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Publication date
1994

Published in
Archives of Environmental Contamination and Toxicology

[Link to publication](#)

Citation for published version (APA):

Postma, J. F., Buckert-de Jong, M. C., Staats, N., & Davids, C. (1994). Chronic toxicity of cadmium to *Chironomus riparius* (Diptera: Chironomidae) at different food levels. *Archives of Environmental Contamination and Toxicology*, 26, 143-148.

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Chronic Toxicity of Cadmium to *Chironomus riparius* (Diptera: Chironomidae) at Different Food Levels

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Received: 3 June 1993/Revised: 1 September 1993

Abstract. The interacting effects of cadmium toxicity and food limitation on the midge, *Chironomus riparius*, were studied during chronic exposure in laboratory experiments. If the food was supplied *ad libitum*, both larval developmental time and mortality of the larvae were negatively affected by cadmium concentrations of 2.0–16.2 µg/L. The number of eggs deposited per female and the mean life span of the imagines were not affected by cadmium. Integration of these separate effects into a population growth rate showed a clear reduction with increasing cadmium concentrations. Food limitation of unexposed larvae at high population density reduced fitness, judged on all parameters studied and consequently reduced the population growth rate (up to 85%).

The effects on larvae, which were exposed to both cadmium and food limitation, differed considerably from the response to the individual stress factors. Exposure to cadmium increased mortality among food-limited first and second instar larvae. Consequently, the amount of food available for each surviving larva increased. At the two lowest concentrations studied (2.0 and 5.6 µg Cd/L), these indirect positive effects of cadmium overruled the direct negative effects and caused an increase of the fitness of the food-limited exposed larvae compared to the food-limited, unexposed controls. At a concentration of 16.2 µg Cd/L, the negative effects of cadmium on food-limited midges balanced the positive effects of reduced food limitation. At this concentration, the population growth rate did not differ significantly from the food-limited control any more. It is concluded that the indirect positive effects of cadmium on food limitation could eliminate negative, direct effects of low cadmium concentrations on food-limited chironomid populations.

Before results of ecotoxicological laboratory experiments can successfully be extrapolated to the field situation, information is needed regarding factors that influence the toxicity of chemicals. Besides physical or chemical factors such as temperature

(Fisher 1986) and pH (Krantzberg and Stokes 1988), biological factors are considered to be of great importance. Krantzberg (1989), for example, found that the age and body weight of chironomids influenced the accumulation of metals significantly. The amount of food available to the animals is another important factor (Lanno *et al.* 1989). In general, food-limited animals are more susceptible to toxicants than well-fed animals (Chandini 1988a; Verriopoulos and Moraitou-Apostolopoulou 1989, working on crustaceans and the effects of the metals Cd and Zn, respectively). In contrast Pascoe *et al.* (1990) observed that the presence of food increased the toxicity of cadmium to *Chironomus riparius* (Diptera, Chironomidae), a result that was partly explained by a rapid binding of cadmium to the food.

Most of these studies involve acute toxicity experiments lasting for at most 4 days, and the influence of different food concentrations on the chronic toxicity of chemicals has rarely been studied. Chandini (1988b, 1989) determined the chronic toxicity of cadmium to two species of Cladocera at three different food levels and Ramakrishna-Rao and Sarma (1986) determined the chronic toxicity of DDT to the rotifer *Brachionus patulus* at both high and low food levels. From these experiments, as well as from acute experiments, it was concluded that food-limited animals are more sensitive to toxicants than are well-fed animals. However, the food in these experiments was not very limited, because mortality was not or only slightly increased compared to the unexposed animals. Koivisto *et al.* (1992) studied the chronic toxicity of copper to five cladoceran species at two food levels. For two species food limitation was so severe that it increased the mortality of the unexposed, food-limited animals. For one of these species, an increased population growth rate was found when food-limited animals were exposed to low concentrations of copper, while the population growth rate showed a steady decrease when well-fed animals were used. The other species showed a decrease of the population growth rate in both cases. These contrasting results of experiments on the long-term interaction of food limitation and exposure to toxicants suggest that the amount of food limitation is crucial for the outcome of the interaction.

Especially after the mass emergence of chironomids in spring, large numbers of first and second instar larvae are competing with each other and are probably affected by limited amounts of food or space. This suggests that food limitation

could be of great importance in a field situation and influence the toxicity of chemicals. Considering *C. riparius* (Meigen), it has already been proved that the growth rate of larvae can be lowered due to an increased intraspecific competition (Rasmussen 1985) and, as has been demonstrated by Gower and Buckland (1978) and Koehn and Frank (1980), *C. riparius* is an indicator of enriched conditions in the ecosystem (for example, due to sewage input). It can therefore be hypothesized that among chironomids *C. riparius* is particularly susceptible to low amounts of food.

First and second instar larvae of *C. riparius* are not only exposed to limited amounts of food and space, but they are also more sensitive to the effects of cadmium than the third or fourth instar larvae (Williams *et al.* 1986). To study the interactions between cadmium toxicity and food limitation, we determined the chronic toxicity of cadmium to the chironomid, *Chironomus riparius*, in both a situation of food limitation as well as with an excess of food, taking care that mortality will occur due to the food limitation.

Materials and Methods

All experiments were made in a climate room at $20 \pm 1^\circ\text{C}$. A 16:7 light:dark regime was provided, with a twilight zone of 30 min before and after the light period, to stimulate reproduction. In plastic aquaria ($1^{\circ}\text{w}^{\circ}\text{h} = 34^{\circ}17^{\circ}21^{\circ}\text{cm}^3$) shredded paper to a depth of 1 cm (14 g dry weight) was provided as a substrate and the overlying water (4 L) was aerated constantly. A cage ($1^{\circ}\text{w}^{\circ}\text{h} = 39^{\circ}21^{\circ}30^{\circ}\text{cm}^3$) was placed on top of the aquaria. Larvae of *C. riparius*, originating from a laboratory culture, were provided with a mixture of ground Trouvit and Tetra-phyll® as food.

The experiments were carried out in Lake Maarsseveen I water, an oligo-mesotrophic lake in which trace metal concentrations are near the detection limits. Specific information on water quality is provided by Timmermans *et al.* (1989). Larvae were exposed to four concentrations of cadmium (added as a solution of cadmiumchloride) and two concentrations of food. Each cadmium–food combination was tested in triplicate. The water was renewed once a week, and water samples were taken before and directly after water renewal. Acidified water samples were analyzed with graphite furnace atomic absorption spectrometry (Perkin Elmer 5100) equipped with Zeeman background correction. The actual concentrations of cadmium were 0.09 ± 0.10 (control), 2.0 ± 0.64 , 5.6 ± 1.68 , and 16.2 ± 6.12 $\mu\text{g Cd/L}$ (mean and standard deviation) (0.8, 17.6, 49.9, and 144.0 nmol Cd/L, respectively). There were no significant differences in the cadmium concentrations between the two food conditions.

Before the experiments started, the aquaria and the cellulose were equilibrated with the corresponding cadmium concentrations, by soaking 14 g of dry cellulose in 4 L lake water with 0.09, 2.0, 5.6, and 16.2 $\mu\text{g Cd/L}$. Cadmium-loaded food was prepared by soaking 10 g of Trouvit and 0.5 g of Tetra-phyll® in 200 ml of the corresponding cadmium solution. Spiking of the aquaria, the cellulose, and the food lasted for 1 week during which all solutions were renewed twice. Before the beginning of the experiment, all solutions were renewed once again. While the aquaria and the cellulose were fully loaded at the beginning of the experiment, it is likely that the food continued to bind cadmium during the experiment, causing a gradual decrease of the water concentrations.

The food supply was limited by increasing the number of larvae per aquarium instead of lowering the absolute amount of food. Two densities were used: 865 animals/m² allowing an excess of food and 3,460 animals/m² to create food limitation. These densities were chosen on the basis of a pilot experiment in which we tested five different densities. In a second pilot experiment, it was proven that the effects of increasing the larval density were mainly caused by a reduced food

availability. Experiments were started by adding 50 (equivalent to a density of 865/m²) or 200 (equivalent to a density of 3,460/m²) newly hatched first instar larvae to each aquarium and adding 2.5 ml of food twice a week. Experiments ended when all surviving larvae had emerged. This took about 4–8 weeks, depending on the combination of cadmium concentration and population density.

At the onset of emerging the following parameters were assessed daily:

The number of newly emerged males and females by sexing the pupal exuviae. On the basis of these data, the mortality as well as the mean larval developmental time were determined.

The number of male and female midges in the cage, to estimate the mean life span of an imago.

The number of dead males and females midges, to check the first two parameters. Dead imagines were removed, and some of them were collected for analysis of dry weight and accumulated cadmium.

The number of dead larvae or pupae.

The number of deposited egg-ropes as well as the number of eggs per egg-rope, to provide an estimation of the average number of eggs per female midge. In addition, the hatchability of the egg-ropes was assessed after hatching in a petridish at 20°C .

These parameters were integrated into a population growth rate, estimated as the mean number of larvae of the second generation which were produced per larvae of the first generation per day (using the mean larval developmental time).

All materials used in the experiments and analysis were cleaned by soaking them in 0.1 N HNO₃ (Merck Pro Analysis) for at least 24 h and then rinsing three times with double distilled water. Lyophilized organisms were weighed and digested individually, in HNO₃ and H₂O₂, using a microdestruction method (Timmermans *et al.* 1989). Samples were analyzed with graphite furnace atomic absorption spectrometry (Perkin Elmer 5100) equipped with Zeeman correction. Quality control of the trace 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).

Routine statistical analysis (ANOVA, Kruskal and Wallis) were applied according to Sokal and Rohlf (1981). Unless stated otherwise, the significance was tested at the $p < 0.05$ level.

Results

Mortality

If well-fed larvae are exposed to cadmium, only exposure to 16.2 $\mu\text{g Cd/L}$ gave a significant increase in mortality (ANOVA $p < 0.001$) (Figure 1a). The mortality of food-limited larvae was about 30% higher at all cadmium concentrations, indicating that food limitation did not influence the effects of cadmium on survival. However, the combination of cadmium exposure and food limitation did influence the moment at which mortality occurred. If the larvae were well fed, 80% of the mortality in the control, in the 2.0 $\mu\text{g Cd/L}$, and in the 5.6 $\mu\text{g Cd/L}$ concentrations was due to death occurring among the fourth instar larvae or pupae. If the larvae were starved, the percentages of the total mortality, which could be ascribed to dead fourth instar larvae or pupae, decreased by about 30% in the 5.6 $\mu\text{g Cd/L}$ treatment compared to the food-limited control. Consequently, death occurred sooner (probably among the first or second instar larvae) in these cadmium exposed, high density

Fig. 1. The effects of cadmium exposure and food-limitation on the following parameters of *Chironomus riparius*: (a) mortality; (b) larval developmental time; (c) number of eggs per female; (d) life span of the imagines; (e) population growth rate; (f) dry weight of the males; (g) accumulated cadmium in the males. Presented are the mean values together with their standard error. Open bars represent the high larval density and hatched bars the low larval density

experiments leaving more food available to the surviving, exposed larvae than to food-limited, control animals.

Larval Developmental Time

The growth of well-fed larvae is significantly reduced by cadmium (Kruskal Wallis $p < 0.001$). The mean day of emergence is delayed by more than 10 days if the well-fed larvae are exposed to 16.2 µg Cd/L and by 3 and 4 days, if they are exposed to 2.0 and 5.6 µg Cd/L, respectively (Figure 1b). Food limitation of the larvae also increased the larval developmental time. However, cadmium exposure had no effect on the larval developmental time in the case of food-limited larvae.

Eggs per Female

Female midges, reared as well-fed larvae, deposited, on average, 400 eggs in the control situation. Low cadmium concentrations (2.0 and 5.6 µg/L) seemed to reduce the mean number of

eggs deposited per well-fed female (ca. 25%). However, this decrease was not present at the highest cadmium concentration (16.2 µg/L), where even a small increase was observed (Figure 1c). In the food-limited situation, the main effect of cadmium, as observed in a two-way ANOVA ($p < 0.001$), was more pronounced. While starved control females deposited on average 100 eggs, this number increased significantly when food-limited midges were exposed to cadmium. At a concentration of 16.2 µg Cd/L, food-limited females deposited almost 400 eggs per female, which was comparable to the well-fed chironomids. The effect of cadmium differed, therefore, significantly between the well-fed and food-limited midges, as expressed by a significant interaction term in the analysis of variance ($p < 0.001$). Although the egg-ropes were somewhat smaller in the food-limited situation, the difference between well-fed and food-limited midges was mainly caused by effects on the average number of egg-ropes deposited per female. Both food limitation and exposure to cadmium did not have any significant effect on the hatchability of the egg-ropes (about 90% in all cases). These results seem to indicate that cadmium exposure can reduce the negative effects of food limitation on

the reproductive capacity of *C. riparius* by increasing the mortality of the larvae.

Life Span of an Imago

Imagines that emerged from well-fed larvae lived on average for 4 days, whether or not they were exposed to cadmium (Figure 1d). In contrast, imagines, emerged from food-limited larvae, had a life span only half of this, but the life span increased significantly when food-limited midges were exposed to cadmium (ANOVA $p < 0.001$). Unexpectedly, starved *C. riparius*, exposed to 16.2 $\mu\text{g Cd/L}$, lived even longer than the unexposed well-fed controls.

Population Growth Rate

We calculated the population growth rate as a way of integrating all the different effects of cadmium exposure and food limitation on population dynamics. Even very low concentrations of cadmium (2.0 $\mu\text{g/L}$) are capable of decreasing the population growth rate (30%) of well-fed midges (Figure 1e). This was mainly caused by a reduced reproductive effort. Exposure of well-fed larvae to 16.2 $\mu\text{g Cd/L}$ decreased the population growth rate by as much as 66%. Completely different results are obtained if the animals are starved and exposed to low concentrations of cadmium at the same time. The reduction of the population growth rate due to food limitation (85%) is lowered by the simultaneous exposure to cadmium. Exposure of food-limited larvae to 2.0 $\mu\text{g Cd/L}$ doubled the population growth rate, and exposure to 5.6 $\mu\text{g Cd/L}$ tripled the population growth rate compared to the unexposed, food-limited controls. When food-limited larvae were exposed to 16.2 $\mu\text{g Cd/L}$, the population growth rate decreased again and did not differ from the control.

Dry Weight of the Imagines and Cadmium Accumulation

The dry weight of the female imagines was rather variable, probably due to the fact that some females had not deposited their egg-rope. The cadmium accumulation in the female midges was consequently also rather variable. These data are therefore not presented and are discarded in further analyses. The dry weight of male imagines, emerged from well-fed larvae, was around 330 μg and was not influenced by exposure to cadmium (Figure 1f). If the larvae were starved, the male midges were considerably smaller in the control (dry weight of $\pm 240 \mu\text{g}$), but the dry weight increased significantly by exposure to cadmium (Kruskal Wallis $p < 0.001$). If the food-limited larvae were exposed to 16.2 $\mu\text{g Cd/L}$, the dry weight of the imagines increased by 70 μg compared to the food-limited control and almost reached the dry weight of well-fed imagines.

The concentration of cadmium in the male midges was not significantly influenced by food limitation, although starved animals exposed to 2.0 $\mu\text{g Cd/L}$ had accumulated relatively more cadmium (Figure 1g). Although food limitation caused an increased larval developmental time and thus an increased exposure time, this did not increase the accumulation of cadmium by the larvae. Therefore, the larvae seemed to be in equilibrium

with the cadmium in the aquaria (in sediment, food, and water) and accumulation of cadmium in the imagines cannot be used as a explanation for the differences between the effects of cadmium on well-fed and food-limited larvae.

Discussion

The present observations on the influence of chronic cadmium toxicity on well-fed *C. riparius* are in good agreement with the literature. For example, we did not find any significant effects on the growth, reproduction, or mortality of well-fed midges exposed to 2.0 $\mu\text{g Cd/L}$. The same was reported by Pascoe *et al.* (1989) using a concentration of 1.5 $\mu\text{g Cd/L}$. At higher cadmium concentrations (both 5.6 and 16.2 $\mu\text{g Cd/L}$), however, negative effects were observed. This is in accordance with data from Pascoe *et al.* (1989) and Timmermans *et al.* (1992), who observed a reduction of the growth rate and an increased mortality at 15 and 10 $\mu\text{g Cd/L}$, respectively. Hatakeyama (1987, 1988) reported that the number of egg-rope deposited per female *Polypedilum nubifer* (Diptera, Chironomidae) is insensitive to both copper and cadmium, as we found in our cadmium experiments with well-fed *C. riparius*. The number of eggs per egg-rope seemed to be a more sensitive parameter (Hatakeyama and Yasuno 1981). In our experiments the hatchability of the egg-rope was unaffected. This is probably caused by the low cadmium concentrations, since Williams *et al.* (1987) found only negative effects on the hatchability of egg-rope of *C. riparius* at cadmium concentrations above the 0.3 mg/L.

Food limitation had a severe effect on all parameters studied and resulted in a decrease of the population growth rate by about 85%. Rasmussen (1985) demonstrated similarly that the growth rate of *C. riparius* decreased when the density of the larvae increased. The dry weight of the imagines was also negatively affected by food limitation in our experiments. This explains at least partly the effects of food limitation on the mean number of eggs per female, because Butler and Walker (1992) found that pupal mass accounted for 76% of the variation in fecundity of *Chironomus cucullini*. These effects of food limitation are possibly caused by a changing energy budget. Macchiusi and Baker (1992) demonstrated that food-limited larvae of *Chironomus tentans* were more active than well-fed larvae and consequently spent more energy in food searching.

From both acute and chronic experiments on a range of species, it has become clear that well-fed animals are usually less susceptible to toxicants than are their food-limited conspecifics (Chandini 1988a, 1988b, 1989; Ramakrishna Rao and Sarma 1986; Verriopoulos and Moraitou-Apostolopoulou 1989). These authors found negative effects of food limitation on both growth and reproduction, although mortality was not or only slightly affected by food limitation. In our experiments, food limitation was more severe than in previous studies and caused high mortality in the unexposed control treatment. Furthermore, food limitation was introduced by increasing the number of larvae, maintaining the same amount of food added. Under these circumstances, the addition of cadmium resulted in an increased mortality among the first and second instar larvae compared to the food-limited unexposed larvae. This means that the exposed, food-limited larvae had, on average, more food available to them than the unexposed, food-limited larvae. The outcome of this experiment with food-limited larvae is

consequently a mixture of direct effects of cadmium on the chironomids and indirect effects of cadmium causing a change in the food limitation.

The direct effects of cadmium exposure and the indirect effects resulting from food limitation vary in strength depending on the cadmium–food combination. At all parameters studied, except for the mortality, increasing cadmium exposure of food-limited larvae resulted in a higher fitness of the midges compared to the unexposed food-limited animals. The negative direct effects of cadmium were compensated for by the positive effects of a reduced food limitation, and the difference between food-limited and well-fed animals for all parameters decreased with increasing cadmium concentrations. Simkiss *et al.* (1993) obtained comparable results in experiments on the effects of population density and cadmium on blowflies. Both the pupal weight as well as the adult emergence weight of well-fed animals was significantly reduced by cadmium exposure. This effect of cadmium was, however, less pronounced or not present at all, when the animals were food-limited. Because eight animals were maintained in one tub, and the highest cadmium concentration used was lethal to 50% of the larvae, interactions between the direct effects of cadmium and the indirect effects due to a reduced food limitation are, therefore, again a possible explanation.

Due to this interaction, the fitness of food-limited chironomid larvae exposed to 16.2 $\mu\text{g Cd/L}$ did not differ much from well-fed larvae exposed to the same concentration. This means that in this treatment the mortality reached a level at which the food for the surviving larvae is provided almost *ad libitum*. Due to these positive effects the population growth rate of food-limited midges increased significantly by exposure to 2.0 and 5.6 $\mu\text{g Cd/L}$ compared to the food-limited control. In experiments with five cladoceran species Koivisto *et al.* (1992) obtained comparable results determining the effects of copper on the intrinsic rate of increase at two food levels. Only for *Daphnia galeata* and *Bosmina longirostris* was food limitation so severe that mortality increased due to food limitation. For *Daphnia galeata* an increased population growth rate was found when food-limited animals were exposed to low concentrations of copper, while the population growth rate showed a decrease when well-fed animals were used. In these experiments, however, all daphnids were exposed individually, excluding the possibility of indirect effects of a toxicant, as observed in our experiments.

Mortality of the food-limited larvae increased by exposure to cadmium reaching a level twice as high as the food-limited control when exposed to 16.2 $\mu\text{g Cd/L}$. At this concentration the positive effects of the reduced food-limitation were in balance with the increased mortality. Therefore, the population growth rate was on the average unaffected at this cadmium concentration compared to the food-limited control. At cadmium concentrations above the 16.2 $\mu\text{g Cd/L}$, the direct effects of cadmium exposure would probably overrule the indirect effects, causing the population growth rate to decrease compared to the food-limited control.

The effect of cadmium exposure and food limitation on the average life span of an imago are more complicated. On the basis of the experiment with well-fed midges, there seem to be no direct effects of cadmium on this parameter, although it was clearly affected by food limitation. If exposure of food-limited larvae to increasing cadmium concentrations only resulted in a reduced food limitation, it would be expected that the food-

limited animals exposed to 5.6 or 16.2 $\mu\text{g Cd/L}$ would, at best, reach the level of the well-fed larvae. However, the larvae exposed to 16.2 $\mu\text{g Cd/L}$ performed significantly better than both the well-fed controls and the well-fed larvae exposed to 16.2 $\mu\text{g Cd/L}$. The long life span of exposed, food-limited larvae is maybe caused by a strong selective pressure due to the combination of food limitation and exposure to cadmium. The larvae, which survived this treatment (only 20%), can consequently have a longer life span than the average of a well-fed population.

After the mass emergence of chironomids in spring, leading to similar or even higher densities of first instar larvae as in our experiment, large numbers of first and second instar larvae are competing with each other and are probably affected by limited amounts of food or space. It seems realistic to assume that both food limitation and cadmium exposure interferes with the population dynamics of chironomids in the field in a way such as found in our study. This makes the amount of food available to the animals a crucial factor in determining the toxicity of cadmium in the field. If the compensating mechanisms of intoxication and food limitation occur on a large scale, it is less likely negative effects of low concentrations of cadmium can be assessed in the field.

Acknowledgments. The authors thank Chris de Groot and Dick Lindenaar for practical assistance, Prof. Dr. Wim Admiraal, and Dr. Michiel H. S. Kraak for their comments, and Prof. Dr. B. L. Roberts for improving the text.

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