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Site specific differentiation in metal tolerance in the midge *Chironomus riparius* (Diptera, Chironomidae)

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Abstract

Metal tolerance in *Chironomus riparius* (Diptera) populations from contaminated streams was studied by comparing the effects of cadmium, zinc and iron on first generation laboratory reared midges. First instar larvae were exposed for four days, after which surviving larvae were counted and their length measured. Larvae from two highly polluted sites, kept under control conditions, grew substantially slower than those from other populations. All populations showed the same growth responses to increased zinc concentrations, but differences were found in the responses to both cadmium and iron. Since population differentiation was demonstrated in first generation laboratory animals, it is suggested that the differences between populations of *C. riparius* have a genetic basis.

Introduction

Metal pollution often reduces the fitness of organisms to such an extent that species diversity in polluted environments is strongly reduced (e.g. Brown, 1977; Clements *et al.*, 1988). Species which do survive often have an increased tolerance to the metals involved, as shown in reviews on bacteria (Trevors *et al.*, 1985), plants (Antonovics *et al.*, 1971; Macnair, 1993) and animals (Klerks & Weis, 1987; Posthuma & Van Straalen, 1993).

Since chironomids are still present in rivers heavily polluted with metals (Clements *et al.*, 1988; Janssens de Bisthoven *et al.*, 1992), it is apparent that they are able to tolerate pollution. Yet, observations on increased metal tolerance in chironomids have shown contradictory trends. Wentzel *et al.* (1978) demonstrated that larvae of *Chironomus tentans* from a metal-polluted site were less affected by exposure to metal-polluted sediment than their conspecifics from a clean location (based on differences in survival, growth and avoidance response). Furthermore, selection experiments in the laboratory showed that *Chironomus riparius* can increase its tolerance to cadmium within a few generations (Postma & Davids, 1995). In con-

trast, Klerks & Levinton (1993) did not detect any differences in tolerance between larvae of the chironomid *Tanytus neopunctipennis* from metal polluted and unpolluted areas, while the oligochaete *Limnodrilus hoffmeisteri* did show an increased tolerance at the polluted site.

At several sampling sites in the present study, cadmium and zinc concentrations in the water exceeded No Observed Effect Concentrations (NOEC) for growth, reproduction, or mortality during chronic experiments (Postma *et al.*, 1994; Postma *et al.*, 1995; Timmermans *et al.*, 1992). In addition, selection experiments performed in the laboratory have shown that cadmium tolerance can increase, when populations are continuously exposed to concentrations in the water, comparable to the field situation (Postma & Davids, 1995). It remains, however, questionable if population differentiation has occurred in the sampling sites studied, since metal concentrations in the water are highly fluctuating and the chironomid population is exposed to other stresses as well.

In this study, *Chironomus riparius* larvae were collected at several sites, differing in metal contamination. Both essential (iron, zinc) and non-essential (cadmium) metals were studied. Short-term experiments on

the reduction of growth rate and increased mortality of first instar larvae were performed to assess metal tolerance. Young larvae were used because of their high sensitivity to metals compared to later instar larvae (Gauss *et al.*, 1985; Williams *et al.*, 1986).

Materials and methods

Site description

Chironomus riparius was collected from three 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 to the city of Brussels: Peer (B), 10 km upstream of a zinc melter; Neerpelt (B), 0.5 km downstream of the factory (in operation since 1888) and Borkel (NL), 7 km downstream. The relatively clean river IJse was sampled in the village Neerijse (20 km east of Brussels) and served as a (second) reference site. The river Grote Nete was sampled near Westerloo (50 km east of Brussels). This part of the river Grote Nete has an extremely high iron content and most sediments, plants and animals were covered by a red layer of iron precipitate. Midges obtained from a laboratory culture were used as an additional control. This laboratory culture had been started about five years ago with larvae from one of the experimental ponds of the University of Amsterdam. Egg-ropes had frequently been exchanged with other laboratories as to reduce inbreeding.

Field sampling

Sampling took place in sedimentation areas of the rivers, which are a suitable habitat for *C. riparius*. Larvae were collected in February and September 1993. The upper 5–10 cm mud-layer was scraped over several metres using a nylon net with a mesh size of 300 μm . Sediment was sieved and larvae belonging to the genus *Chironomus* (>90% of the total amount of chironomids) were collected and taken into the laboratory. Larvae were stored at 4 °C in natural river water and sediment until use (up to one month later) and the water was aerated constantly.

Water samples and samples of the top sediment layer were taken for metal analyses. The mixture of sediment and organic material was sieved twice using mesh size 0.6 mm. Thereafter, sand and other large particles were allowed to settle during one minute. This pro-

cedure was repeated twice. The resulting suspension was carefully suctioned off and the particles sedimenting from this suspension were collected after a day and stored freeze-dried until analyses. This procedure increased the amount of organic carbon in the samples to about 30% and this material will be called detritus hereafter. Samples were digested in HNO_3 using a microwave equipped with a temperature and pressure control program. Although this procedure also reduces the concentration of metals in detritus, it was thought that metal concentrations in detritus (which is used as food by chironomids) will characterize the exposure of the different populations better than that in water or sediment.

Experimental conditions

Field sampled larvae were cultured in the laboratory using plastic aquaria with a cage on top, according to Postma *et al.* (1994). Larvae were kept in clean, artificial sediment (cellulose), and were fed a solution of grounded Trouvit and Tetraphyll®. Egg-ropes were collected and allowed to hatch in clean water. Experiments were started by using larvae originating from at least 10 different egg-ropes, except for the exposed populations whose larvae did not survive well in clean sediment (between 5–10 egg-ropes were used). Male imagoes were collected and all identified as *C. riparius*, using Pinder (1978). First generation laboratory reared animals were used in the experiments to examine the presence of a genetic component for population differentiation, although maternal effects can not be ruled out. Culturing more than one generation, however, was avoided to minimize inbreeding and artificial selection by laboratory conditions. All experiments as well as the culturing of midges were carried out in a climate room at 20 °C $\pm$ 1 °C. A 16:7 h light:dark regime was provided, with a twilight zone of 30 min. before and after the light period, to stimulate reproduction. Experiments were carried out in water obtained from the oligo-mesotrophic lake Maarsseveen I (pH = 7.8; Na and Ca content 18.8 and 63.6 mg l⁻¹, respectively; Timmermans *et al.*, 1989).

Short-term tests for metal tolerance were performed by determining the growth rate of first instar larvae. Since no significant differences were observed between experiments performed in February and in September, experimental data were pooled. Thirty larvae, less than 24 hours old, were exposed during four days to concentration series of cadmium, zinc or iron in 200 ml lake water. Chloride salts were used for all metals and Fe^{3+}

Table 1. Metal concentrations and organic carbon in detritus samples.

	Cd ($\mu\text{mol g}^{-1}$)		Zn ($\mu\text{mol g}^{-1}$)		Fe ($\mu\text{mol g}^{-1}$)		Cu ($\mu\text{mol g}^{-1}$)		Org.C. %
	aver.	std.	aver.	std.	aver.	std.	aver.	std.	
Neerpelt	1.95	0.10	102.1	4.15	0.47	0.05	4.12	0.50	33.0
Borkel	2.19	0.16	93.2	23.2	0.42	0.12	3.40	0.90	35.4
Westerloo	0.42	0.03	37.0	2.32	1.93	0.52	2.97	0.11	35.7
Peer	0.12	0.03	19.9	2.52	1.13	0.29	1.71	0.39	39.3
Neerijse	0.01	0.01	6.18	2.15	0.19	0.10	0.49	0.35	20.9

was used in the tests with iron. The pH of the two highest iron concentrations had to be adjusted with NaOH to the control level of 7.8, after which iron started to precipitate. Water samples were taken at the start and at the end of the experiments and were directly acidified. Actual metal concentrations were: 2.7, 113.2, 197.8, 458.2, 1009.8 and 1832.7 nM Cd; 0.03, 1.91, 4.05, 7.89, 11.7 and 15.9 μM Zn; <0.001, 0.18, 1.64, 17.9, 183.6, 1743 μM Fe. The food (a mixture of grounded Trouvit and Tetraphyll®) was added at the start of the experiments and provided a suitable substrate for tube building. Therefore, no cellulose fibers were added. The initial length was measured in a similar sample of 30 animals, using an ocular micrometer. After 96 hours, the surviving larvae were counted and their length measured. Population differences in the growth rates of larvae in the control situation were assessed. Since these differences were present, metal toxicity was studied by calculating the percentage reduction of the growth relative to the control. In addition, differences in larval mortality were assessed by calculating linear regressions between mortality and metal concentrations. Since some assumptions for ANOVA were violated (even after transformation of the data), non parametric statistical analyses (Kruskal & Wallis) were applied according to Sokal & Rohlf (1981).

All materials used in the experiments and analyses were cleaned by soaking in 0.1 N HNO_3 (Merck Pro Analysis) for at least 24 hours and then rinsed three times with double distilled water. Water and detritus samples were analysed with Graphite Furnace Atomic Absorption Spectrometry (Perkin Elmer 5100) equipped with Zeeman background correction or Flame Atomic Absorption Spectrometry (Perkin Elmer 1100B). Quality control of trace metal analyses was carried out by analysing destruction blanks and reference material (IAEA MAA-3/TM shrimp homogenate). Measured values were in good agree-

ment with certified values (less than 10% deviation) and destruction blanks near detection limits.

Results

Metals in water and detritus

Routine measurements in the river Dommel near Neerpelt, performed by the Vlaamse Milieumaatschappij, showed that yearly averages of cadmium and zinc concentrations were 117 nM (range: 10–667 nM) and 11.2 μM (range: 0.92–110 μM), respectively. About the same was found for the sampling site Borkel (measurements performed by waterschap 'de Dommel'), with average concentrations of 113 nM Cd and 10.7 μM Zn. The single determinations of metal concentrations carried out during the sampling of chironomids were within the wide ranges of these regular measurements. Cadmium and zinc concentrations in the water at the other sampling sites were low with ranges of <1–7.8 nM Cd and 0.9–4.2 μM Zn. The concentrations of iron in the water did not differ significantly between sampling sites (range: 2.7–8.6 μM Fe). Metal concentrations in detritus samples reflect, however, metal exposure more effectively than the highly fluctuating levels in the water. Both cadmium and zinc concentrations were highest at the sampling sites Neerpelt and Borkel, but concentrations (compared to the clean reference sites) were also increased at the sampling site Westerloo (Table 1). Iron concentrations were highest at Westerloo, where about 10% of the detritus consisted of iron. Compared to the polluted sites, cadmium and zinc concentrations in both control sites were low, but the river IJse contained a consistently lower amount of metals than the Dommel, where especially iron contents were quite high.

Table 2. Linear regressions between mortality (%) and cadmium conc. (μM)

Population	Slope	Y-intercept	F-value	p-value
Neerpelt	10.12	4.12	4.86	$0.05 < p < 0.10$
Borkel	18.73	39.78	14.43	$0.01 < p < 0.025$
Westerloo	37.40	11.38	14.75	$0.01 < p < 0.025$
Neerijse	30.14	19.06	65.11	$0.001 < p < 0.005$
Peer	25.21	11.82	9.41	$0.025 < p < 0.05$
Culture	20.86	-1.68	67.68	$0.001 < p < 0.005$

Fig. 1. Growth (mm) of first instar larvae cultured in a clean environment during four days. Larvae were obtained from several field populations and a laboratory culture. Average values, pooled over several experiments, and standard errors are presented.

Larval growth rate

The growth rate of larvae, grown under control conditions, differed significantly between populations (Kruskal Wallis, $p < 0.001$) (Fig. 1). Larvae from the populations Borkel and Westerloo (two highly polluted sites) had a lowered average growth rate, while larvae from the population Neerpelt (also highly polluted) did not differ from larvae of the two reference sites (Peer & Neerijse). Larvae from the laboratory culture grew at a somewhat lower rate than larvae from the reference sites. Because of these differences, growth rates of metal exposed larvae were calculated as a percentage of the growth rate under control conditions.

Cadmium

First instar larvae obtained from the population Neerpelt (close to a zinc melter) were less sensitive to the

Fig. 2. Growth (as percentage of control) of first instar larvae exposed to cadmium. Larvae were obtained from several field populations and a laboratory culture. Average values and standard errors are presented. The following symbols were used: ■ = Neerpelt, ● = Borkel, ◆ = Westerloo, □ = Neerijse, ○ = Peer, ◇ = Laboratory culture.

effects of cadmium than larvae from other populations (Kruskal Wallis, $p < 0.001$). The growth even increased 50% when these larvae were exposed to low cadmium concentrations (Fig. 2). Effects of cadmium on larval mortality also differed among populations. Linear regressions between mortality and cadmium concentrations were significant for all populations, but the slope was very low (and not significantly different from zero) for the population Neerpelt (Table 2). Larvae from the population Borkel did not show any sign of increased cadmium tolerance although cadmium levels in water and detritus were high at this field site.

Zinc

Even at a low concentration of $1.9 \mu\text{M}$, zinc was inhibiting larval growth, but there were no differences between populations (Fig. 3). This uniform response did not reflect the major differences in zinc concentrations in water and detritus at the field sites. None of the test concentrations increased larval mortality significantly (pers. obs.).

Iron

The three lowest iron concentrations tested did not affect the larval growth rate of most populations (Fig. 4), and inhibitory effects were only observed at concentrations, leading to precipitation. However, the growth of larvae from the population Westerloo increased significantly at the two lowest iron con-

Fig. 3. Growth (as percentage of control) of first instar larvae exposed to zinc. Larvae were obtained from several field populations and a laboratory culture. Average values and standard errors are presented. The same symbols were used as in Fig. 2.

Fig. 4. Growth (as percentage of control) of first instar larvae exposed to iron. Larvae were obtained from several field populations and a laboratory culture. Average values and standard errors are presented. The same symbols were used as in Fig. 2.

centrations and stayed high at the two highest iron concentrations (Kruskal Wallis, $p < 0.001$). No significant effects were observed on larval mortality (pers. obs.).

Discussion

The different responses of the test populations to metals matched our expectations on adaptation at polluted sites partly. Two populations were indeed less sensitive to iron or cadmium, but increased tolerance to cadmium was not observed at the highly polluted sampling site near Borkel. Furthermore, despite the sensitivity to zinc, no differences in zinc tolerance were found. Also in these cases, increased tolerance was expected since

both cadmium and zinc concentrations in the water exceeded chronic LOEC values for growth, survival or mortality (e.g. Postma & Davids, 1995; Postma *et al.*, 1995; Timmermans *et al.*, 1992). On the other hand, the relative increase (compared to background levels) of zinc concentrations in detritus was much lower (about factor 15) than that of cadmium (factor 200), which made increased tolerance to zinc less likely than to cadmium. Furthermore, selection for site characteristics other than metal concentrations could have interfered with selection for metal tolerance, since several site characteristics (like stream velocity, depth, sediment type) differed between sampling sites. It is, however, likely that the selected metals were the main causative agent for the observed population differentiation, since low concentrations of both cadmium and iron stimulated the growth rate in the two tolerant populations. In addition, several studies demonstrated that metal concentrations as found in our sampling sites can affect the growth, reproduction and survival of *C. riparius* (Pascoe *et al.*, 1989; Postma & Davids, 1995; Postma *et al.*, 1995; Timmermans *et al.*, 1992), thereby creating a selective pressure. In addition, selection experiments in the laboratory (Postma & Davids, 1995) proved that at least in the case of cadmium, concentrations in the field were high enough to cause increased cadmium tolerance. Differences in cadmium tolerance among the two exposed populations in the river Dommel require further analysis: the input of genes from (unexposed) upstream populations as well as a decrease of metal availability downstream of the source are main variables in future studies.

The mechanism causing increased tolerance in *C. riparius* is not yet known, but literature indicates several possibilities. Krantzberg & Stokes (1989), for example, observed that *Chironomus* species from a metal exposed field population had an increased capacity to regulate the body concentration of some essential metals (Cu, Ni, and to some extent Mn). Metallothionein-like proteins could be involved, since both Yamamura *et al.* (1983) and Seidman *et al.* (1986) found that *Chironomus* species can synthesize these proteins when exposed to cadmium.

Since maintenance of a tolerance mechanism may be energetically expensive, 'costs of tolerance' have frequently been suggested as a possible consequence of being tolerant (Holloway *et al.*, 1990; Klerks & Levinton, 1989; Postuma & Van Straalen, 1993; Wilson, 1988). As in other cases of lowered fitness for tolerant individuals cultured in clean environments (Cook *et al.*, 1972; Cox & Hutchinson, 1981; Hickey &

McNeilly, 1975; Weis & Weis, 1989), the lowered larval growth rate of the iron tolerant population Westerlo could also indicate such costs. However, the larval growth rate of the cadmium tolerant population Neerpelt did not differ from the reference populations. These results, therefore, do not give any conclusive sign of increased costs, causing a lowered growth rate. Results by Postma *et al.* (1995), however, seemed to indicate that the lowered larval growth rate of cadmium tolerant *C. riparius* cultured in the absence of cadmium, was not a direct consequence of higher energetic expenses but a consequence of zinc shortage, since addition of low concentrations of zinc stimulated the growth. The stimulation of larval growth rate by low cadmium or iron concentrations, as observed in the present experiments, seemed to accord with these results, although no stimulation was observed at low zinc concentrations.

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References

- Antonovics, J., A. D. Bradshaw & R. G. Turner, 1971. Heavy metal tolerance in plants. *Adv. Ecol. Res.* 7: 1–85.
- Brown, B. E., 1977. Effects of mine drainage on the river Hayle, Cornwall a) factors affecting concentrations of copper, zinc and iron in water, sediments and dominant invertebrate fauna. *Hydrobiologia* 52: 221–233.
- Clements, W. H., D. S. Cherry & J. Cairns, 1988. Impact of heavy metals on insect communities in streams: A comparison of observational and experimental results. *Can. J. Fish. aquat. Sci.* 45: 2017–2025.
- Cook, S. C. A., C. Lefebvre & T. McNeilly, 1972. Competition between metal tolerant and normal plant populations on normal soil. *Evolution* 26: 366–372.
- Cox, R. M. & T. C. Hutchinson, 1981. Multiple and co-tolerance to metals in the grass *Deschampsia cespitosa*: adaptation, preadaptation and 'costs'. *J. Plant Nutrition* 3: 731–741.
- Gauss, J. D., P. E. Woods, R. W. Winner & J. H. Skillings, 1985. Acute toxicity of copper to three life stages of *Chironomus tentans* as affected by water hardness-alkalinity. *Envir. Pollut. Ser. A* 37: 149–157.
- Hickey, D. A. & T. McNeilly, 1975. Competition between metal tolerant and normal plant populations; a field experiment on normal soil. *Evolution* 29: 458–464.
- Holloway, G. J., R. M. Sibly & S. R. Povey, 1990. Evolution in toxin-stressed environments. *Funct. Ecol.* 4: 289–294.
- Janssens de Bisthoven, L. G., K. R. Timmermans & F. Ollevier, 1992. The concentration of cadmium, lead, copper, and zinc in *Chironomus gr. thummi* larvae (Diptera, Chironomidae) with deformed *versus* normal menta. *Hydrobiologia* 239: 141–149.
- Klerks, P. L. & J. S. Levinton, 1989. Rapid evolution of metal resistance in a benthic oligochaete inhabiting a metal-polluted site. *Biol. Bull.* 176: 135–141.
- Klerks, P. L. & J. S. Levinton, 1993. Evolution of resistance and changes in community composition in metal-polluted environments: a case study on Foundry Cove. In R. Dallinger and P. S. Rainbow (eds). *Ecotoxicology of metals in invertebrates*, Lewis Publishers, Boca Raton, USA: 223–241.
- Klerks, P. L. & J. S. Weis, 1987. Genetic adaptation to heavy metals in aquatic organisms: a review. *Envir. Pollut.* 45: 173–205.
- Krantzberg, G. & P. M. Stokes, 1989. Metal regulation, tolerance and body burdens in the larvae of the genus *Chironomus*. *Can. J. Fish. aquat. Sci.* 46: 389–398.
- Macnair, M. R., 1993. Transley review No. 49; The genetics of metal tolerance in vascular plants. *New Phytol.* 124: 541–559.
- Pascoe, D., K. A. Williams & D. W. J. Green, 1989. Chronic toxicity of cadmium to *Chironomus riparius* – effects upon larval development and adult emergence. *Hydrobiologia* 175: 109–115.
- Pinder, L. C. V., 1978. A key to adult males of British Chironomidae. *Freshwater Biological Association, Scientific Publ.*, No. 37, Vols 1 and 2.
- Postma, J. F., M. C. Buckert-de Jong, N. Staats & C. Davids, 1994. Chronic toxicity of cadmium to *Chironomus riparius* (Diptera: Chironomidae) at different food levels. *Arch. Envir. Contam. Toxicol.* 26: 143–148.
- Postma, J. F. & C. Davids, 1995. Tolerance induction and life-cycle changes in cadmium exposed *Chironomus riparius* (Diptera) during consecutive generations. *Ecotox. Environ. Safety*, 30: 195–202.
- Postma, J. F., S. Mol, L. Larsen & W. Admiraal, 1995. Life-cycle changes and zinc shortage in cadmium tolerant midges, *Chironomus riparius* (Diptera) reared in the absence of cadmium. *Envir. Toxicol. Chem.* 14: 117–122.
- Posthuma, L. & N. M. Van Straalen, 1993. Heavy-metal adaptation in terrestrial invertebrates: a review of occurrence, genetics, physiology and ecological consequences. *Comp. Biochem. Physiol.* 106C: 11–38.
- Seidman, L. A., G. Bergtrom, D. J. Gingrich & C. C. Remsen, 1986. Accumulation of cadmium by the fourth instar larva of the fly *Chironomus thummi*. *Tissue and Cell* 18: 395–405.
- Sokal, R. R. & F. J. Rohlf, 1981. *Biometry*, 2nd edn. Freeman, NY.
- Timmermans, K. R., B. van Hattum, M. H. S. Kraak, & C. Davids, 1989. Trace metals in a littoral foodweb: concentrations in organisms, sediment and water. *Sci. Tot. Envir.* 87/88: 477–494.
- Timmermans, K. R., W. Peeters & M. Tonkes, 1992. Cadmium, zinc, lead and copper in *Chironomus riparius* (Meigen) larvae (Diptera, Chironomidae): uptake and effects. *Hydrobiologia* 241: 119–134.
- Trevors, J. T., K. M. Oddie & B. H. Belliveau, 1985. Metal resistance in bacteria. *FEMS Microbiol. Rev.* 32: 39–54.
- Weis, J. S. & P. Weis, 1989. Tolerance and stress in a polluted environment; the case of the mummichog. *Bioscience* 39: 89–95.
- Wentzel, R., A. McIntosh & G. Atchison, 1978. Evidence of resistance to metals in larvae of the midge *Chironomus tentans* in a metal contaminated lake. *Bull. envir. Contam. Toxicol.* 20: 451–455.
- Williams, K. A., D. W. J. Green, D. Pascoe & D. E. Gower, 1986. The acute toxicity of cadmium to different larval stages of *Chironomus riparius* (Diptera: Chironomidae) and its ecological significance for pollution regulation. *Oecologia (Berlin)* 70: 362–366.

Wilson, J. B., 1988. The cost of heavy-metal tolerance: an example. *Evolution* 42: 408–413.

Yamamura, M., K. T. Suzuki, S. Hatakeyama & 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.