Persistence of benthic invertebrates in polluted sediments.
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Benthic communities in flood plain lake ecosystems are often exposed to varying levels of both food and toxicants. Inhibition through toxicants of sensitive species and stimulation through higher amounts of food of opportunistic species have been observed in separate studies. The aim of this study was, therefore, to assess the responses of benthic invertebrates to combined food and contamination input in floodplain lake sediments. Seven flood plain lakes located along the River Waal, a branch of the River Rhine, the Netherlands, with different trophic status (phytoplankton [low food] or macrophyte dominated [high food]) and toxicants were selected. The responses of the sensitive mayfly *Ephoron virgo* and the opportunistic midge *Chironomus riparius* to these sediments were assessed in 10-day growth bioassays with both species, and a 28-day emergence experiment with *C. riparius*. Lower survival and growth rates of *E. virgo* were observed at higher contaminant levels, independent on the amount of food in the sediments. In contrast, *C. riparius* responded to the food quantity and quality in the sediments, in spite of the toxicants present. Therefore, we conclude that the midge *C. riparius* is not a suitable test organism for the assessment of sediment toxicity. Alternatively, it proved to be an appropriate test organism to determine the nutritional value of sediments. The mayfly *E. virgo* turned out to be a much more appropriate test organism for sediment toxicity bioassays, because it responds to the toxicant levels in the sediments, rather than to the nutritional value. Our results demonstrate that the trophic state of an ecosystem (macrophyte or plankton dominated) influences the ecological risk of toxicants to benthic invertebrates in a species-specific way. It is concluded that not the toxicant load, but the combination of food and contaminants determines the persistence of benthic invertebrates and therewith the benthic invertebrate composition in complexly polluted ecosystems.
INTRODUCTION

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). Analysing the consequences of varying food and toxicant availability to the benthos therefore requires an inventory of the positive and negative terms in the regulation of benthic processes. Inhibition through toxicants of sensitive species (SIBLEY et al. 1997) and stimulation through higher amounts of food of opportunistic species (DUBÉ & CULP 1996, STUIJFZAND et al. 2000) have been observed in separate studies. Opportunistic species, however, are not necessarily more tolerant to toxicants, but may exploit at higher rate the higher food levels (DUBÉ & CULP 1996, STUIJFZAND et al. 2000). The aim of this study is, therefore, to disentangle the mechanisms that determine the response of benthic invertebrates to combined toxicant and food input.

Because of the opposite responses of sensitive and opportunistic species, a representative of both groups has been selected: the sensitive mayfly *Ephoron virgo* and the opportunistic midge *Chironomus riparius*. The mayfly *E. virgo*, a benthic species present in the River Rhine, proved to be a sensitive test organism in acute and chronic toxicity tests (VAN DER GEEST et al. 2000a, 2000b, 2001). The early instar nymphs of *E. virgo* live freely on the sediment, feeding on fine particulate organic matter. In later stages they burrow U-shaped tubes in the sediment and filter food, such as detritus and algae, from the water by generating wavelike movements with their feathered tracheal gills (KURECK & FONTES 1996). Chironomid larvae, such as *C. riparius*, are widely used test organisms in acute and chronic (sediment) toxicity tests (HARKEY et al. 1994b, KEMBLE et al. 1994, RISTOLA et al. 1996, 1999). The larvae of *C. riparius* are opportunistic tube-dwelling detritivores, which prefer eutrophic and organic enriched waters (ARMITAGE et al. 1995). They feed mainly on detritus and organic matter present in the sediment.

During the past few decades, water quality of the large western European rivers has improved (ADMIRAAL et al. 1993), but the floodplain lake sediments of these rivers still contain high concentrations of xenobiotic compounds (KOELMANS & MOERMOND 2000). Therefore, the floodplain lake sediments may nowadays act not only as a sink, but also as a source of a wide range of chemical substances such as nutrients, metals, polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs), which were deposited in
high concentrations in the 1960s and 1970s (Beurskens et al. 1993). The benthic communities of these floodplain lakes are thus exposed to a diffuse flux of sediment-bound toxicants, nutrients, and organic matter. This makes these lakes an ideal area to study the combined effects of contaminants and available food. Furthermore, floodplain lakes tend to be either dominated by phytoplankton or by macrophytes, which affects the supply of food to the sediment.

The objective of this study was to compare the responses of the mayfly E. virgo and the midge C. riparius to floodplain lake sediments with varying food and contamination levels. To this purpose whole-sediment bioassays with both species were conducted, being an appropriate tool to assess the effects of contaminated sediments on benthic invertebrates (Giesy et al. 1990, Ankley et al. 1994a, Kemble et al. 1994, Liber et al. 1996, Ristola et al. 1996, 1999, Huuskonen et al. 1998). In such bioassays, adverse effects on the test organisms are usually attributed to the presence of contaminants in the sediments. However, if effects are observed in whole-sediment bioassays, this will be the joint effect of all sediment characteristics (e.g. contaminants, physical-chemical parameters, and food quantity and quality parameters). Food availability is a major factor in the development of benthic invertebrates (Suedel & Rodgers 1994, Vos 2001). Thus, if food is deficient, the test organisms may suffer from starvation, which may lead to a false interpretation of sediment toxicity. On the other hand, feeding of the test organisms during exposure to sediments may mask the toxic effects, also resulting in a false interpretation (Ankley et al. 1994a, Day et al. 1994, Harkey et al. 1994a, Lacee et al. 1999). We therefore exposed both test species to sediments in the presence and absence of an additional food source, in order to determine the effects of sediment-bound chemicals and exclude mortality and reduction in growth due to food deficiency.

For this study, seven floodplain lakes located along the River Waal, a branch from the River Rhine, with different levels of contamination and trophic states were selected (Koelmans & Moermood 2000). The species-specific preferences for these sediments were assessed using 10-day whole-sediment bioassays with both species and a 28-day emergence experiment with C. riparius. Survival, growth, and emergence were related to contaminant levels (metals, PAHs, PCBs) and food quantity and quality parameters (organic matter content [OM], chlorophyll a [chl a], labile fraction of the OM [CO₂ production], fatty acids [FAs], and polyunsaturated fatty acids [PUFAs]). This enabled us to specify eco(toxico)logical profiles of these benthic invertebrates and to gain insight in key factors structuring benthic macro fauna communities in the field.
MATERIALS AND METHODS

Sample collection, storage, and treatment
From seven floodplain lakes located along the River Waal, the Netherlands (Figure 1.2), sediment and water were collected. Sediments for chemical analyses were collected in September 2000. Sediments were sampled at three sites in each lake using a core sampler. The upper 5 cm of the core sample was isolated and frozen at -20°C within 6 h after sampling in either glass jars for organic contaminant analyses or polyethylene bottles for metal analyses.

Sediments for bioassays and food quantity and quality analyses were collected in December 2000, using an Ekman-Birdge grab, which was adjusted to sample the upper 5 cm of the sediment. The sediments were transported to the laboratory, where large debris was picked out by hand. Next the sediment was homogenized and stored at -20°C in 500-ml polyethylene bottles within 6 h after sampling in order to eliminate autochthonous organisms. 25 L lake water was collected in jerry cans. The water was filtered twice over a 30-μm filter in order to remove the zooplankton and was stored at 4°C in the dark under constant aeration.

Sediment analyses

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. in triplicate. 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 already partly degraded into phaeophytin.

Lipids were extracted from 1 g wet sediment with 6 ml methanol containing 2.5% H2SO4 for 90 min at 80°C. 400 μl hexane and 1 ml 0.9 % NaCl were added to the samples and placed for 1 min on a shaker and centrifuged at 12,000 rpm for 1 min. 200 μl of the supernatant was transferred to a 200-μl vial. Fatty
acid methyl esters (FAMEs) were measured using a gas chromatographer (GC) 8000 top (CE Instruments, Milan, Italy) by injecting a 2-μl aliquot in a polar 30-m DB WAX column (0.25 mm I.D.; 0.5-μm film). Peaks were identified using a 37 component FAME Mix (Supelco, Zwijndrecht, the Netherlands).

The labile fraction of the sediment organic matter was measured using microbial mineralization as a value for CO₂ production according to Vos (2001). A bacterial inoculum was prepared from the surface layer of a sediment containing a decaying bacterial mat. Bacteria were detached by ultrasonification, and large particles were removed by centrifuging for 5 min at 5000 rpm. Four ml of homogenized wet sediment (duplicate) were suspended in 11 ml of a 55-mM phosphate buffer in a 60-ml serum bottle, and 1 ml of the inoculum was added. After 30 min of aeration, pH was measured, and the bottle was capped airtight. The bottles were placed on a rotary shaker at 20°C in the dark, and after 1 h the CO₂ in the headspace was measured using a Carlo Erba® GC (Milan, Italy). After 24 and 48 h of incubation, the CO₂ concentration and the pH were again measured.

**Bioassays**

All experiments were conducted in 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. Test systems were not aerated during the experiments. Every 5 days temperature, oxygen, and pH were analyzed using a pH/Oxi 340i oxygen meter (WTW, Weilheim, Germany). NH₄⁺ and NO₃⁻ concentrations were determined with Quantofix® (Düren, Germany) test kits. During the experiments, water quality conditions were satisfactory in all test systems.

**Ephoron virgo**

Survival and growth of the mayfly *Ephoron virgo* on floodplain lake sediments were determined in a 10-day experiment. First instar nymphs (< 48 h) were obtained from field-collected eggs, kept in artificial diapause at 4°C in our laboratory. Seven days prior to the start of the experiments, several glass slides containing *E. virgo* eggs were placed in petri dishes containing Elendt-M7 medium (OECD 2001) and transferred to 20°C in order to terminate the artificial diapause (GREVE et al. 1999). Four days prior to the experiment sediments were thawed at 4°C.

One day before the start of the experiment, three replicate glass jars (150-ml) with 25 ml wet homogenized sediment and 100 ml filtered site water were prepared and aerated overnight. Control treatments contained 25 ml quartz sand (Sibelco® M32, Antwerp, Belgium) with a 100- to 400-μm grain size and 100 ml
Elendt-M7 medium. Twenty nymphs were randomly transferred into each test vessel using a blunt Pasteur pipette. In addition, the body length of twenty nymphs was measured using an automatic image analyzer [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).

The control treatments were fed $1.0 \times 10^7$ μm$^3$ of small diatoms (Navicula atomus:Nitzschea perminuta, 1:1) per cm$^2$ and two drops of an Urtica suspension (0.75 g Urtica in 25 ml Elendt-M7 medium) at day 0. At days 3, 5, and 7 the controls were fed $0.5 \times 10^7$ μm$^3$ of diatoms per cm$^2$ and one drop of Urtica suspension. At day 7, the controls were additionally fed $3.0 \times 10^7$ μm$^3$ Chlamydomonas monoica per ml. The experiment was repeated with addition of food, according to the control-feeding regime.

At the end of the test, nymphs were collected from the sediment using a floating technique according to BOIVIN et al. (2001). Surviving nymphs were counted and body length was measured with an automatic image analyzer. Growth was calculated by subtracting the average initial length from the individual final length.

**Chironomus riparius**

Two bioassays were performed: larval survival and growth was determined in a 10-day bioassay, larval development and adult emergence in a 28-day emergence experiment. Both tests were started with first instar Chironomus riparius larvae (< 24 h). Four days before the start of the experiments sediments were thawed at 4°C. Three days prior to the test, three newly deposited egg ropes were removed from the culture maintained in our laboratory and transferred into a petri dish with Elendt-M7 medium and placed at 20°C.

For the 10-day bioassay, five replicate 400-ml glass beakers with 75 ml homogenized wet sediment and 300 ml filtered site water were prepared one day before the start of the experiment and aerated overnight. Control treatments contained 75 ml quartz sand (Sibelco M32) with a 100- to 400-μm grain size and 300 ml Elendt-M7 medium. Five larvae were carefully transferred into each test vessel with a blunt Pasteur pipette. Twenty larvae were additionally measured for body length using an automatic image analyzer. The control experiments were fed 1 ml of a Trouvit® (Trouw, Fontaine-les-Vervins, France) - Tetraphyll® (Tetrawerke, Melle, Germany) (20:1, wt/wt) suspension (250 mg Trouvit®-Tetraphyll® in 100 ml of Elendt-M7 medium) daily. After 10 d, the larvae were collected from the sediment using a 250-μm sieve. Surviving larvae were counted and body length was measured. Growth was calculated by subtracting the average initial length from the individual final length.
Chapter 2 - Combined effects of food and toxicants

The first 10 days, the emergence experiment was carried out exactly as the 10-day bioassay. At day 10, a net trap was placed on the test beakers and emerged adults were removed, counted, and sexed daily. The control experiments were fed 1 ml of a Trouvit®-Tetraphyll® (20:1, wt/wt) suspension (250 mg Trouvit®-Tetraphyll® in 100 ml of Elendt-M7 medium) daily. After 28 days, the experiment was terminated and surviving larvae and pupae were removed from the sediment by sieving over a 250-μm sieve, counted, and measured for body length. Growth of non-emerged larvae was calculated by subtracting the average initial length from the individual final length. Both experiments were repeated with addition of food to all sediments, according to the feeding regime in the controls.

Data analyses

One-way analysis of variance (ANOVA) tests followed by a Scheffé’s post hoc test were conducted to test for significant differences between treatments and controls for survival, growth, or emergence. If data were not homogenous or normally distributed, a Kruskall-Wallis was performed following a Student’s t test when permitted. Significant differences between the absence and presence of food were analyzed with a Student’s t test.

Emergence is given as the time (days) at which 50% of the adults had emerged (EmT50) and was calculated using a logistic response model adopted from HAANSTRA et al. (1985):

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

in which: \( Y \) = non-emerged midges (%), \( c \) = emergence in control (%), \( a = \log \) EmT50 (days), \( b = \) slope, and \( X = \log \) time (days).

Correlations between survival, growth, time of emergence, and sediment characteristics were determined with Pearson’s correlation, using the average survival, growth, and EmT50 and the mean of the repeated analyses for the different sediment characteristics.

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).

RESULTS

Sediment characteristics

The sediment characteristics of the seven floodplain lake sediments are listed in Table 2.1, in which the sediments are ranked from relatively clean to contaminated. Concentrations of metals, PAHs, and PCBs were lowest in D2.
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Metal concentrations were highest in 3A, 3B, and D4, PAH concentrations were highest in D4 (10.5 mg/kg) and PCB concentrations were highest in 3B (133 μg/kg). The concentrations of Cd, Zn, PAHs, and PCBs all correlated positively with each other \( (P < 0.05) \), and Cu was positively correlated with Cd, Zn, and PCBs \( (P < 0.05) \). Hence, a clear gradient in contaminant concentrations was observed, allowing a ranking of the sediments from relatively clean (D2) to contaminated (D4).

Table 2.1. Sediment characteristics: Cd, Cu, Zn, sum of polycyclic aromatic hydrocarbons (ΣPAHs), total phosphorus (P), chl a, sum of fatty acids (ΣFAs), and sum of polyunsaturated fatty acids (ΣPUFAs) in mg/kg, sum of polychlorinated biphenyls (ΣPCBs) in μg/kg, percentage organic matter content (OM), and CO\(_2\) production (CO\(_2\)) in μmol CO\(_2\)/kg/d. Sediments are ranked from relatively clean to contaminated.

<table>
<thead>
<tr>
<th></th>
<th>D2</th>
<th>G1</th>
<th>G3</th>
<th>O2</th>
<th>3A</th>
<th>3B</th>
<th>D4</th>
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<tr>
<td>Cd</td>
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<td>Zn(^{A})</td>
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<td>386</td>
<td>199</td>
<td>322</td>
<td>507</td>
<td>544</td>
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<td>ΣPAHs(^{A})</td>
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<td>2.97</td>
<td>5.76</td>
<td>5.87</td>
<td>10.5</td>
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<tr>
<td>ΣPCBs(^{A})</td>
<td>4.4</td>
<td>7.2</td>
<td>20.2</td>
<td>33.4</td>
<td>74.3</td>
<td>133</td>
<td>109</td>
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<tr>
<td>OM(^{A})</td>
<td>2.7</td>
<td>3.9</td>
<td>9.7</td>
<td>11.0</td>
<td>8.3</td>
<td>9.0</td>
<td>9.5</td>
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<tr>
<td>P(^A)</td>
<td>434</td>
<td>1226</td>
<td>1137</td>
<td>1312</td>
<td>1458</td>
<td>1703</td>
<td>1198</td>
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<td>chl a</td>
<td>19.3</td>
<td>16.4</td>
<td>76.5</td>
<td>74.1</td>
<td>30.1</td>
<td>43.3</td>
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<td>ΣFAs</td>
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<td>11.7</td>
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<td>CO(_2)</td>
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<td>30.8</td>
<td>19.5</td>
<td>20.2</td>
<td>24.3</td>
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<td>vegetation</td>
<td>A(^B)</td>
<td>A</td>
<td>M(^C)</td>
<td>M</td>
<td>A</td>
<td>A</td>
<td>M</td>
</tr>
</tbody>
</table>

\(^{A}\) From KOELMANS \& MOERMOND (2000).

\(^{B}\) A = phytoplankton dominated lake,

\(^{C}\) M = macrophyte dominated lake.

Sediment organic matter content was lowest in D2 (2.7%) and G1 (3.9%), while the organic matter content in the other sediments ranged from 8.3 to 11.0%. The lowest total P concentration was also measured in D2 (434 mg/kg) and varied from 1137 to 1703 mg/kg in the other sediments. Macrophytes were present in the lakes D4, G3, and O2. Concentrations of chl a, FAs, PUFAs, and the labile fraction of the organic matter (CO\(_2\) production) in these macrophyte dominated lakes were higher than in phytoplankton dominated lakes, with the lowest concentration of chl a (16.4 mg/kg) in G1 and the highest (76.5 mg/kg) in G3. The labile fraction of the organic matter ranged from 9.10 μmol CO\(_2\)/kg/d in G1 to 30.8 μmol CO\(_2\)/kg/d in O2. The lowest FA and PUFA concentrations were
found in G1 (87.3 and 6.21 mg/kg, respectively), and the highest concentrations were found in O2 (358 and 36.4 mg/kg, respectively).

The CO₂ production was positively correlated to OM, chl a, FAs, PUFAs, and macrophyte abundance. Chl a correlated positively with OM, FAs, PUFAs, and the presence of macrophytes. Organic matter content was positively correlated to FAs and PUFAs. Macrophyte abundance was positively correlated to chl a, CO₂ production, FAs, and PUFAs ($P < 0.05$). Therefore, the sediments can be grouped in either sediments with a low food quantity and quality (phytoplankton dominated lakes) or sediments with a high food quantity and quality (macrophyte dominated lakes).

No correlations were found between contaminant levels and food quantity and quality parameters, expressing the contaminant levels based on OM did not change these results. However, the relatively clean sediment (D2) is low in food quantity and quality and the most heavily polluted sediment (D4) is high in food quantity and quality.

**Survival and growth of *Ephoron virgo***

Average control survival was 86.3% and the average growth of control nymphs was 220 μm. At higher contamination levels, both survival and growth rates of the nymphs was lower (Figure 2.1A-B). In the most polluted sediments (3A, 3B, and D4), significant ($P < 0.01$) lower survival and growth was observed compared to the control. In the moderate polluted sediments (G3 and O2) only survival was significantly lower ($P < 0.05$) control survival. Growth in D2 sediment was also significantly lower ($P < 0.01$) than control growth. Survival was negatively correlated with Cd, Cu, Zn, PAHs, and PCBs (Table 2.2) and growth was negatively correlated with PAHs and PCBs.

Additional feeding did not alter survival or growth of the *E. virgo* nymphs, except for growth in D2, which was significantly higher in the presence of food ($P < 0.01$). Again, in the presence of food, both survival and growth were negatively correlated with Cd, Cu, Zn, PAHs, and PCBs. (Table 2.2). No correlations were found with any of the food quality parameters.

**Survival and growth of *Chironomus riparius***

Average control survival was 99% and the average growth of control larvae was 7.8 mm. Survival of *C. riparius* was above 90% in the three macrophyte dominated lakes (Figure 2.1C). In contrast, significant lower survival ($P < 0.05$) compared to the control was observed in three of the four phytoplankton dominated lakes (G1, 3A, and 3B). In all sediments, growth was significantly lower than control growth($P < 0.05$) (Figure 2.1D), except for G3, the least polluted macrophyte dominated lake. Larval growth in the sediments positively
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correlated with chl $a$, PUFAs, CO$_2$ production, and the presence of macrophytes.

Addition of food diminished mortality in G1, 3A, and 3B. As a result, significant lower survival in comparison to the control was only found for 3B and, unexpectedly, O2 ($P < 0.05$). In the presence of food, growth was higher in all sediments except for G3. Growth negatively correlated with chl $a$, FAs, PUFAs, CO$_2$ production, and the abundance of macrophytes. No correlations were found with any of the contaminant concentrations.

**Figure 2.1.** Survival (%) and growth ($\mu$m or mm) of *Ephoron virgo* nymphs and *Chironomus riparius* larvae after 10 days on sediments in the absence (white bars) and presence (black bars) of additional food. A = survival of *E. virgo*; B = growth of *E. virgo*; C = survival of *C. riparius*; D = growth of *C. riparius*. Error bars = standard error; * indicates significant difference from control ($P < 0.05$); # indicates significant difference between the absence and presence of additional food ($P < 0.05$).

**Development of Chironomus riparius**
Survival exceeded 80% in the control treatments. Average EmT50 in the control was 20.3 days. In the experiments without additional food, adult emergence was only observed in the sediments from macrophyte dominated lakes (Figure 2.2). In the phytoplankton dominated lake sediments, little (4 and 8% in D2 and 3A, respectively) to no emergence occurred, therefore no reliable EmT50 values for these lakes could be calculated. In the macrophyte dominated lakes, EmT50 values were significantly higher ($P < 0.05$) than control EmT50 values.
Emergence was positively correlated with chl $\alpha$, FAs, PUFA$s$, CO$_2$ production, and macrophyte abundance ($P < 0.05$). Growth of non-emerged larvae (not shown) was positively correlated with growth after 10 days on sediments without additional food and with chl $\alpha$, FAs, and PUFA$s$ ($P < 0.05$).

Addition of food resulted in faster development in all sediments (Figure 2.2); time of 50% emergence ranged from 17.5 days in D2 to 21.5 days in O2, and all midges emerged within 28 days. The EmT50 value in D2 was significantly lower ($P < 0.05$) than control EmT50, while the EmT50 values in all other sediments were comparable to the control EmT50. No correlations were found between the EmT50 in the presence of additional food and any of the sediment characteristics.

Figure 2.2. Median emergence time (EmT50 [days]) of Chironomus riparius in sediments in the absence ($\bigcirc$) and presence ($\bullet$) of additional food. Error bars = 95% confidence limits; no emergence = EmT50 could not be calculated because of low or zero emergence.

DISCUSSION

The present study demonstrated that the sensitive mayfly Ephoron virgo and the opportunistic midge Chironomus riparius responded completely opposite to the varying food and toxicant levels. E. virgo was far more sensitive to the toxicants present in the sediments than C. riparius. An increase in contaminant loading in the sediments resulted in a decrease of both survival and growth of E. virgo. Addition of food did not enhance survival and growth of E. virgo in any of the sediments, except for growth in D2 sediment. These results indicate that reduction in survival and growth observed in the sediments without additional food is caused by the toxicants present in the sediment rather than to food deficiency. The reduction in growth on D2 sediment in the absence of additional food, however, can be explained by deficiency of food due to the low OM of this sediment since growth of the nymphs in the presence of additional food was
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significantly higher. As reported in several studies, mayflies are sensitive to many classes of contaminants (GIESY et al. 1990, DIAMOND et al. 1992, LOWELL et al. 1995, SIBLEY et al. 1997). In addition, the species used in the present study, \textit{E. virgo}, is very sensitive to several contaminants in water-only bioassays (VAN DER GEEST et al. 2000a, 2000b, 2002). Our results clearly demonstrated that it is also a sensitive test organism in sediment toxicity bioassays. This sensitive response of \textit{E. virgo} to sediment-bound toxicants can partly explain their slow re-colonization in the River Rhine (BU DE VAA TE et al. 1992) since the onset of water quality improvement.

\textbf{Table 2.2.} Pearson’s correlation of survival and growth of \textit{Ephoron virgo} and growth of \textit{Chironomus riparius} after 10 days and 28 days (\textit{growth} \textit{28}) and \textit{EmT50} of \textit{C. riparius} with sediment characteristics. Unfed = absence of additional food source, fed = presence of additional food source, $\Sigma$PAHs = sum of polycyclic aromatic hydrocarbons, $\Sigma$PCBs = sum of polychlorinated biphenyls, $\Sigma$FAs = sum of fatty acids, $\Sigma$PUFAs = sum of polyunsaturated fatty acids, CO$_2$ = CO$_2$ production; M = macrophyte abundance; $\pm$ = positive correlation with $P < 0.05$; $-$ = negative correlation with $P < 0.05$, $--$ = negative correlation with $P < 0.01$.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>E. virgo</th>
<th>C. riparius</th>
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<tbody>
<tr>
<td>Cd</td>
<td>unfed</td>
<td>fed</td>
</tr>
<tr>
<td>Cu</td>
<td>unfed</td>
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<td>Zn</td>
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<td>$\Sigma$PAHs</td>
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<td>$\Sigma$PCBs</td>
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<td>$\Sigma$FAs</td>
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<td>$\Sigma$PUFAs</td>
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<td>CO$_2$</td>
<td>unfed</td>
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<td>M</td>
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In contrast to \textit{E. virgo}, \textit{C. riparius} responded mainly to the food quantity and quality in the sediments rather than to the toxicants present. A reduction in both survival and growth was observed in the phytoplankton dominated lake sediments in the absence of additional food. In the presence of additional food, however, survival and growth were acceptable in almost all sediments. Thus, it can be assumed that the reduced survival and growth was not caused by toxicants but by deficiency of food. However, not only food quantity but also food quality may play an important role in the development of \textit{C. riparius} as indicated by the positive correlation of larval growth in the absence of additional
food with chl α, PUFAs, and CO₂ production. The composition and nutritional value of the organic matter can indeed influence the growth of chironomids (JOHNSON et al. 1989, VOS et al. 2000) and lower survival, growth, and reproduction of chironomids in nutritionally poor substrates has been observed in several studies (HARKEY et al. 1994a, LACEY et al. 1999, RISTOLA et al. 1999). In the presence of additional food, growth of larvae in phytoplankton dominated lake sediments was higher than growth of larvae in macrophyte dominated lake sediments. Growth of chironomids has been shown to correlate well with the availability of algae or detritus from algal origin in their food (KAJA & WARD 1968), indicating that the principle food source of C. riparius larvae is detritus derived from algal and plant origin (VOS 2001). Hence, in the sediments where food was abundant, the larvae could have fed selectively on the organic matter present in the sediment instead of the highly nutritive food added to the test system (PINDER 1995).

Food deficiency of C. riparius larvae was even more profound in the 28-day emergence test in the absence of additional food, where emergence was only observed in macrophyte dominated lake sediments, whereas in the presence of an additional food source, no differences were found between phytoplankton and macrophyte dominated lake sediments. In an experiment with natural sediments and varying food quantities, RISTOLA et al. (1999) found no emergence of C. riparius without addition of food and only larvae in the sediment with the highest nutritional value survived. With increasing food levels added, a decrease in time of emergence was observed, and no differences were observed between sediments with different quantities of natural food. Thus, food limitation or poor food quality in natural sediments can reduce survival, growth, and development of C. riparius, which can be counteracted by adding an additional food source (ANKLEY et al. 1994a, LIBER et al. 1996, BRIDGES et al. 1997, VOS 2001).

When food was not the limiting factor, survival, growth, and development of C. riparius were satisfactory in spite of the toxicants present. However, C. riparius is not necessarily very tolerant to toxicants (PHIPPS et al. 1995, VAN DER GEEST et al. 2000b). Apparently, in the present set of sediments, the advantages of organic enrichment prevail against the potential adverse effects of the toxicants (DUBÉ et al. 1996, STUIJFZAND et al. 2000). Therefore, the amount and quality of available food, either natural or added to the test system, may affect the response of the test organism to sediment-bound toxicants or might even neutralize their effects (HARKEY et al. 1994b). This will cause undesired side effects in sediment bioassays using C. riparius. Addition of food in midge bioassays is needed to exclude reduction of survival, growth, and reproduction due to food deficiency, but may lead to masking of toxic effects. Therefore, we
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conclude that the midge *C. riparius* is not a suitable test organism for the assessment of sediment toxicity. Alternatively, it proved to be an appropriate test organism to determine the nutritional value of sediments. The mayfly *E. virgo* turned out to be a much more appropriate test organism for sediment toxicity bioassays, because it responds to the toxicant levels in the sediments rather than to the nutritional value.

In conclusion, *C. riparius* performs best on macrophyte dominated lake sediments, because it exploits the higher food levels at higher rates, in spite of the toxicants present. On the other hand, *E. virgo* performs best on uncontaminated sediments containing relatively low amounts of food, where the performance of *C. riparius* would be low because of starvation. Thus, the trophic state of an ecosystem influences the ecological risk of toxicants to benthic invertebrates in a species-specific way. It is concluded that not the toxicant load, but the combination of food and contaminants determine the persistence of benthic invertebrates and therewith the benthic community composition in complexly polluted ecosystems.

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