UNIVERSITY OF AMSTERDAM

UvA-DARE (Digital Academic Repository)

Increased cadmium excretion in metal-adapted populations of the midge Chironomus riparius (Diptera).

Postma, J.F.; van Nugteren, P.; Buckert-de Jong, M.C.

Link to publication

INCREASED CADMIUM EXCRETION IN METAL-ADAPTED POPULATIONS OF THE MIDGE *CHIRONOMUS RIPARIUS* (DIPTERA)

JAAP F. POSTMA,* PAUL VAN NUGTEREN and MARION B. BUCKERT-DE JONG
University of Amsterdam, Section of Aquatic Ecotoxicology, Kruislaan 320, 1098 SM Amsterdam, The Netherlands

Abstract—Cadmium kinetics were studied in cadmium-adapted and nonadapted field populations of the midge *Chironomus riparius*. Accumulation and elimination experiments were carried out using first-generation laboratory-reared animals. Differences between populations were, therefore, assumed to have a genetic basis. Larvae were dissected to analyze the guts and the remainder of the larvae separately. First-order one-compartment models were not always successful in describing accumulation processes, probably due to acclimation. No interpopulation differences were observed in larval development based on dry weights, whereas some differences existed based on pupation rate. In most cases more than 80% of the total amount of cadmium was found in the guts of all populations. Larvae from cadmium-adapted populations showed a decreased net accumulation rate as well as higher equilibrium values (15–20%) compared to nonadapted populations. In addition, cadmium excretion efficiency was increased for cadmium-adapted larvae, which was due to an increased elimination rate from the guts. It was concluded that exposure to high cadmium concentrations in the field resulted in populations of *C. riparius* with an increased storage capability and an increased excretion efficiency, especially regarding the guts.

Keywords—Chironomids   Cadmium tolerance   Excretion efficiency   Storage capability

INTRODUCTION

The acquisition of tolerance in environments polluted with metals has been established for several animal species [1,2], including a few dipterans [3–7]. However, the physiological mechanisms causing such increased metal tolerance are poorly understood. Possible mechanisms include induction of metal-binding proteins [8] and formation of metal-containing granules [9].

Among insects, induction of metal-binding proteins by metal exposure has been demonstrated in the caterpillar *Galleria mellonella* [10], the housefly *Musca domestica* [11], the fruitfly *Drosophila melanogaster* [12,13], and several chironomid species [14,15]. Experiments with *D. melanogaster* further indicated that a duplication of the metallothionein gene could be involved in adaptation processes [16]. Other authors demonstrated the presence of metal-containing mineral concretions in the midgut epithelium of several insect species [17–20]. A central role of insect midgut in detoxification of increased metal concentrations is further indicated by the high amounts of metals accumulated by the midgut [21–26]. For some species it was found that a large portion of accumulated metal in the midgut was bound to an inducible, possibly metallothionein-like protein [10,27,28].

It has been suggested that metal-loaded metallothioneins are picked up by lysosomal vesicles in the midgut epithelium where the metal can be retained after degradation of the protein [29]. Because these granules may be expelled into the gut lumen by exocytosis or degeneration of complete cells, increased efficiency of these processes can increase metal excretion and, thereby, tolerance to metals. Such an increased metal excretion efficiency has been demonstrated in collembola [30–32]. However, increased metal excretion has not been found for all metal-tolerant organisms. Copper-tolerant *Nereis diversicolor,* for example, may contain 100 times more copper compared to non-

tolerant worms and absorption of copper is more rapid in tolerant than in nontolerant *Nereis* [33]. Adaptation of *N. diversicolor* to zinc, however, involved a decrease in permeability of the body surface and probably an improved ability to excrete the metal [33]. Furthermore, Klerks and Bartholomew [34], working with the cadmium-adapted oligochaete *Limnodrilus hoffmeisteri,* showed that resistance was not achieved by reduced net accumulation. These studies indicate that there is no uniform mechanism of regulating high metal concentrations among invertebrates.

Since cadmium adaptation has already been studied for populations of the midge *Chironomus riparius* [7,35], the present study tested the hypothesis that cadmium kinetics differed between cadmium-adapted and nonadapted populations of this midge. Larvae were collected at field sites studied by Postma et al. [7,35], who demonstrated that especially the growth of larvae from cadmium-exposed populations was less sensitive to the toxic effects of cadmium compared to nonexposed populations, while larval mortality remained substantial. Laboratory-reared offspring was used to conduct cadmium accumulation and elimination experiments. Possible differences between populations of *C. riparius* were, therefore, assumed to have a genetic basis, although maternal effects cannot be ruled out completely. A first-order one-compartment model was fitted to the experimental data to obtain estimates for accumulation and elimination rates as well as for equilibrium concentrations.

MATERIALS AND METHODS

Site description

In May 1994 *C. riparius* larvae were collected from two small lowland rivers in The Netherlands and Belgium. In the river Dommel, flowing from Belgium to The Netherlands, three sampling sites were selected, each located about 100 km east of the city of Brussels: Peer, 10 km upstream of a zinc smelter in operation since 1888; Neerpelt, 0.5 km downstream of the

---

* To whom correspondence may be addressed.

smelter and Borkel, 7 km downstream. During the 1980s yearly production of this factory averaged 120,000 tons zinc and 600 tons cadmium. In 1992 this primary production of zinc and cadmium was stopped and the factory switched to recycling and the production of zinc alloys, but until now no change of metal concentrations in the Dommel are noted. The relatively clean river IJse was sampled in the village Neerijse (20 km east of Brussels) and served as a (second) reference site, based on previous metal analysis [7].

Field sampling took place in sedimentation areas of the rivers, which are a suitable habitat for *C. riparius.* The upper 5-cm mud-layer was scraped over several meters using a nylon net with a mesh size of 300 μm. Sediment was sieved and larvae belonging to the genus *Chironomus* were collected and taken into the laboratory. Previous metal analysis of both water and detritus demonstrated that at the Neerpelt and Borkel sites, cadmium and zinc were the main pollutants, reaching concentrations exceeding chronic NOEC values for survival, growth, or reproduction of reference *C. riparius* [7].

Experimental conditions

Accumulation and elimination experiments were started with first-generation laboratory-reared animals. Larvae from the field were cultured in the laboratory according to Postma et al. [36]. Larvae were kept in clean sediment (fine sand) and were fed a solution of ground Trouvit and Tetraphyll®. After emergence, egg masses were collected and allowed to hatch in clean water. Experiments were started using larvae originating from at least 10 different egg masses. Male imagoes were collected and identified as *C. riparius* using Pinder's key [37]. Experiments were carried out in a climate-controlled room at 20 ± 1°C, with a 16:7-h light : dark regime and a twilight zone of 30 min before and after the light period. Experiments were carried out in water obtained from the oligomesothrophic lake Maarsseveen I (pH = 7.8; Na and Ca content were 18.8 and 63.6 mg/L, respectively [38]). Metal concentrations in this lake water were as follows: <0.2 to 1.8 nM Cd, <0.03 μM Zn, <1.0 nM Pb, and <4.7 to 12.6 nM Cu [39].

Because molting may interfere with both accumulation and elimination kinetics, all experiments were started with fourth-instar larvae (which is the oldest instar). To obtain sufficient numbers of fourth-instar larvae, newly hatched first-instar larvae were precultured for each population in plastic aquaria, supplied with a 1-cm layer of cellulose (4 g dry weight) as a substrate. Overlying water was aerated constantly. Per population three aquaria with 400 larvae each were used for the preculturing of larvae, to be used in the accumulation experiments. These larvae were grown for 12 d in a clean environment (0.4 nM Cd; ±0.04 μg/L). Larvae, which were to be used in elimination experiments, were precultured for a 16-d accumulation period, while exposed to 176.2 nM Cd; ±19.8 μg Cd/L). Cadmium was added as a solution of cadmium chloride. Surplus food was provided.

After reaching the fourth instar, 200 exposed and 200 unexposed larvae were selected randomly from each population and experiments were started by placing exposed larvae in a clean environment and unexposed larvae in a cadmium-polluted aquarium. During experiments, water and cadmium were renewed daily and 1-ml water samples were taken before and directly after water renewal. Samples were analyzed with Graphite Furnace Atomic Absorption Spectrometry (Perkin Elmer 5100) equipped with Zeeman background correction. The actual concentrations of cadmium (average ± standard error) during the accumulation experiment were 742.6 ± 26.5 nM (Neerpelt),

744.6 ± 28.7 (Peer), 736.1 ± 28.6 (Neerijse) and 743.0 ± 33.0 (Borkel). The actual concentrations of cadmium during the preculture for the elimination experiment were 171.6 ± 6.9 nM (Neerpelt), 180.3 ± 8.3 (Peer) and 174.7 ± 5.4 (Borkel), while the actual concentrations of cadmium during the elimination experiment itself were 1.66 ± 0.33 nM (Neerpelt), 1.40 ± 0.29 (Peer) and 1.74 ± 0.37 (Borkel). There were no significant differences in these cadmium concentrations in the water between the different populations or between different sampling periods during the experiments as well as during the preculturing periods (tested by ANOVA). The cadmium concentrations used were based on previous experiments [6,7,35,38]. In order to prevent a rapid decrease of metal concentrations in the water during accumulation experiments, the aquaria, cellulose, and food were spiked with the corresponding cadmium concentrations by preexposure during 1 week, in which solutions were renewed three times. Although aquaria and cellulose were saturated at the beginning of experiments, food continued to bind cadmium, causing a gradual decrease of water concentrations. During the experiments, 2.5 ml of suspended food were added for every 50 larvae twice a week, providing an excess of food.

The experiments lasted 2 weeks. During the first week 10 exposed and 10 unexposed larvae per population were randomly sampled daily. The sampling interval was three times a week during the second week. After sampling larvae were kept for 1 d in a fresh solution of 741.6 nM Cd (accumulation experiments) or clean lake water (elimination experiments), without food or sediment to remove gut contents. This water was refreshed regularly (six times a day) to prevent coprophagy. Hereafter all larvae were rinsed in acidified lake water (pH = 2) for 15 min to eliminate the cadmium bound to the exoskeleton. Five larvae from each sample were frozen. The other five larvae were dissected to analyze the guts (including the Malpighian tubules) and the remainder of the larvae separately. As this procedure extended the accumulation or elimination period for 1 d, corrected exposure times were used analyzing the data. Lyophilized organisms were weighed and digested individually, in concentrated HNO₃ and H₂O₂, using a microdestruction method [38] and cadmium concentrations were analyzed with Graphite Furnace Atomic Absorption Spectrometry (Perkin Elmer 5100) equipped with Zeeman background correction.

All materials used in experiments and analyses were cleaned by soaking in 0.1 N HNO₃ (Merck) for at least 24 h and then rinsing three times with double-distilled water. Quality control of metal analysis was carried out by analyzing destruction blanks and reference material (IAEA MAA-3/TM shrimp homogenate). Measured values were in good agreement with certified values (less than 10% deviation) and destruction blanks were near detection limits (<0.4 nM Cd).

Compartment models

The concentration of cadmium in the guts, larvae minus guts, or intact larvae at time t was calculated using first-order one-compartment models:

$$C_{\text{chir},t} = \frac{k_1}{k_2} C_w [1 - e^{(-k_2 t)}]$$

for the accumulation experiments

$$C_{\text{chir},t} = C_{\text{chir},t=0} e^{(-k_2 t)}$$

for the elimination experiments

with

$C_{\text{chir},t}$ = cadmium concentration in the larvae at time t
        (mmol/kg dry weight);

C_w = cadmium concentration in the water (μM);

k_1 = rate constant for uptake (d^{-1});

k_2 = rate constant for elimination, combined with growth
        (d^{-1});

t = time (d).

Parameters were estimated from the data using the Quasi-Newton method for least squares as implemented in the SYSTAT software program. Differences between populations were tested by comparing the residual sum of squares (RSS), assuming equal parameter values for all populations with the RSS, allowing each population to have its own parameter values. This is part of a standard analysis of covariance (ANCOVA) [40]. Statistical analyses of larval dry weights were applied according to Sokal and Rohlf [40]. When assumptions for ANOVA were violated, data transformations were executed logarithmically. If assumptions were still violated, nonparametric tests were performed (Kruskal–Wallis). Differences between groups were tested using Student–Newman–Keuls (SNK) procedures when possible. Unless stated otherwise, significance was tested at the $p < 0.05$ level.

RESULTS

Accumulation experiments

Larval development. Larval dry weights during the experiments differed significantly between populations (ANOVA: $F = 6.30$, $p < 0.001$). Larvae from the Borkel population had the highest average dry weight, whereas larvae from the Peer population had the lowest, but especially at the end of the experiment differences between populations were small (Fig. 1a). Furthermore, larval dry weights gradually decreased during the experiment for all populations and differences existed between sampling times (ANOVA: $F = 3.40$, $p < 0.001$). There was, however, no significant interaction between the factor population and the factor sampling time (ANOVA: $F = 1.56$, $p = 0.52$). Dry weights of larvae minus guts (Fig. 1c) showed the same effects of population and sampling time compared to intact larvae (KW = 16.8, $p < 0.001$; KW = 28.5, $p < 0.001$, respectively) but effects on dry weights of the guts differed (Fig. 1b). Decrease in dry weight of guts during experiments was more pronounced (KW = 103.3, $p < 0.001$), but differences between populations were not significant (KW = 6.1, $p = 0.11$). During the experiment, pupae were found in all populations from the third day onward. However, for all populations pupation ceased almost completely after the first week. Pupation was most pronounced in the adapted Borkel population, where 50% of the larvae had pupated after 7 d. In the nonadapted Peer population only 20% of the larvae pupated. Pupation rate in the Neerpelt and Neerijse populations was intermediate. Larval mortality was low for all populations (<5%).

Cadmium kinetics. Cadmium concentrations in both larvae and guts peaked after about 3 d (Fig. 2a,b). Therefore, one-compartment models with fixed parameter values did not fit properly to the experimental data and consequently estimates of parameter values are absent. During the first 3 to 4 d of the experiment 80 to 90% of the accumulated cadmium was found in the gut for all populations. Thereafter this percentage gradually decreased to 50% at the end of the experiment. This coincided with a gradual increase in cadmium concentrations in larvae minus guts and for most populations an equilibrium was reached at the end of the experiment (Fig. 2c). For the larvae
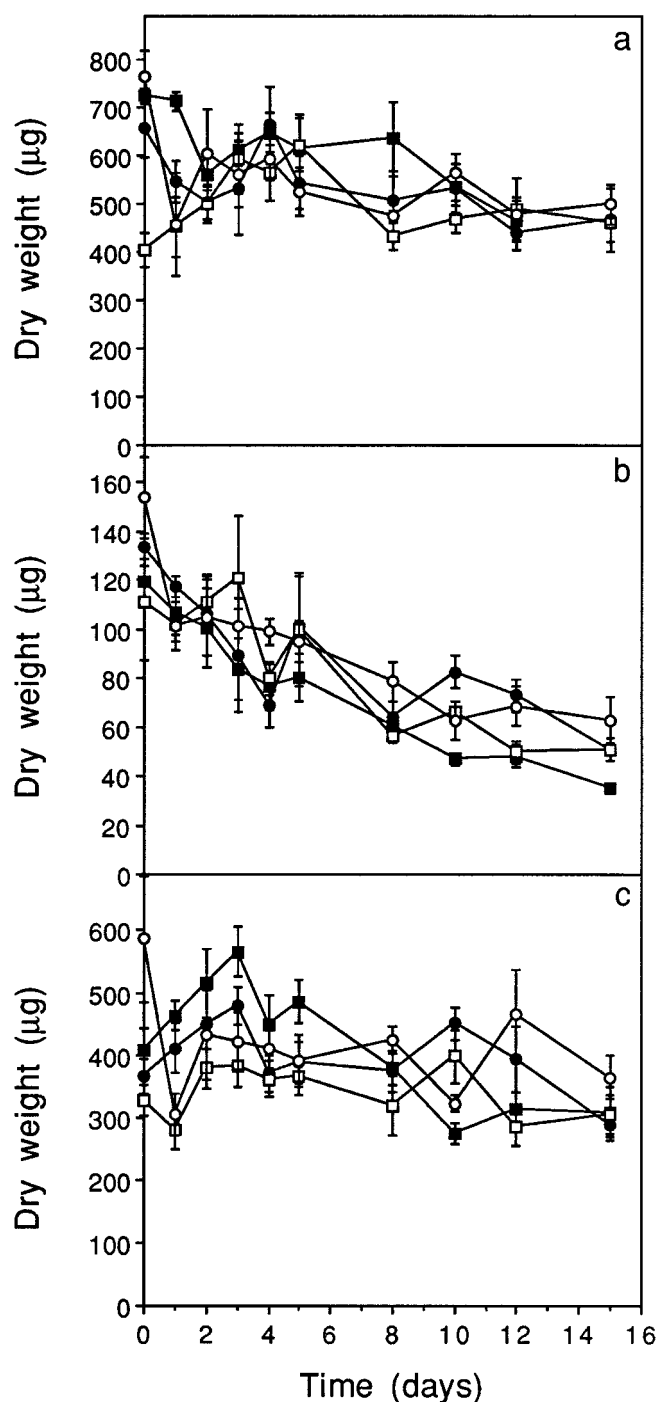

Fig. 1. Dry weights (μg) of larvae from two nonadapted populations (open symbols: Neerijse, $\bigcirc$ and Peer, $\square$) and two adapted populations (closed symbols: Neerpelt, $\bullet$ and Borkel, $\blacksquare$) during the accumulation experiments: (**a**) dry weight of the larvae; (**b**) dry weight of the guts; (**c**) dry weight of larvae minus guts. Presented are the mean values together with their standard errors.

minus guts, first-order one-compartment models described the actual cadmium concentration accurately and differences between populations were found (ANCOVA, $F = 17.3$, $p < 0.001$). Net accumulation rate in the adapted Borkel population was significantly lower compared to the other populations, including Neerpelt. In addition, models indicated that equilibrium values were highest for the Neerpelt population (Fig. 2d). Estimated parameter values are presented in Table 1.
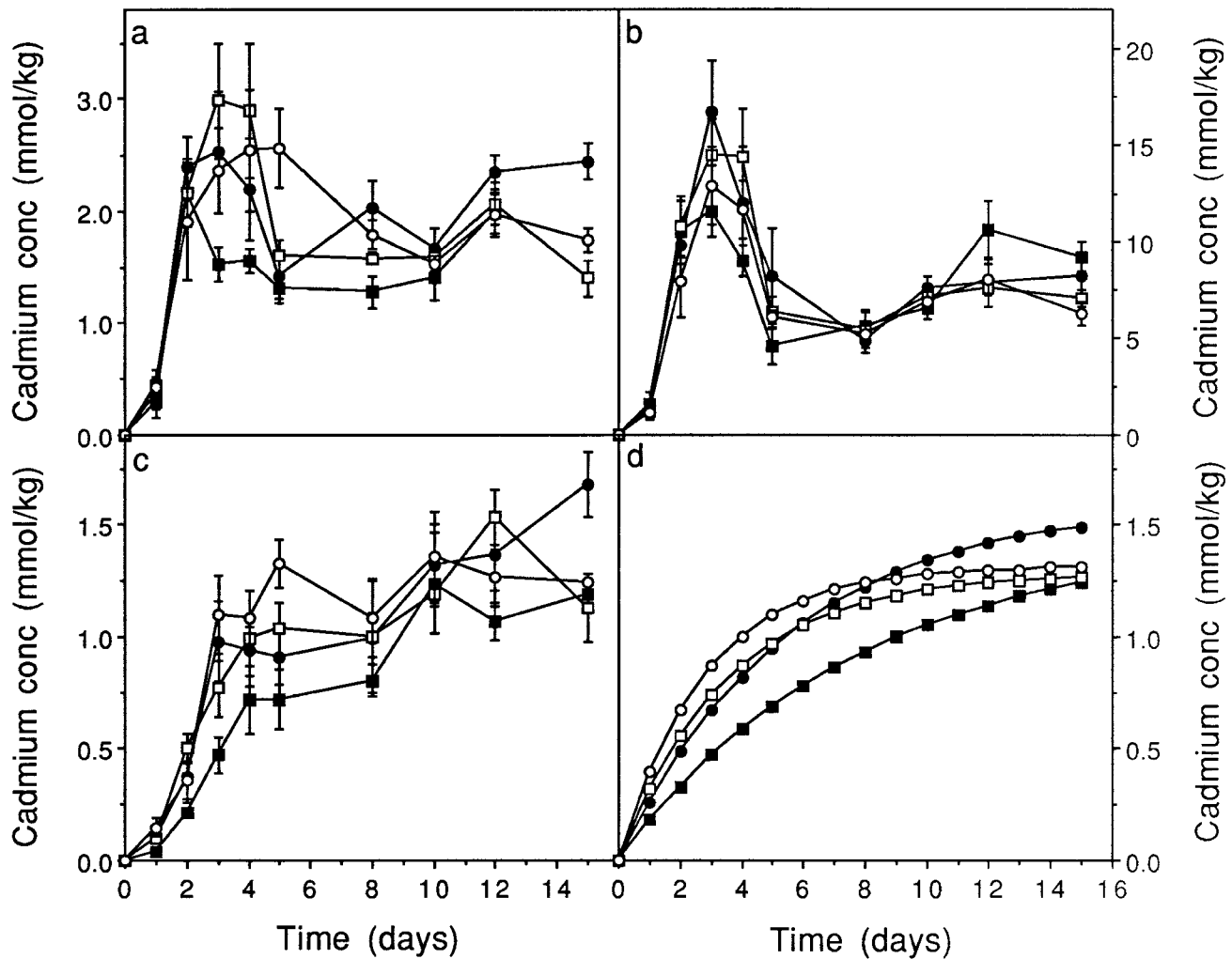
Fig. 2. Cadmium concentrations (mmol/kg) for two nonadapted populations (open symbols: Neerijse, ○ and Peer, □) and two adapted populations (closed symbols: Neerpelt, ● and Borkel, ■) during the accumulation experiments: (**a**) larvae; (**b**) guts; (**c**) larvae minus guts; (**d**) fitted one-compartment models for larvae minus guts. Presented are the mean values together with their standard error.

Elimination experiments

Elimination experiments with larvae from the Neerijse population failed due to a low larval growth rate in the precultures. Larvae were mainly third instar at the start of the experiment and molting during the experiment would certainly interfere with the elimination kinetics. Preculturing larvae from Neerijse during a longer period of time was avoided, because this would have increased the cadmium concentration at the start of the experiment and may have interfered with the cadmium kinetics.

Larval development. During elimination experiments no interpopulation differences were found in dry weight of intact

Table 1. Cadmium accumulation (k_1; d^{-1}) and elimination of (k_2; d^{-1}) rates, estimated for larvae minus guts for four populations during the accumulation experiment, together with sum of squares (SS) and degrees of freedom (*d.f.*) values

	k_1	k_2	$SS_{regression}$	*d.f.*	$SS_{residual}$	*d.f.*
Borkel	0.254	0.131	320,782.7	2	34,686.6	45
Neerpelt	0.390	0.181	592,210.0	2	55,552.4	47
Peer	0.490	0.283	520,691.2	2	56,281.6	47
Neerijse	0.637	0.359	625,324.3	2	53,628.1	46

larvae (KW = 3.89, p = 0.14), dry weight of the larvae minus guts (KW = 4.58, p = 0.10), or dry weight of guts (ANOVA, F = 1.66, p = 0.20) (Fig. 3). However, larvae, larvae minus guts and guts showed a significant increase in weight across sampling times (KW = 58.19, $p < 0.001$; KW = 82.02, $p < 0.001$; ANOVA, F = 4.71, $p < 0.001$, respectively). Consequently, larvae pupated during the experiments but no differences were found between populations (about 60% for all populations, data not shown). Larval mortality was low for all populations (<5%).

Cadmium kinetics. Although larval growth increased elimination rates due to dilution, population comparisons were made based on cadmium concentration instead of cadmium burden to allow comparison with accumulation experiments.

At all sampling times, more than 90% of the cadmium was found in guts and elimination of cadmium by intact larvae closely resembles elimination from guts (Fig. 4a,c). In both cases cadmium kinetics were properly described by a first-order one-compartment model (Fig. 4b,d) and estimated parameter values are given in Table 2. Cadmium kinetics differed significantly between populations (ANCOVA, larvae: F = 13.7, $p < 0.001$; guts: F = 18.16, $p < 0.001$) caused by both an increased cadmium concentration at the start of the experiments as well as
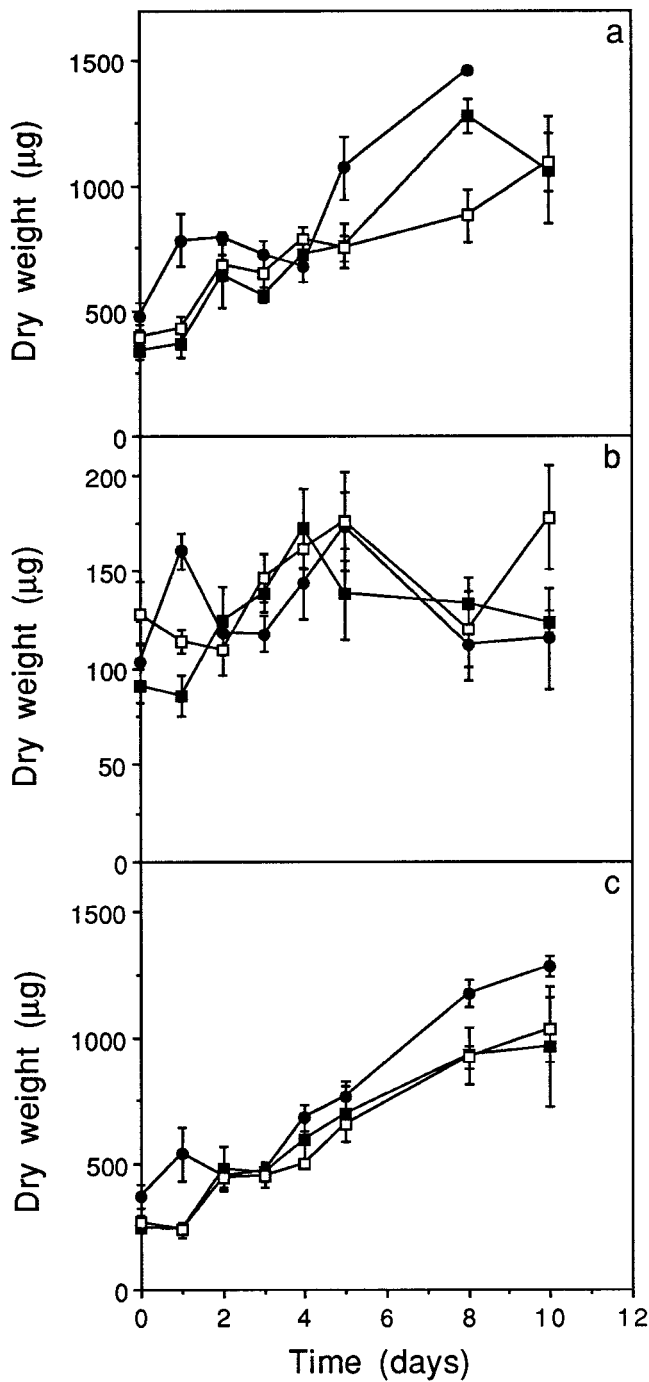
Fig. 3. Dry weights (μg) of larvae from a nonadapted population (open symbols: Peer, □) and two adapted populations (closed symbols: Neerpelt, ● and Borkel, ■) during the elimination experiments: (**a**) dry weight of larvae; (**b**) dry weight of guts; (**c**) dry weight of larvae minus guts. Presented are the mean values together with their standard error.

Fig. 4. Cadmium concentrations (mmol/kg) in larvae from a non-adapted population (open symbols: Peer, □) and two adapted populations (closed symbols: Neerpelt, ● and Borkel, ■) together with the fitted one-compartment models during the elimination experiments: (**a**) larvae, data; (**b**) larvae, models; (**c**) guts, data; (**d**) guts, models; (**e**) larvae minus guts, data; (**f**) larvae minus guts, models. Presented are the mean values together with their standard error.

DISCUSSION

Larval development

Differences in larval development between accumulation and elimination experiments are explained by cadmium exposure. Larval growth ceased completely during accumulation experiments, but steady growth was observed during elimination experiments. These results differ from cadmium accumulation experiments performed by Timmermans et al. [41], in which larvae of *C. riparius* continued to grow while exposed to 0.1 mg Cd/L ($\pm$890 nM Cd, compared to 742 nM during the present ex-

by an increased elimination rate for the two cadmium-adapted populations. These differences were not due to differences in growth rate, because patterns were similar when models were fitted on the cadmium burden. In larvae minus guts, cadmium concentrations were low and although a significant fit was found for the models, differences between populations were small and insignificant (ANCOVA, $F = 2.97$, $0.10 > p > 0.05$) (Fig. 4e,f).
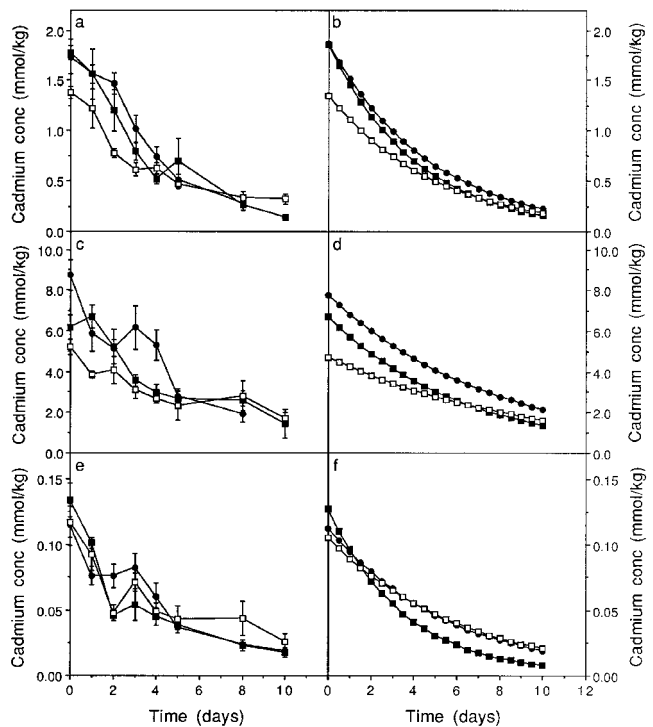
Table 2. Cadmium concentrations at the beginning of the experiment (C_0; mmol/kg), cadmium elimination rates (k_2; d^{-1}), sum of squares (SS) and degree of freedom (*d.f.*) values, estimated for intact larvae, guts and larvae minus guts in three populations during the elimination experiment

	C_0	k_2	$SS_{regression}$	*d.f.*	$SS_{residual}$	*d.f.*
Larvae						
Borkel	1.853	0.245	38.85	2	2.67	33
Neerpelt	1.864	0.211	44.52	2	2.71	30
Peer	1.347	0.200	25.55	2	1.55	38
Guts						
Borkel	6.687	0.159	719.43	2	56.11	35
Neerpelt	7.733	0.127	1078.20	2	115.06	34
Peer	4.704	0.106	447.66	2	47.17	37
Larvae minus guts						
Borkel	0.127	0.281	0.182	2	0.014	35
Neerpelt	0.112	0.177	0.179	2	0.017	33
Peer	0.105	0.162	0.178	2	0.022	38

periments). Differences in pupation rates were in accordance with larval growth patterns because larvae in accumulation experiments started to pupate after 2 to 3 d, but pupation ceased after 6 d. In elimination experiments pupation was more gradual (caused by a higher variation in larval dry weights at the start of the experiment) but continued until all larvae were either sampled or pupated.

Before chironomid larvae pupate, they enter a prepupal phase in which physiology and morphology of the gut changes. These prepupal larvae are easily recognized by the integration of the three thoracal segments and were not used for sampling. A direct influence of pupation on the observed cadmium kinetics was most likely eliminated. This is further illustrated by the dry weights of the guts in the elimination experiments. Although pupation was frequently observed in the elimination experiments, dry weights of the guts did not decrease as would have been expected when prepupal larvae were sampled. It is, therefore, unlikely that the decrease in dry weights of the guts during the accumulation experiments was caused by pupation. A direct or indirect effect of the high cadmium exposure seemed more likely.

Indirect effects of pupation might occur also, because pupation will not occur randomly in the population. Unfortunately, the strength of such indirect effects is not known. Consequently, this might have influenced interpopulation differences during the accumulation experiments. Differences in pupation rate between populations were absent in elimination experiments and differences in cadmium excretion efficiency were consequently unaffected although absolute values might have been influenced.

Cadmium kinetics

In the present study first-order one-compartment models did not always provide a good description of experimental data. Exceptions were found for the cadmium accumulation by intact larvae and their guts, because a clear maximum was present for the cadmium concentrations in guts after 3 to 4 d. This maximum was most likely caused by physiological acclimation processes in the larvae, by which means either the cadmium accumulation rate or the elimination rate changed. Such physiological acclimation processes are described for several metals and invertebrates [1,42,43].

Although extensive literature is available on short-term effects of metals on chironomids, comparisons with present observations are hard to make. For example, several authors demonstrated that the midgut of chironomids accumulates large amounts of metals [21,24,44]. Detailed data on the rate of such accumulation are, however, lacking. Furthermore, accumulation experiments have been performed with several species but experimental time is often limited to a few days [15,45,46]. For *C. riparius* cadmium kinetics are described in detail by Timmermans et al. [41], who demonstrated that the accumulation rate of cadmium was high and that equilibrium concentrations (420 mg Cd/kg or 3.74 mmol Cd/kg) were reached in about 10 d. In the present experiments, equilibrium concentrations were reached even faster (4–6 d) but values were somewhat lower (±2 mmol Cd/kg). In addition to lower exposure levels, these differences may partially be due to the fact that Timmermans et al. started their experiments with younger instar larvae and significant growth was observed during the experiments. In addition, Krantzberg [47] demonstrated that cadmium concentrations in chironomid larvae were influenced by age and body weight of larvae.

Accumulation of cadmium in the larvae minus guts seemed

to be unaffected by possible acclimation processes and a good fit was obtained using first-order one-compartment models. Furthermore, differences between populations were found, because larvae from the adapted Borkel population were characterized by a low net accumulation rate, whereas larvae from the adapted Neerpelt population had an increased equilibrium concentration. We expect that larvae from the Borkel population would also have reached increased equilibrium values, if the experimental period had been longer.

Postma et al. [35] found differences in life-history patterns between the Borkel and Neerpelt population and they suggested that differences in genetic stability could be involved. Because the Neerpelt population is situated only a few hundred meters away from the point source of cadmium, drifting larvae originating from unpolluted sites further upstream can easily reach this population and possibly interbreed. The Borkel population is situated 7 km downstream limiting the influence of gene flow from presumably nonadapted populations.

For accumulation experiments, larvae were precultured for 2 weeks in a clean environment and a selection for cadmium-adapted larvae was absent. Consequently as argued above, the accumulation experiment with the Neerpelt population might have been started with a mixture of adapted and nonadapted larvae. The ratio between these two groups would consequently determine the mean cadmium accumulation rate. Furthermore, differences in the pupation rate during the experiment could change this ratio and thus the cadmium accumulation pattern. Consequently, the cadmium kinetics found in the adapted Borkel population more likely represented the situation for adapted populations. According to this hypothesis on the genetic stability of populations, it is expected that differences between the Borkel and Neerpelt populations were smaller during the elimination experiments because larvae were exposed to cadmium for 2 weeks before the experiments started. During the preculture of the Neerpelt population, selection acted in favor of cadmium-adapted larvae because a higher percentage would reach the fourth instar compared to nonadapted larvae. Experimental data were in accordance with this hypothesis as the elimination rates estimated for the Neerpelt population were intermediate between the reference population and Borkel.

Furthermore, differences were observed in estimated elimination rates between accumulation and elimination experiments. This is most likely due to differences in larval development and possible acclimation processes during accumulation experiments. Elimination rates, estimated from elimination experiments, are therefore more accurate. In general, these rates are in accordance with those of Timmermans et al. [41], who estimated a biological half life time (ln $2/k_2$) of 2.1 d. In the present experiments this value varied between 2.8 and 3.5. Experiments with other insects also demonstrated that elimination of cadmium could be rather fast, but parameter values were not estimated [48].

The present experiments demonstrated significant differences in elimination rates between populations because guts of two cadmium-adapted populations were characterized by an increased excretion efficiency compared to the Peer population. An increased excretion efficiency by the gut has also been found for metal-adapted collembola [30–32]. In both cases, the increase in excretion efficiency seemed to be a gradual process. In addition to the increased elimination rates, the present experiments demonstrated an increased storage capability in the gut of cadmium-adapted larvae, because more cadmium was accumulated during the preculture period compared to non-

adapted larvae. An increased storage capability could be caused by the amount or the efficiency of metal-containing granula in the gut epithelium, as such concretions have been found in the midgut epithelium of several insects among which are chironomids [17,18,44]. As these granula may be expelled into the gut lumen by exocytosis or degeneration of complete cells, an increased elimination efficiency is to be expected. Indications for this were obtained by Seidman et al. [14], who found high amounts of cadmium in degenerating cells or debris from cells from the midgut epithelium, which has been sloughed into the gut lumen.

Cadmium-adapted populations of the midge *C. riparius* had increased cadmium excretion efficiency in gut tissue. This reduced the net accumulation rate for the larvae minus guts, but equilibrium values in both guts and larvae minus guts were higher in cadmium-adapted larvae than in larvae from a nonadapted population. Because differences were demonstrated in laboratory-reared F_1 larvae, a genetic basis for this differential response may exist.

REFERENCES

1. **Klerks, P.L.** and **J.S. Weis.** 1987. Genetic adaptation to heavy metals in aquatic organisms: A review. *Environ. Pollut.* **45**:173–205.
2. **Posthuma, L.** and **N.M. Van Straalen.** 1993. Heavy-metal adaptation in terrestrial invertebrates: A review of occurrence, genetics, physiology and ecological consequences. *Comp. Biochem. Physiol. C* **106**:11–38.
3. **Blackmann, G.G.** and **J.A.F. Bakker.** 1975. Resistance of the sheep blowfly, *Lucilia cuprina,* to insecticides in the republic of South Africa. *J. S. Afr. Vet. Assoc.* **46**:337–339.
4. **Magnusson, J.** and **C. Ramel.** 1986. Genetic variation in the susceptibility to mercury and other metal compounds in *Drosophila melanogaster. Teratog. Carcinog. Mutagen.* **6**:289–305.
5. **Nassar, R.** 1979. Genetics of resistance to tetraethyllead. *Aust. J. Biol. Sci.* **32**:127–132.
6. **Postma, J.F.** and **C. Davids.** 1996. Tolerance induction and life-cycle changes in cadmium exposed *Chironomus riparius* (Diptera) during consecutive generations. *Ecotoxicol. Environ. Saf.* **30**:195–202.
7. **Postma, J.F., M. Kyed** and **W. Admiraal.** 1996. Site specific differentiation in metal tolerance in the midge *Chironomus riparius* (Diptera: Chironomidae). *Hydrobiologia* (in press).
8. **Roesijadi, G.** 1992. Metallothioneins in metal regulation and toxicity in aquatic animals. *Aquat. Toxicol.* **22**:81–114.
9. **Brown, B.E.** 1982. The form and function of metal-containing granules in invertebrate tissues. *Biol. Rev.* **57**:621–667.
10. **Polek, B., J. Godocikova, R. Batora** and **M. Ursinyova.** 1993. Cu-binding proteins of digestive tract of *Galleria mellonella* caterpillars (Lepidoptera; Pyralidae). *Biologia* **48**:631–636.
11. **Kasai, T., T. Watanabe, K. Inoue** and **T. Hasegawa.** 1993. Purification and some properties of metal-binding proteins in housefly larvae (*Musca domestica*). *Biosci. Biotechnol. Biochem.* **57**:1873–1876.
12. **Maroni, G., D. Lastowski-Perry, E. Otto** and **D. Watson.** 1986. Effects of heavy metals on *Drosophila* larvae and a metallothionein cDNA. *Environ. Health Perspect.* **65**:107–116.
13. **Mokdad, R., A. Debec** and **M. Wegnez.** 1987. Metallothionein genes in *Drosophila melanogaster* constitute a dual system. *Proc. Natl. Acad. Sci. USA* **84**:2658–2662.
14. **Seidman, L.A., G. Bergtrom, D.J. Gingrich** and **C.C. Remsen.** 1986. Accumulation of cadmium by the fourth instar larva of the fly *Chironomus thummi. Tissue & Cell* **18**:395–405.
15. **Yamamura, M., K.T. Suzuki, S. Hatakeyama** and **K. Kubota.** 1983. Tolerance to cadmium and cadmium-binding proteins induced in the midge larva, *Chironomus yoshimatsui* (Diptera, Chironomidae). *Comp. Biochem. Physiol. C* **75**:21–24.
16. **Maroni, G., J. Wise, J.E. Young** and **E. Otto.** 1987. Metallothi-

17. **Lauverjat, S., C. Ballan-Dufrancais** and **M. Wegnez.** 1989. Detoxification of cadmium. Ultrastructural study and electron-probe microanalysis of the midgut in a cadmium resistant strain of *Drosophila melanogaster. Biol. Metals* **2**:97–107.
18. **Sohal, R.S.** and **R.E. Lamb.** 1977. Intracellular deposition of metals in the midgut of the adult housefly, *Musca domestica. J. Insect Physiol.* **23**:1349–1354.
19. **Sohal, R.S., P.D. Peters** and **T.A. Hall.** 1977. Origin, structure, composition and age-dependence of mineralised dense bodies (concretions) in the midgut epithelium of the adult housefly, *Musca domestica. Tissue & Cell* **9**:87–102.
20. **Tapp, R.L.** and **A. Hockaday.** 1977. Combined histochemical and X-ray microanalytical studies on the copper-accumulating granules in the mid-gut of larval *Drosophila. J. Cell Sci.* **26**:201–215.
21. **Krantzberg, G.** and **P.M. Stokes.** 1990. Metal concentrations and tissues distribution in larvae of *Chironomus* with reference to X-ray microprobe analysis. *Arch. Environ. Contam. Toxicol.* **19**:84–93.
22. **Sohal, R.S.** and **R.E. Lamb.** 1979. Storage-excretion of metallic cations in the adult housefly, *Musca domestica. J. Insect Physiol.* **25**:119–124.
23. **Hare, L., A. Tessier** and **P.G.C. Campbell.** 1991. Trace element distributions in aquatic insects: Variations among genera, elements and lakes. *Can. J. Fish. Aquat. Sci.* **48**:1481–1491.
24. **Sumi, Y., T. Suzuki, M. Yamamura, S. Hatakeyama, Y. Sugaya** and **K.T. Suzuki.** 1984. Histochemical staining of cadmium taken up by the midge larva, *Chironomus yoshimatsui* (Diptera, Chironomidae). *Comp. Biochem. Physiol. A* **79**:353–357.
25. **Waterhouse, D.F.** 1945. A histochemical examination of the distribution of copper in *Lucilia cuprina. Bull. Counc. Sci. Ind. Res. Melb.* **191**:5–20.
26. **Waterhouse, D.F.** 1945. A quantitative investigation of the copper content of *Lucilia cuprina. Bull. Counc. Sci. Ind. Res. Melb.* **191**:21–39.
27. **Aoki, Y., K.T. Suzuki** and **K. Kubota.** 1984. Accumulation of cadmium and induction of its binding protein in the digestive tract of fleshfly (*Sarcophaga peregrina*) larvae. *Comp. Biochem. Physiol. C* **77**:279–282.
28. **Maroni, G.** and **D. Watson.** 1985. Uptake and binding of cadmium, copper, and zinc by *Drosophila melanogaster* larvae. *Insect Biochem.* **15**:55–63.
29. **Dallinger, R.** 1993. Strategies of metal detoxification in terrestrial invertebrates. In R. Dallinger and P.S. Rainbow, eds., *Ecotoxicology of Metals in Invertebrates.* Lewis, Boca Raton, FL, USA, pp. 245–289.
30. **Posthuma, L., R.F. Hogervorst** and **N.M. van Straalen.** 1992. Adaptation to soil pollution by cadmium excretion in natural populations of *Orchesella cincta* (L.) (Collembola). *Arch. Environ. Contam. Toxicol.* **22**:146–156.
31. **Posthuma, L., R.F. Hogervorst, E.N.G. Joosse** and **N.M. van Straalen.** 1993. Genetic variation and covariation for characteristics associated with cadmium tolerance in natural populations of the springtail *Orchesella cincta* (L.). *Evolution* **47**:619–631.
32. **van Straalen, N.M., et al.** 1987. Efficiency of lead and cadmium excretion in populations of *Orchesella cincta* (Collembola) from various contaminated forest soils. *J. Appl. Ecol.* **24**:953–968.
33. **Bryan, G.W.** and **L.G. Hummerstone.** 1973. Adaptation of the polychaete *Nereis diversicolor* to estuarine sediments containing high concentrations of zinc and cadmium. *J. Mar. Biol. Assoc. UK* **53**:839–857.
34. **Klerks, P.L.** and **P.R. Bartholomew.** 1991. Cadmium accumulation and detoxification in a Cd-resistant population of the oligochaete *Limnodrilus hoffmeisteri. Aquat. Toxicol.* **19**:97–112.
35. **Postma, J.F., A. van Kleunen** and **W. Admiraal.** 1995. Alterations in life-history traits of *Chironomus riparius* (Diptera) obtained from metal contaminated rivers. *Arch. Environ. Contam. Toxicol.* **29**:469–475 .
36. **Postma, J.F., M.C. Buckert-de Jong, N. Staats** and **C. Davids.** 1994. Chronic toxicity of cadmium to *Chironomus riparius* (Diptera: Chironomidae) at different food levels. *Arch. Environ. Contam. Toxicol.* **26**:143–148.
37. **Pinder, L.C.V.** 1978. A key to adult males of British Chironomidae. *Freshwater Biol. Assoc. Sci. Publ. 37.*
38. **Timmermans, K.R., B. van Hattum, M.H.S. Kraak** and **C. Da-**

vids. 1989. Trace metals in a littoral food web: Concentrations in organisms, sediment and water. *Sci. Total Environ.* **87/88**:477–494.

39. **van Hattum, B., K.R. Timmermans** and **H.A. Govers.** 1991. Abiotic and biotic factors influencing in situ trace metal levels in macro invertebrates in freshwater ecosystems. *Environ. Toxicol. Chem.* **10**:275–292.

40. **Sokal, R.R.** and **F.J. Rohlf.** 1981. *Biometry,* 2nd ed. W.H. Freeman, New York, NY, USA.

41. **Timmermans, K.R., W. Peeters** and **M. Tonkes.** 1992. Cadmium, zinc, lead and copper in *Chironomus riparius* (Meigen) larvae (Diptera, Chironomidae): uptake and effects. *Hydrobiologia* **241**:119–134.

42. **Maltby, L.** and **M. Crane.** 1994. Responses of *Gammarus pulex* (Amphipoda, Crustacea) to metalliferous effluents: Identification of toxic components and the importance of interpopulation variation. *Environ. Pollut.* **84**:45–52.

43. **Stuhlbacher, A., M.C. Bradley, C. Naylor** and **P. Calow.** 1993. Variation in the development of cadmium resistance in *Daphnia*

magna Straus; effect of temperature, nutrition, age and genotype. *Environ. Pollut.* **80**:153–158.

44. **Seidman, L.A., G. Bergtrom** and **C.C. Remsen.** 1986. Structure of the larval midgut of the fly *Chironomus thummi* and its relationship to sites of cadmium sequestration. *Tissue & Cell* **18**:407–418.

45. **Heinis, F., K.R. Timmermans** and **W.R. Swain.** 1990. Short-term sublethal effects of cadmium on the filter feeding chironomid larva *Glyptotendipes pallens* (Meigen) (Diptera). *Aquat. Toxicol.* **16**:73–86.

46. **Kosalwat, P.** and **A.W. Knight.** 1987. Acute toxicity of aqueous and substrate-bound copper to the midge, *Chironomus decorus. Arch. Environ. Contam. Toxicol.* **16**:275–282.

47. **Krantzberg, G.** 1989. Metal accumulation by chironomid larvae: The effects of age and body weight on metal body burdens. *Hydrobiologia* **188/189**:497–506.

48. **Clubb, R.W., A.R. Gaufin** and **J.L. Lords.** 1975. Acute cadmium toxicity studies upon nine species of aquatic insects. *Environ. Res.* **9**:332–341.