Persistence of benthic invertebrates in polluted sediments.
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CHAPTER 3
COMBINED EFFECTS OF COPPER AND FOOD ON THE MIDGE CHIRONOMUS RIPARIUS IN WHOLE-SEDIMENT BIOASSAYS

E.M. de Haas, M. León Paumen, A.A. Koelmans, M.H.S. Kraak
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Effects observed in whole-sediment bioassays must be seen as the joint effect of all sediment characteristics. In whole-sediment bioassays, however, adverse effects on test organisms are usually attributed to the presence of contaminants and the effects of food are often ignored. The aim of this study was to analyze the response of the midge Chironomus riparius to sediment spiked with different combinations of artificial food and a model toxicant, copper. The responses of C. riparius to these spiked sediments were assessed in 10-day whole-sediment bioassays. Decreases in survival, dry weight, and length of C. riparius were observed with increasing copper concentrations. However, an increase in the amount of food resulted in an increase of larval dry weight and length until copper concentrations reached a critical threshold of 200 mg/kg. In addition, an increase in the amount of food resulted in a decrease of accumulated copper in the larvae. The present study demonstrated that the combination of copper and food in the sediment determines the performance of C. riparius in whole-sediment bioassays. The dependency of C. riparius on high feeding levels, which masks toxic effects, questions its suitability as a test organism for whole-sediment bioassays. Because benthic communities in polluted ecosystems are often exposed to varying levels of both food and toxicants it is concluded that the trophic state of the ecosystem may alter the ecological risk of sediment-bound toxicants to opportunistic benthic invertebrates, such as C. riparius.
INTRODUCTION

Benthic invertebrates in polluted ecosystems are often exposed to varying levels of both food and toxicants (DE HAAS et al. 2002). The trophic state of an ecosystem influences the amount of food available for the benthic detritivorous community. Simultaneously, the trophic state also influences the sedimentation of abiotic and biotic particles and therewith the deposition of xenobiotic compounds due to sorption of organic toxicants (KOELMANS et al. 2001) and metals (ADMIRAAL et al. 1993).

In whole-sediment bioassays adverse effects on test organisms are usually only attributed to the amount of sediment-bound toxicants. However, effects observed in whole-sediment bioassays must be seen as the joint effect of all sediment characteristics, like contaminants, physical-chemical parameters, and food quantity and quality parameters (PEETERS et al. 2000a, VOS 2001, DE HAAS et al. 2002).

Food availability is an important factor in the development of benthic invertebrates (SUEDEL & RODGERS 1994, PEETERS et al. 2000, VOS 2001). Food limitation or poor food quality in natural sediments can reduce survival, growth, and development of opportunistic species such as the midge Chironomus riparius (RISTOLA et al. 1999, VOS 2001, DE HAAS et al. 2002). If food is deficient, the test organisms may suffer from starvation, which may lead to an overestimation of sediment toxicity. Addition of food in midge bioassays is therefore often needed to exclude reduction of survival, growth, and development due to food deficiency (ANKLEY et al. 1994a, LIBER et al. 1996, BRIDGES et al. 1997, VOS 2001, DE HAAS et al. 2002). On the other hand, feeding of the test organisms during exposure to sediments may mask toxic effects resulting in an underestimation of sediment toxicity (ANKLEY et al. 1994a, DAY et al. 1994, HARKEY et al. 1994b, LACEY et al. 1999). In addition, feeding can modify the bioavailability of sediment-bound toxicants resulting in either an increase or a decrease in the accumulation of toxicants in test organisms and, therewith, alter toxicity (HARKEY et al. 1994b). Nevertheless, chironomid larvae, such as C. riparius, are still widely used as a test organism in acute and chronic (sediment) toxicity and accumulation tests in which an excess of food is supplied (HARKEY et al. 1994a, KEMBLE et al. 1994, RISTOLA et al. 1999, HARRAHY & CLEMENTS 1997).

The high risk of false interpretations of the results of whole-sediment bioassays with chironomids requires a systematic analysis of the combined effects of food and toxicants under controlled laboratory conditions. To determine the effects of feeding level on the response of C. riparius to toxicants we conducted 10-day whole-sediment bioassays. C. riparius larvae were
exposed to clean natural sediment spiked with different concentrations of copper and artificial food. We hypothesize, based on our previous findings in a study with several flood plain lake sediments varying in natural food and toxicant concentrations (DE HAAS et al. 2002), that *C. riparius* is able to better withstand toxicants at higher food availability. Our data are provident to emphasize the importance of dietary control in sediment toxicity testing.

**MATERIALS AND METHODS**

**Sediment collection, storage, and treatment**
Sediment was collected from a relatively clean floodplain lake located along the River Waal, the Netherlands, in September 2001. About 50 L of sediment were collected using a stainless steel Ekman-Birdge grab, which was adjusted to sample the upper 5 cm of the sediment.

The sediment was transported to the laboratory in polythene buckets where large debris was picked out by hand and homogenized in a 75-L container. The sediment was stored until use at -20°C in 500-ml polyethylene containers within 6 h after sampling in order to eliminate autochthonous organisms.

**Sediment preparation**
Three days before the spiking procedure, the sediment was thawed at 4°C. Sediment was spiked by diluting the appropriate amount of copper [CuCl$_2$.2H$_2$O (J.T. Baker®, Phillipsburg, NJ, USA)] in 300 ml Elendt-M7 medium (OECD 2001) using a copper stock solution (1 or 10 g/L depending on the final concentration). This copper solution was added to 75 ml of homogenized wet sediment in 500-ml polyethylene bottles together with the appropriate amount of food [Trouvit® (Trouw, Fontaine-les-Vervins, France) - TetraPhyll® (Tetrawerke, Melle, Germany) mixture (20:1, wt/wt)].

Sediment was spiked with copper in order to achieve nominal copper concentrations of 25, 50, 100, 150, 200, 300, 400, 500, and 600 mg/kg dry weight (mg/kg). Because the amount of sediment was insufficient to test nominal copper concentrations of both 500 and 600 mg/kg at all food levels, 500 mg/kg was only performed at the two lowest food levels and 600 mg/kg only at the two highest food levels. The midges were fed 0, 0.05, 0.25, or 0.5 mg/larvae/d of ground fish food. According to the OECD guideline 218 (OECD 2001), 0.25-0.5 mg/larvae/d of a finely ground fish food is adequate for larvae younger than 10 days. For each food-copper treatment 12 test systems were prepared, except for 300, 400, 500, and 600 mg/kg of which six test systems were prepared. Twelve controls (without added copper) for each food concentration were prepared and treated as the spiked sediments.
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The sediment and medium were homogenized by placing the bottles on a roller bank (30 rpm), after 24 h the suspension was poured into 400-ml glass beakers and the sediment was allowed to settle for 72 h to facilitate partitioning of copper between sediment and water. During the experiment the overlying water was not replaced. For each copper-food treatment two test systems were removed at the start of the experiment for measurement of actual copper concentrations in sediment and water, except for 300, 400, 500, and 600 mg/kg of which one test system was removed.

Bioassays

All experiments were conducted in a 20 ± 1°C climate room with moderate light (~ 10 μmol/m²/s) and a 16:7-h light:dark regime with 30 min of twilight (~ 5 μmol/m²/s) between each period. During the experiments the test systems were constantly aerated. At the beginning and end of each experiment temperature, oxygen, and pH were analyzed using a pH/Oxi 340i meter (WTW, Weilheim, Germany). NH₄⁺ and NO₂⁻ concentrations were determined with Quantofix® (Düren, Germany) test kits.

First instar Chironomus riparius larvae were exposed to sediments with different concentrations of copper and food. Larval survival, length, dry weight, and copper accumulation were determined in a 10-day whole-sediment bioassay. Experiments were started with < 24 h old C. riparius larvae, which were obtained from a culture maintained in our laboratory. Three days prior to the start of the experiment, five newly deposited egg ropes were removed from the culture and transferred into a petri dish with Elendt-M7 medium and held at 20°C. Five larvae were carefully transferred into each test system at the start of the experiment using a blunt Pasteur pipette.

After 10 days, larvae were obtained from the sediment by sieving the sediment using a 200-μm sieve. Surviving larvae were counted and body length was recorded using a Leica® MZ 8 Microscope equipped with a Leica® DC100 Digital Camera (Leica Geosystems Products, Rijswijk, the Netherlands) using the computer program Research Assistant 3 (RVC, Hilversum, the Netherlands). After length measurements larvae (per replicate treatment) were placed at 20°C in clean Elendt-M7 medium under constant aeration for 24 h. After 24 h of gut clearance, dry weight of the larvae was determined by freeze-drying the larvae until constant weight. Pooled larvae per replicate were weighed on a microbalance to the nearest ng. Average length and dry weight per copper-food treatment were determined using the average length or dry weight per replicate treatment.
Copper analysis

Duplicate water samples (1 ml) were taken at the start and at the end of the experiments to measure total copper concentrations in the water. The samples were acidified with 20 μl 70% HNO₃ Ultrex® (J.T. Baker®, Phillipsburg, NJ, USA) and stored at -20°C until analysis. The samples were analyzed by air-acetylene Flame Atomic Absorption Spectrometry (Perkin-Elmer 1100B, Norwalk, CT, USA) or by Furnace Atomic Absorption Spectrometry (Perkin-Elmer 5100PC/HGA600/AS60 equipped with Zeeman background correction, Norwalk, CT, USA). Quality of the copper analysis was ascertained by analyzing blanks and reference material (NIST: SRM 1643, National Institute of Standards and Technology, Gaithersburg, MD, USA).

Before analysis the sediments were freeze dried until constant weight. A sample of ~ 5 mg of sediment was weighed (triplicate) and placed in a 3-ml Teflon digestion vessel and 50 μl 70% HNO₃ Ultrex® (J.T. Baker®) was added. Every 30 samples a blank (no sediment) and a reference (NIST: SRM 2704) were digested for quality control. The vessels were sealed and placed in a lined digestion vessel assembly and digested using a CEM® MD-2000 microwave system (CEM laboratories, Matthews, NC, USA). The vessels were heated to 175°C within 15 min and maintained at 175°C for another 45 min. The samples were diluted with 2 ml deionized water and analyzed by air-acetylene Flame Atomic Absorption Spectrometry (Perkin-Elmer 1100B).

Weighed larvae were placed in a 3-ml Teflon digestion vessel and 25 μl 70% HNO₃ Ultrex® (J.T. Baker®) was added. Every 30 samples a blank (no larvae) and a reference (MA-A-3/TM shrimp homogenate, IAEA, Monaco) were digested for quality control. The vessels were sealed and placed in a lined digestion vessel assembly and digested using a CEM® MD-2000 microwave system. The vessels were heated to 175°C within 15 min and maintained at 175°C for another 30 min. The samples were diluted with 1 ml deionised water and analysed by air-acetylene Flame Atomic Absorption Spectrometry (Perkin-Elmer 1100B). The measured values of reference material were in good agreement with the certified values (< 10% deviation); recovery of the reference materials SRM 2704 and MA-A-3/TM shrimp homogenate were 99.9 ± 5.9% and 105.9 ± 2.1%, respectively. Blanks were below detection limit (~ 10 μg/L).

Sediment analysis

Metals (Cd, Cu, and Zn), Σ13PAHs (phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[e]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylenone, dibenzo[ah]anthracene, and indeno[123]pyrene), and Σ15PCBs (PCB18, PCB20, PCB28, PCB31, PCB44, PCB52, PCB101, PCB105, PCB118, PCB138, PCB149, PCB153,
PCB170, PCB180, and PCB194) were measured by KOELMANS & MOERMOND (2000).

The organic matter content (OM) in the sediment was determined as loss-on-ignition by combusting 1 g dry sediment at 550°C for 6 h (LUCKZAK et al. 1997) in triplicate. Chlorophyll \(a\) (chl \(a\)) and phaeophytin were measured according to LORENZEN (1967) in triplicate using 1 g dry sediment. Three ml of a 90% acetone solution was added and the test tubes were sonificated for 10 min and placed for 18 h at 4°C after which the samples were centrifuged for 15 min at 3000 rpm in closed test tubes to avoid optical disturbance by suspended sediment. Chl \(a\) and phaeophytin contents were summed, because in sediments chl \(a\) is partly degraded into phaeophytin.

**Data analyses**

Comparisons within and among food levels for survival, length, dry weight, and copper accumulation were performed by One-way analysis of variance (ANOVA), followed by Sheffé's post hoc test. No observed effect concentrations (NOECs) for survival, dry weight, and length were determined by statistically significant differences relative to controls obtained from the Scheffé's post hoc test. Additionally, a Two-way ANOVA was performed with the General Linear Model Univariate procedure using a full factorial model with the nominal copper concentrations and food levels as the independent variables, in order to assess the contribution of the variables and their interaction to explain the variation in survival, dry weight, length, and copper accumulation.

Median effect concentrations for survival (LC50), dry weight (EC50_{DRY WEIGHT}), and length (EC50_{LENGTH}) at each food level were calculated by a non-linear curve fit using the logistic response model after HAANSTRA et al. (1985):

\[
Y = \frac{c}{1 + e^{b(X-a)}}
\]

in which: \(Y = \text{effect (\%)}, c = \text{effect in control (\%)}, a = \log \text{EC50 (mg/kg)}, b = \text{slope}, \) and \(X = \log \text{concentration (mg/kg)}.\) The differences in effect concentrations determined at the different food levels were tested for significance by fitting the toxicity data of the treatments simultaneously to logistic models that differed in their slope parameters but had the same effect concentration parameter. A likelihood ratio test was used to test the hypothesis of similarity of effect concentrations by comparing these results to those obtained when each model had its own effect concentration parameter (VAN GESTEL & HENSBERGEN 1997).

Differences were considered significant between the test categories at the 0.05 probability level. All statistical analyses were conducted using the computer program SPSS® 10.0 for Windows (SPSS, Chicago, IL, USA).
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RESULTS

Sediment characteristics
The sediment characteristics are listed in Table 3.1. The concentrations of Cd, Cu, Zn, PAHs, and PCBs in the sediment are low according to sediment quality criteria (CIW 2000).

The organic matter content (OM content) and chl a concentration in the sediment were much higher than measured in our previous study (DE HAAS et al. 2002). The OM content slightly increased with increasing food level (ranging from 6.2 to 6.9%), but these values did not differ significantly from each other. Concentrations of chl a of the food-spiked sediments also did not significantly differ among food levels (53.4 to 56.7 mg/kg). Actual copper concentrations in the sediment at the start of the experiments for each food level are given in Table 3.2.

Table 3.1. Sediment characteristics. Cd, Cu, Zn, sum of polycyclic aromatic hydrocarbons (ΣPAHs), and chl a in mg/kg, sum of polychlorinated biphenyls (ΣPCBs) in μg/kg, and percentage organic matter content (OM).

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>0.17</td>
</tr>
<tr>
<td>Cu</td>
<td>12</td>
</tr>
<tr>
<td>Zn</td>
<td>42</td>
</tr>
<tr>
<td>ΣPAHs</td>
<td>0.55</td>
</tr>
<tr>
<td>ΣPCBs</td>
<td>4.37</td>
</tr>
<tr>
<td>OM</td>
<td>6.2</td>
</tr>
<tr>
<td>chl a</td>
<td>53.4</td>
</tr>
</tbody>
</table>

A From KOELMANS & MOERMOND (2000).

Water quality
Total aqueous copper concentrations increased with increasing sediment copper concentrations (not shown). Total water concentrations ranged from < 10 to 110 μg/L, and were less than 0.25% than the copper initially added to the test systems. Because these aqueous copper concentrations were < 10-day LC50 values (1502 μg/L) obtained for Chironomus tentans in water-only exposures (SUEDEL et al. 1996), sediment-bound copper can account for the observed adverse effects.

Dissolved oxygen ranged between 7.51 and 9.04 mg/L; pH ranged from 7.72 to 8.33; ammonium concentrations were < 10 mg/L; and nitrite concentrations were < 1 mg/L. Above mentioned water quality parameters were all within the boundary conditions set for whole-sediment bioassays with C. riparius (MAAS et al. 1993).
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Survival
Mean survival in all controls was above 98% (Figure. 3.1\textsubscript{A}). More than 80% of the larvae survived nominal copper concentrations up to 200 mg/kg (Figure. 3.1\textsubscript{B-F}). Above that concentration survival of the larvae decreased with increasing sediment copper concentration (Figure. 3.1\textsubscript{G,H}). Significant differences ($P < 0.05$) from control treatments (no copper added) were found at nominal copper concentrations of $\geq 300$ mg/kg at all food levels, hence the NOECs were the same for all food levels (Table 3.3). Within each copper concentration an increasing food level did not result in higher survival. However, the LC50 values increased slightly with increasing food level (Table 3.4) and LC50 values calculated for the 0 and 0.5 mg/larvae/d food level were significantly different ($P < 0.05$). However, the Two-way ANOVA showed that only sediment copper concentration could account for the major part of the variance observed between the different treatments ($P < 0.001$). The food level was not significant in explaining the observed variation. Also the interaction term of copper and food was not significant, this indicates that the effect of copper on survival was independent on the food level.

Table 3.2. Mean sediment copper concentrations (mg/kg) at the start of the experiment at different food levels. Standard deviations (not given) were within 10%; $n$ = number of analyses.

<table>
<thead>
<tr>
<th>nominal [Cu] (mg/kg)</th>
<th>food level (mg/larvae/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>control\textsuperscript{A}</td>
<td>25.0</td>
</tr>
<tr>
<td>25\textsuperscript{A}</td>
<td>31.9</td>
</tr>
<tr>
<td>50\textsuperscript{A}</td>
<td>62.6</td>
</tr>
<tr>
<td>100\textsuperscript{A}</td>
<td>133.4</td>
</tr>
<tr>
<td>150\textsuperscript{A}</td>
<td>156.4</td>
</tr>
<tr>
<td>200\textsuperscript{A}</td>
<td>223.2</td>
</tr>
<tr>
<td>300\textsuperscript{B}</td>
<td>375.1</td>
</tr>
<tr>
<td>400\textsuperscript{B}</td>
<td>448.3</td>
</tr>
<tr>
<td>500\textsuperscript{B}</td>
<td>531.6</td>
</tr>
<tr>
<td>600\textsuperscript{B}</td>
<td>-</td>
</tr>
</tbody>
</table>

\textsuperscript{A} $n = 6$, \textsuperscript{B} $n = 3$

Dry weight
In the control treatments dry weight of the midge larvae increased with increasing food level. Larval dry weight in the control without additional food was significantly ($P < 0.05$) lower than the controls with the two highest feeding levels, but not significantly different from the lowest feeding level ($0.05$
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Figure 3.1. Survival of Chironomus riparius larvae after 10 days of exposure to copper-spiked sediments at different food levels. A = control, B = 25 mg/kg, C = 50 mg/kg, D = 100 mg/kg, E = 150 mg/kg, F = 200 mg/kg, G = 300 mg/kg, H = 400 mg/kg. Bars = standard errors. No significant differences are detected among feeding levels.
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mg/larvae/d). No significant differences were detected among the three feeding controls (Figure 3.2A). Dry weight of copper exposed larvae increased with increasing food level \((P < 0.05)\) until copper concentrations of 200 mg/kg (Figure 3.2B-F). At higher copper concentrations no significant differences between food levels were observed (Figure 3.2G, H). With increasing copper concentration a decrease in dry weight of \textit{C. riparius} larvae was observed. Significant differences \((P < 0.05)\) from control treatments (no copper added) were found at nominal copper concentrations of \(\geq 150\) mg/kg at the food levels 0, 0.05, and 0.25 mg/larvae/d. At the food level of 0.5 mg/larvae/d significant differences with the control were found at the nominal copper concentrations of \(\geq 200\) mg/kg. Hence, at the highest food level a higher NOEC was observed (Table 3.3). No differences were observed between the calculated EC\(_{50}\) values for dry weight at the different food levels (Table 3.4). A Two-way ANOVA showed that dry weight was significantly affected by both sediment copper concentration and food level \((P < 0.001)\). The statistical interaction term between copper and food was not significant, which indicates that the effect of copper on larval dry weight was independent on the food level.

Table 3.3. No observed effect concentrations for survival (NOEC\textsubscript{SURVIVAL}), dry weight (NOEC\textsubscript{DRYWEIGHT}), and length (NOEC\textsubscript{LENGTH}) of \textit{Chironomus riparius} larvae after 10 days of exposure to copper-spiked sediments at different food levels.

<table>
<thead>
<tr>
<th>food level (mg/larvae/d)</th>
<th>NOEC\textsubscript{SURVIVAL} (mg/kg)</th>
<th>NOEC\textsubscript{DRYWEIGHT} (mg/kg)</th>
<th>NOEC\textsubscript{LENGTH} (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>223.2</td>
<td>133.4</td>
<td>62.6</td>
</tr>
<tr>
<td>0.05</td>
<td>227.7</td>
<td>119.9</td>
<td>55.0</td>
</tr>
<tr>
<td>0.25</td>
<td>211.1</td>
<td>109.9</td>
<td>60.1</td>
</tr>
<tr>
<td>0.50</td>
<td>231.8</td>
<td>151.4</td>
<td>132.0</td>
</tr>
</tbody>
</table>

Length

In the control without additional food, length of the midge larvae was significantly lower \((P < 0.001)\) than in the feeding controls. The length of the larvae did not differ significantly among the three feeding controls (Figure 3.3A). Length of the copper exposed larvae increased with increasing food level \((P < 0.01)\) until nominal copper concentrations of 200 mg/kg (Figure 3.3 B-F). At higher copper concentrations no significant differences between food levels were observed (Figure 3.3G, H). Significant differences \((P < 0.05)\) from control treatments (no copper added) were found at nominal copper concentrations of \(\geq 100\) mg/kg at the food levels 0, 0.05, and 0.25 mg/larvae/d. At the food level of 0.5 mg/larvae/d, significant differences with the control were found at the nominal copper concentrations of \(\geq 150\) mg/kg. Hence, at the highest food level
Figure 3.2. Dry weight of *Chironomus riparius* larvae after 10 days of exposure to copper-spiked sediments at different food levels. A = control, B = 25 mg/kg, C = 50 mg/kg, D = 100 mg/kg, E = 150 mg/kg, F = 200 mg/kg, G = 300 mg/kg, H = 400 mg/kg. Bars = standard errors; food levels sharing the same letter are not significantly different.
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A higher NOEC was observed (Table 3.3). Calculated EC50 values increased with increasing food level (Table 3.3) and the EC50 values of the 0 and 0.05 mg/larvae/d food level were significantly lower than the EC50 value of the 0.5 mg/larvae/d food level \( (P < 0.05) \). A Two-way ANOVA showed that copper and food, as well as their statistical interaction term were significant in explaining the observed variation of length \( (P < 0.001) \). This interaction indicates that the length of the larvae was dependent on both copper concentration and the food level.

**Copper accumulation**

Total copper concentrations in control larvae ranged from 131.7 to 155.2 µg/g dw. With increasing sediment copper concentrations, an increased accumulation of copper in the midge larvae was observed (Figure 3.4). Significant differences \( (P < 0.05) \) in copper accumulation compared to controls (no copper added) were found at nominal copper concentrations of ≥ 25mg/kg at the food level 0 mg/larvae/d, at nominal copper concentrations of ≥ 50 mg/kg at the food levels 0.05 and 0.25 mg mg/larvae/d, and at nominal copper concentrations of ≥ 100 mg/kg at the food level 0.5 mg mg/larvae/d.

A Two-way ANOVA demonstrated that both copper and food could account for the observed variation of copper accumulation \( (P < 0.001) \) and also the statistical interaction term between copper and food was significant \( (P < 0.001) \). This interaction indicates that copper accumulation was dependent on both copper concentration and food level.

**Table 3.4.** Median effect concentrations for survival (LC50), dry weight (EC50\_DRYWEIGHT), and length (EC50\_LENGTH) of *Chironomus riparius* larvae after 10 days of exposure to copper-spiked sediments at different food levels; 95% confidence limits are given in parentheses; effect concentrations sharing the same letter are not significantly different \( (P < 0.05) \).

<table>
<thead>
<tr>
<th>Food level (mg/larvae/d)</th>
<th>LC50 (mg/kg)</th>
<th>EC50_DRYWEIGHT (mg/kg)</th>
<th>EC50_LENGTH (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>426.1 (401-453) (^A)</td>
<td>186.8 (168-208) (^A)</td>
<td>304.5 (267-347) (^A)</td>
</tr>
<tr>
<td>0.05</td>
<td>432.5 (400-468) (^AB)</td>
<td>180.4 (163-199) (^A)</td>
<td>308.5 (269-353) (^A)</td>
</tr>
<tr>
<td>0.25</td>
<td>443.7 (395-498) (^AB)</td>
<td>174.6 (151-202) (^A)</td>
<td>354.2 (293-429) (^AB)</td>
</tr>
<tr>
<td>0.50</td>
<td>487.3 (442-538) (^B)</td>
<td>210.3 (189-234) (^A)</td>
<td>412.4 (346-492) (^B)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Both dry weight and length of *Chironomus riparius* larvae increased with increasing food level as has been observed for chironomids in many other studies (ANKLEY et al. 1993, SIBLEY et al. 1997, RISTOLA et al. 1999). Larval
Figure 3.3. Length of *Chironomus riparius* larvae after 10 days of exposure to copper-spiked sediments at different food levels. A = control, B = 25 mg/kg, C = 50 mg/kg, D = 100 mg/kg, E = 150 mg/kg, F = 200 mg/kg, G = 300 mg/kg, H = 400 mg/kg. Bars = standard errors; food levels sharing the same letter are not significantly different.
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Survival was not affected by increasing food level. Other studies also observed that survival was not or only slightly affected to the food concentration added to the test systems (Ankley et al. 1993, Sibley et al. 1997, Ristola et al. 1999). If food is deficient, midge larvae are able to decrease their development rate and as long as the available food is sufficient to maintain their basic metabolism they are able to remain alive in the sediment (Armitage et al. 1995).

Previous research (De Haas et al. 2002) showed that growth of C. riparius larvae was more influenced by food quantity and quality in the sediment than by sediment-bound toxicants. This suggested a possible compensating effect of the available food for the negative effect of sediment-bound toxicants. The results from this study indeed showed that food could compensate for the negative effects of copper. However, this compensating effect disappears if the copper concentration in the sediment reaches a certain threshold level. Three stages of interaction between copper and food have been observed in this study: (1) at low copper concentrations (≤ 50 mg/kg) no toxic effects of copper were observed and an increase in the amount of food resulted in an increase of larval dry weight and length; (2) at intermediate copper concentrations (100 to 200 mg/kg) copper had a toxic effect on larval dry weight and length but an increase in the amount of food, however, resulted in an increase in both dry weight and length; and (3) at high copper concentrations (≥ 300 mg/kg) copper had a toxic effect on larval survival, dry weight, and length and this negative effect could not be counteracted by an increase of the amount of food.

A high increase in accumulated copper was observed between the controls (no added copper) and the lowest spiked copper concentration (25 mg/kg) at all food levels. Comparable results were obtained for the marine amphipod Paracorophium excavatum (Marsden & Wong 2001). This rapid increase in accumulated copper between the control and the lowest spiked copper concentration can probably be explained by differences in bioavailability of contaminants in aged sediment and freshly spiked sediment (Landrum et al. 1992).

The increase in the amount of food resulted in a decrease of accumulated copper in the larvae. In agreement, bioaccumulation of copper in the oligochaete Lumbriculus variegatus was found to be much higher in sediments with low organic carbon content (Chapman et al. 1999) compared to sediments with higher organic carbon content (Ankley et al. 1994b) in sediments spiked with comparable actual copper concentrations. The decrease in accumulated copper with increasing food level can be caused by an acceleration of the metabolism that allows a higher depuration rate, so the toxicant can be eliminated easier and the negative effect on growth is lower (Ristola 1995). On the other hand,
addition of a high nutritive food to the sediment may result in a reduced ingestion rate by the midge larvae, thus leading to lower bioaccumulation of copper in the larvae (HARKEY et al. 1994b).

Our study demonstrated that intermediate levels of food (0.05 and 0.25 mg/larvae/d) did not affect the outcome of the experiments since both the EC50/LC50 values and the NOEC values were comparable to the unfed treatment. However, the high feeding level (0.5 mg/larvae/d), as recommended in the OECD guideline 218 (OECD 2001), resulted in an increase in the EC50/LC50 and/or the NOEC values. Hence, at this artificial food level the toxic effects are, at least, partly compensated leading to an underestimation of sediment toxicity. Thus, the advantages of increased food availability prevail against the potential adverse effects of copper. Therefore, the amount and quality of available food (either natural or added to the test system) affect the response of the test organism to sediment-bound toxicants. If feeding should be necessary, the amount of added food should be kept at a minimum. It must also be considered that some toxicants may accumulate in food and sediment particles that the larvae feeds on which may influence larval exposure to these toxicants (HARKEY et al. 1994b).

The use of C. riparius in whole-sediment toxicity tests is questionable based on our findings. Our results demonstrate both overestimation of sediment toxicity due to starvation as well as underestimation of sediment toxicity due to masking of toxic effects at high food levels.
Persistence of benthic invertebrates in polluted sediments

Because benthic communities in polluted ecosystems are often exposed to varying levels of both food and toxicants, it is concluded that the trophic state of the ecosystem may alter the ecological risk of sediment-bound toxicants to opportunistic benthic invertebrates such as *C. riparius*.

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