Larvae were not handled further before at least 90% of the gut contents was removed. Hereafter, larvae were rinsed for circa 10 minutes in acidified water to eliminate metals associated with larval integument. All larvae were stored frozen prior to further analysis. It was shown earlier (Postma 1995) that, based on karyotypic identification, probably all *Chironomus* larvae in the Dommel belong to the species *C. riparius*. Furthermore, control identification of randomly sampled male imagoes from each study site using the key of Pinder (1978) confirmed that, in the present study, also the adult *Chironomus* midges from the Dommel belonged to *C. riparius*.

adult midges

Imagoes were collected using a 125 W high pressure mercury lamp emitting a fraction of UV (Philips: HPL-N). The lamp, combined with a white screen, was installed simultaneously on the evening of August 19 at the C2 and P1 sites and on the evening of August 21 at the C1 and the P2 sites during calm (wind velocity < 2 m s^{-1}), cloudless, and warm (21 °C) weather. Collection took place during circa two hours starting at sunset. All *Chironomus*-like midges were caught alive in portions of 10-20 insects using an aspirator and were transported the same night to the laboratory and then stored frozen. The day after, midges were sorted by hand and a maximum of 100 *Chironomus* females and 100 *Chironomus* males for each location were collected separately. All midges were individually stored frozen prior to further analysis.

water and detritus sampling

Water samples were collected on August 21, 1997, in triplicate from the middle of the stream using acid-washed polyethylene bottles (100 ml) at a depth of approximately 10 cm. These water samples were centrifuged for five minutes at 3000 r min^{-1} and were acidified thereafter. Samples of the top sediment layer were collected in acid-washed polyethylene bottles (250 ml) and collected at several spots per sampling location to cover spatial heterogeneity. All samples were stored frozen prior to further preparation and were handled separately during the rest of the procedure. These samples, containing a coarse mixture of sediment and organic material, were sieved using a mesh size of 0.6 mm. Thereafter, sand and other heavy particles were allowed to settle for 60 s. This procedure was repeated twice, and all these sedimentated particles were discarded from the procedure. After an overnight stay, the cleared suspension was carefully siphoned off and the settled particles were collected and stored freeze-dried until further analysis. This material will hereafter be referred to as detritus, which is a common food source for detritivorous chironomids like *C. riparius* (Rasmussen 1984). Therefore, concentrations of metals in detrital particles will be a valuable characterisation of the exposure of detritivores, like chironomids, to the overall metal pollution in sediments (Simkiss 1990; Dorgelo et al 1995).

metal analysis

Detritus samples were digested six-fold per sampling location in HNO_3 (Ultrex, Baker, Philipsburg, NJ, USA) in fractions of about 1-2 mg. Digestion took place in a microwave equipped with a temperature and pressure control programme. Lyophilised *C. riparius* larvae and imagoes were weighed and digested individually in concentrated HNO_3 (Ultrex, Baker, Philipsburg, NJ, USA) and 30% H_2O_2 using the micro-destruction method described in Timmermans et al (1989). Water, detritus, and midge tissue samples were analysed for cadmium and zinc using Graphite Furnace Atomic Absorption Spectrometry (Perkin Elmer 5100) equipped with Zeeman background correction or air-acetylene Flame Atomic Absorption

Spectrometry (Perkin Elmer 1100B), depending on the metal concentration in the samples. Quality control of metal analyses was carried out by analysing destruction blanks and reference material (NIST: 2704 Buffalo River Sediment for detritus and IAEA Shrimp MA-A-3/TM for midge tissue). Recoveries were well within product specifications (less than 10% deviation), and destruction blanks were close to detection limits.

statistical analysis

All data were tested for homogeneity of variances using Bartlett's test and for normality by the Kolmogorov-Smirnov test for goodness of fit. This revealed that assumptions for analysis of variance were repeatedly violated, even after logarithmic transformation of the data. Therefore, non-parametric Mann-Whitney (MW) *U*-tests (two classes) and Kruskal-Wallis (KW) tests (more than two classes) were performed to analyse the data. To avoid a significant influence of individual differences in metal uptake, sample sizes of 100-200 individuals were chosen for accurate and reliable population estimates. Furthermore, to reduce the likelihood of type I errors, H_0 was rejected in all tests if the calculated p-value was equal to or higher than 0.01. All statistical methods are outlined in Sokal & Rohlf (1995).

Results

metal concentrations in water and detritus

Dissolved cadmium and concentrations of cadmium in detritus (figure 4.2) differed significantly among locations (KW = 11.4; $p < 0.01$ and KW = 18.6; $p < 0.001$, respectively). Dissolved zinc (figure 4.3A), however, was not significantly different among locations (KW = 10.4; $p = 0.016$). In contrast, zinc concentrations in detritus (figure 4.3B) showed a marked difference among sampling sites (KW = 19.5; $p < 0.001$). Cadmium and zinc concentrations in both water and detritus were clearly higher downstream from the outlet of the zinc factory, differing roughly one or two orders of

magnitude for cadmium and less than one order of magnitude for zinc between reference and polluted sites.

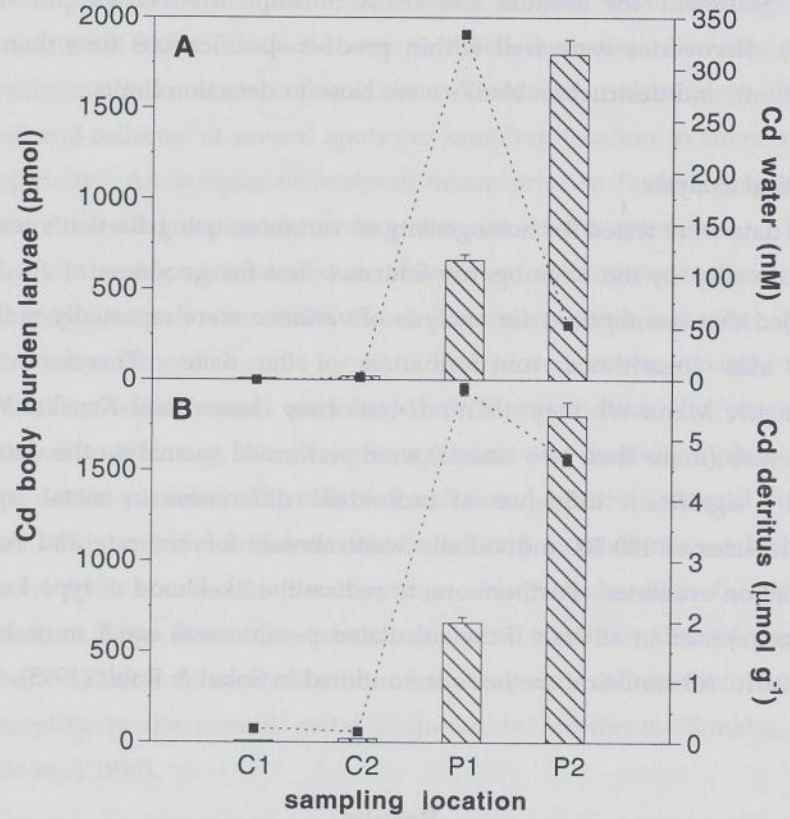

FIGURE 4.2: Cadmium body burdens in larvae (hatched bars) of *Chironomus riparius* and cadmium concentrations (black squares) at four sites in the River Dommel. C1 and C2 represent reference sites, P1 and P2 represent metal-polluted locations. Panel A shows dissolved cadmium concentrations (nM), panel B shows cadmium concentrations in detritus (µmol g^{-1} DW). Presented are the mean values together with their standard error.

body burdens versus concentrations

Cadmium concentrations (nmol g^{-1}) in male midges were a factor of two higher than those in female midges (MWU = 22,595; $p < 0.001$; data not shown). Female midges weighed about twice as much as male imagoes, which corresponds closely with the two-fold difference in cadmium concentrations between male and female midges. This difference due to sex disappeared when body burdens were measured (MWU = 34,870; $p > 0.01$),

and subsequent analyses with cadmium were, therefore, based on body burdens. In agreement, zinc body burdens as well as zinc concentrations differed significantly between male and female adult midges using all populations (body burden: $MWU = 55{,}180$; $p < 0.001$; data not shown; concentrations ($\mu mol\ g^{-1}$): $MWU = 28{,}191$; $p < 0.001$; data not shown). This difference most likely reflects the physiological need of extra zinc in females, which carry developing egg masses. Despite the difference, further analyses were based on body burden levels to make comparison with cadmium measurements possible. No significant differences in weight of larvae and midges among sampling locations were found (larvae: $KW = 6.11$; $p > 0.01$; imagoes: $KW = 5.44$; $p > 0.01$), justifying the use of body burdens in the present analyses.

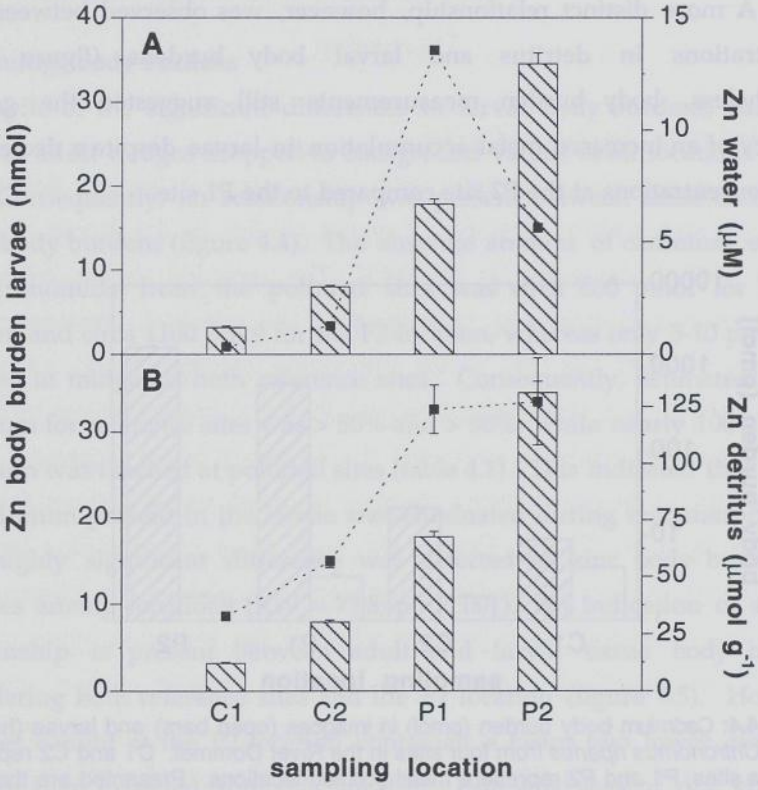

FIGURE 4.3: Zinc body burdens in larvae (hatched bars) of *Chironomus riparius* and zinc concentrations (black squares) at four sites in the River Dommel. C1 and C2 represent reference sites, P1 and P2 represent metal-polluted locations. Panel A shows dissolved zinc concentrations (µM), panel B shows zinc concentrations in detritus ($\mu mol\ g^{-1}$ DW). Presented are the mean values together with their standard error.

larval body burdens

A highly significant difference was observed in larval cadmium body burdens among populations (KW = 351.3; $p < 0.001$). Although cadmium concentrations both in water and detritus were higher at the P1 site compared with the P2 site, midge larvae sampled at the P1 location showed lower cadmium body burdens compared to those sampled at the second polluted site. Consequently, cadmium body burdens did not reflect a consistent relationship with the cadmium concentrations in either water (figure 4.2A) or detritus (figure 4.2B). As observed for cadmium, highly significant interpopulation differences were detected in larval zinc body burden levels (KW = 339.5; $p < 0.001$), but again no relation between zinc larval tissue levels and dissolved zinc at the study sites was observed (figure 4.3A). A more distinct relationship, however, was observed between zinc concentrations in detritus and larval body burdens (figure 4.3B). Nevertheless, body burden measurements still suggested the general tendency of an increased metal accumulation in larvae despite a decrease in metal concentrations at the P2 site compared to the P1 site.

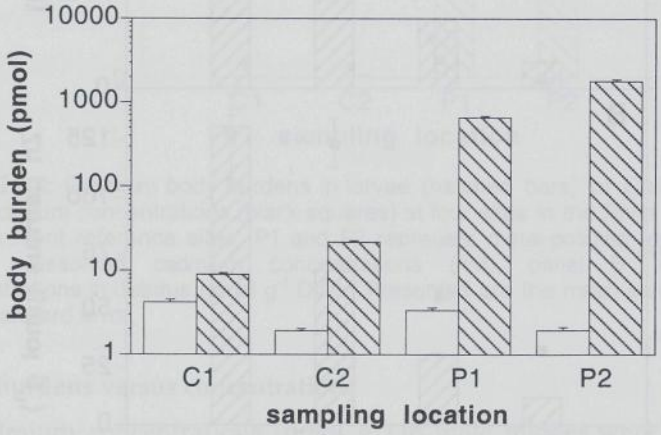

FIGURE 4.4: Cadmium body burden (pmol) in imagoes (open bars) and larvae (hatched bars) of *Chironomus riparius* from four sites in the River Dommel. C1 and C2 represent reference sites, P1 and P2 represent metal-polluted locations. Presented are the mean values together with their standard error.

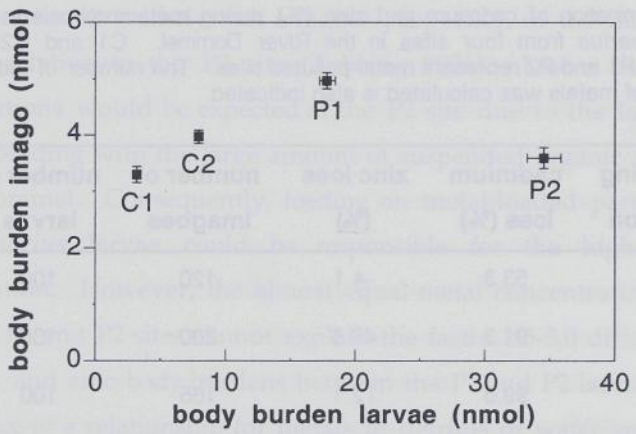

FIGURE 4.5: Relationship between zinc body burden in imagoes (nmol) and zinc larval body burden (nmol) in *Chironomus riparius* from four sites in the River Dommel. C1 and C2 represent reference sites, P1 and P2 represent metal-polluted locations. Presented are the mean values together with their standard error.

adult midge body burdens

In spite of the significant differences in larval body burdens, cadmium levels in adult midges dropped to background values at all locations (figure 4.4). Consequently, no relationship was present between adult and larval tissue body burdens (figure 4.4). The absolute amount of cadmium excreted in chironomids from the polluted sites was circa 600 pmol for the P1 location and circa 1100 pmol for the P2 location, whereas only 5-10 pmol was excreted in midges at both reference sites. Consequently, estimated loss of cadmium for reference sites was > 50% and > 90%, while nearly 100% loss of cadmium was reached at polluted sites (table 4.1). This indicated that almost all cadmium present in the larvae was eliminated during metamorphosis.

A highly significant difference was detected in zinc body burdens in imagoes among locations ($KW = 73.8$; $p < 0.001$). An indication of a linear relationship is present between adult and larval tissue body burdens considering both reference sites and the P1 location (figure 4.5). However, imagoes caught at the P2 location contained a factor two lower zinc body burdens than expected from this linearity. Estimated loss of zinc gradually increased from circa 0% for the reference site C1 to > 80% for the P2 site (table 4.1).

TABLE 4.1: Elimination of cadmium and zinc (%) during metamorphosis in the midge *Chironomus riparius* from four sites in the River Dommel. C1 and C2 represent reference sites, P1 and P2 represent metal-polluted sites. The number of individuals on which the loss of metals was calculated is also indicated.

sampling location	cadmium loss (%)	zinc loss (%)	number of imagoes	number of larvae
C1	53.3	-4.1	120	100
C2	91.3	49.8	200	100
P1	99.5	72.1	165	100
P2	99.9	89.6	112	100

Discussion

metals in larvae, detritus and water

Metal concentrations in water and detritus in the present dataset are consistent with results obtained in earlier studies in the River Dommel (Postma 1995; Groenendijk et al 1996). The recorded values reflect the overall metal pollution and, consequently, the selection pressure on the benthic fauna that has lead to the previously reported metal adaptation (Postma 1995). The observed differences in metal body burdens in larvae among locations generally suggested the tendency of an increased metal accumulation in larvae despite a decrease in metal concentrations at the P2 site compared to the P1 site. Commonly, such a lack of correlation between contaminants in biota and sediment or water is explained by differences in abiotic factors among sites, such as physical (eg sediment type; current velocity) and chemical characteristics (eg pH; organic carbon content). However, no major differences in principal water and sediment characteristics along the River Dommel have been recorded (Postma 1995), although it should be noted that, due to the distance between the P1 and the P2 location, some differences in bioavailability of metals could be expected. Because the zinc factory is located only a few hundred metres upstream from

the P1 location, it is likely that a higher proportion of the metals is still in solution relative to the P2 site (Tubbing 1996). Lower dissolved metal concentrations would be expected at the P2 site due to the larger period of time for binding with the large amount of suspended organic matter present in the Dommel. Consequently, feeding on metal-loaded particulate matter by *C. riparius* larvae could be responsible for the higher net metal accumulation. However, the almost equal metal concentrations in detritus from the P1 and P2 sites cannot explain the factor 2.5-3.0 difference in both cadmium and zinc body burdens between the P1 and P2 larvae. Therefore, the absence of a relationship for metals in detritus or water and in larvae of *C. riparius* may be explained by physiological differences in the midge populations themselves. The recorded cadmium adaptation in *C. riparius* populations from the metal-loaded part of the river has been shown to influence the accumulation and depuration of metals (Postma et al 1995a; 1995b). Adapted midge populations showed an increased storage capability in the guts and an increased excretion efficiency (Postma et al 1996) and, consequently, differences in net metal accumulation may reflect differences in metal adaptation between upstream and downstream midge populations. The difference in metal accumulation between the P1 and the P2 site can be explained by immigration of non-adapted larvae from upstream sites. For example, it was shown that migrating *C. riparius* larvae entering the polluted zone directly after the point source interbreed with the specimens already present at the polluted sites because of their similar life cycles (Groenendijk et al 1996; 1998). Although not demonstrated among invertebrates, crossbreeding has been shown to lower metal tolerance among offspring in copper-tolerant plants (McNeilly 1968). Consequently, the midge population at the P2 site may well accumulate more metals because the larvae present are most likely better adapted to the metal-stressed environment than the midges present at the P1 site due to immigration and crossbreeding. In addition, adult flight can also contribute to gene flow, but this influence is supposed to be much weaker compared with larval drift because of the less pronounced dispersal capacity (less than a few hundred metres) of the short living and relatively weak flying adult

midges (Davies 1976; McLachlan 1983). We conclude therefore, that metal adaptation in *C. riparius* is influencing in situ metal accumulation relationships between larvae (tissue body burdens) and detritus and water.

shedding of accumulated metals

Large differences were measured in cadmium body burdens between larvae from reference and polluted sites (a factor of 30-200). However, these elevated levels dropped to background body burdens in adult midges. To our knowledge, the values for adult midges are comparable to, or even lower than, cadmium levels in chironomid imagoes present in non-polluted environments or habitats containing the same background levels as the reference sites in the present study (Timmermans & Walker 1989; Currie et al 1997). However, when Timmermans & Walker (1989) exposed *C. riparius* larvae from a reference laboratory culture for only ten days to the same cadmium concentration as measured at the P1 location, larval body burdens were comparable with those in the present study, whereas the imagoes showed a hundred-fold increase in cadmium body burdens. This strongly suggests an increased cadmium-elimination efficiency in *C. riparius* midges from metal-contaminated sites in the present study. In agreement with this field observation, Postma et al (1996) found an increased cadmium depuration rate in *C. riparius* larvae sampled at both the P1 and P2 sites. We conclude, therefore, that the cadmium adaptation present in *C. riparius* populations (Postma et al 1995a; 1995b) is most likely attained by both a higher metal-excretion capacity in larvae and a highly efficient capacity to shed accumulated cadmium during metamorphosis.

This increased loss of cadmium as well as the increased net metal accumulation in larvae can be explained by an increased metal storage capability in the guts of metal-exposed chironomids (Postma et al 1996). Such storage in gut tissue is known to contain 90% of the accumulated cadmium. Increased storage capacity could be caused by an increase in metal-handling granules in the gut epithelium of the metal-exposed midges or by a higher capacity or increased efficiency of these granules. The presence of these granules and their possible function in metal handling is

reported in several insects, including a *Chironomus* species (Sohal et al 1977; Seidman et al 1986a; Lauverjat et al 1989). As these granules may be expelled into the gut lumen by exocytosis or degeneration of complete cells, an increased elimination is to be expected. Indications for this mechanism are described by Seidman et al (1986b), who measured high amounts of cadmium in degenerating cells or debris from cells from the midgut epithelium sloughed into the gut lumen of *C. thummi* larvae.

Reported values of zinc body burdens in imagoes grown under clean conditions ranged from 2.0 to 3.5 nmol zinc for three different chironomid species, including *C. riparius* (Timmermans & Walker 1989). Body burdens in imagoes from both reference locations, as well as from the P2 site, corresponded closely to this range. Imagoes from P1, however, showed higher levels of zinc compared with those from P2, although larval body burdens were nearly a factor two lower compared with the P2 larvae. This trade-off cut across the suggested linear relationship between zinc adult midge and larval body burden and consequently the P2 population eliminated circa 20% more zinc than the P1 population. In contrast to the cadmium measurements, these data suggest that not all zinc present is lost during the process of metamorphosis. This indicates that the shedding capacity in *C. riparius* larvae from the River Dommel is used first to eliminate the excess of the non-essential and potentially toxic cadmium to background levels. As far as excretion capacity is available, redundant amounts of zinc will be excreted as well in an attempt to reach the reported background values of circa 2.0 to 3.5 nmol zinc in imagoes (Timmermans & Walker 1989).

The marked difference in zinc loss between the P1 and P2 location can be explained by a lower metal storage capacity in P1 chironomids compared with P2 midges (Postma et al 1996). As argued above, this can reasonably be explained by a positive net immigration of non-tolerant larvae from upstream sites. In particular, this drift can easily result in crossbreeding of non-exposed and metal-exposed subpopulations (Groenendijk et al 1996), and this will consequently result in a less defined adaptation to metals in midges at sites just downstream from the zinc factory. This suggests that

population differentiation due to metal stress is a gradual process rather than an all-or-nothing situation. The possibility for a continuum in metal adaptation has been supported by results obtained with other invertebrates such as the collembolan *Orchesella cincta* (Posthuma et al 1993; van Straalen et al 1987).

conclusions

1) Metal adaptation in *C. riparius* modifies in situ relationships between larval body burdens and concentrations of metals in detritus or water.
2) During pupation, elimination of accumulated metals was highly efficient in *C. riparius*. The efficient shedding of metals in exposed chironomids is most likely explained by an increased metal depuration capacity recorded in genetically adapted midges (Postma & Groenendijk 1999).
3) Metal shedding capacity in *C. riparius* larvae is most likely used first to eliminate the excess of cadmium to background levels. As far as excretion capacity is available, redundant amounts of zinc will be excreted as well.

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