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RESPONSES OF BENTHIC INVERTEBRATES TO COMBINED TOXICANT AND FOOD INPUT IN FLOODPLAIN LAKE SEDIMENTS

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Abstract—Benthic communities in floodplain lake ecosystems are often exposed to varying levels of both food and toxicants. Inhibition through toxicants of sensitive species and stimulation through increased 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. Hence, seven floodplain lakes located along the River Waal, The Netherlands, with different levels of food (being either phytoplankton or macrophyte dominated) 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-d growth bioassays with both species and a 28-d emergence experiment with *C. riparius*. A decrease in both survival and growth of *E. virgo* was observed with increasing contaminant levels. 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.

Keywords—Contamination Eutrophication Combined effects *Chironomus riparius* *Ephoron virgo*

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 [1] and metals [2]. Analyzing 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 [3] and stimulation through increased amounts of food of opportunistic species [4,5] have been observed in separate studies. These opportunistic species, however, are not necessarily more tolerant to toxicants but may exploit at a higher rate the higher food levels [4,5]. 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 Waal, proved to be a sensitive test organism in acute and chronic toxicity tests [6–8]. The first-instar nymphs of *E. virgo* live freely on and in the sediment, feeding on fine particulate organic matter. In later stages they burrow U-shaped tubes in the sediment. They filter food, such as detritus and algae, from the water by generating wavelike movements with their feathered tracheal gills [9]. Chironomid larvae, such as *C. riparius*, are widely used test organisms in acute and chronic (sediment) toxicity tests [10–13]. The larvae of *C. riparius* are opportunistic tube-dwelling deposit feeders, which prefer eutrophic and organic enriched waters [14]. 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 [2], but the floodplain lake sediments of these rivers still contain high concentrations of xenobiotic compounds [15]. Therefore, the floodplain lake sediments may now 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), that were deposited in high concentrations in the 1960s and 1970s [16]. 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 dominated by either phytoplankton or 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.

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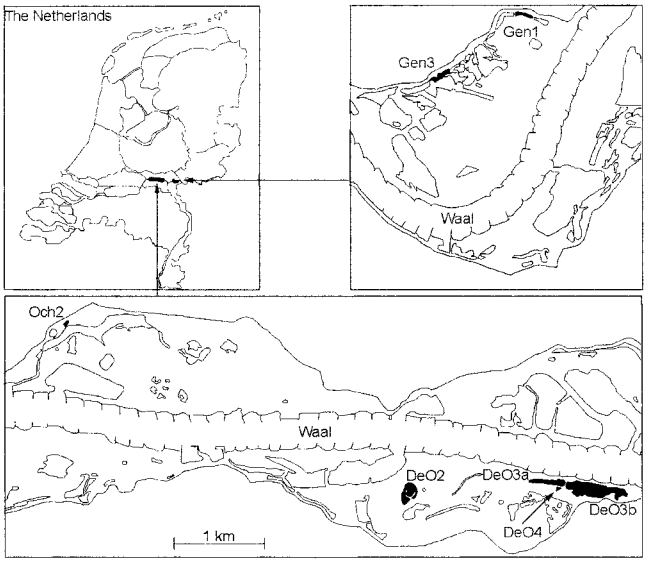

Fig. 1. Sampling locations in the River Waal floodplain areas (The Netherlands).

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 [11–13,17–20]. 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 [21,22]. 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 [18,23–25]. We therefore exposed both test species to sediments in the presence and absence of additional food 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 state were selected [15]. The species-specific preferences for these sediments were assessed using 10-d whole-sediment bioassays with both species and a 28-d 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], chl *a*, labile fraction of the OM [CO_2 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 macrofauna communities in the field.

MATERIALS AND METHODS

Sample collection, storage, and treatment

From seven floodplain lakes located along the River Waal, The Netherlands, sediment and water were collected (Fig. 1). 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 were 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. About 5 L of sediment were collected 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. Twenty to 25 L of lake water were collected in jerry cans. The water was filtered twice over a 30-μm filter in order to remove the zooplankton and stored at 4°C in the dark under constant aeration.

Sediment analyses

Metals (Cd, Cu, and Zn), Σ13PAHs (phenantrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*e*]pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*ghi*]perylene, dibenzo[*a,h*]anthracene, indeno[123]pyrene), Σ15PCBs (PCB18, PCB20, PCB28, PCB31, PCB44, PCB52, PCB101, PCB105, PCB118, PCB138, PCB149, PCB153, PCB170, PCB180, PCB194), and total P were measured by Koelmans and Moermond [15].

The OM content was measured as loss-on-ignition by combustion of dried sediment samples (60°C until constant wt) at 550°C for 6h [26] in triplicate.

Chlorophyll *a* and phaeophytin were measured according to Lorenzen [27] in triplicate using freeze-dried sediment samples. The acetone solution was centrifuged in closed test tubes to avoid optical disturbance by suspended sediment. Chlorophyll *a* and phaeophytin contents were summed because in sediments chl *a* is already partly degraded into phaeophytin.

Lipids were extracted from 1 g of wet sediment with 6 ml methanol containing 2.5% H_2SO_4 for 90 min at 80°C. The 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. The 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).

The labile fraction of the sediment organic matter was measured by using microbial mineralization as a value for CO_2 production according to Vos et al. [22]. A bacterial inoculum was prepared from the surface layer of a sediment containing a bacterial mat. Bacteria were detached by ultrasonification, and large particles were removed by centrifuging for 5 min at 500 rpm. Four milliliters 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 CO_2 in the headspace was measured using a Carlo Erba® GC (Milan, Italy). After 24 and 48 h of incubation, the CO_2 concentration and the pH were again measured.

Bioassays

All experiments were conducted at 20 ± 1°C, moderate light (~10 μm/s) and a 16:7-h light:dark regime with 30 min of twilight before and after each light period. During the ex-

periments, the test systems were not aerated. Temperature, dissolved oxygen, and pH were monitored every 5 d. At the beginning and the end of each experiment, samples were taken for determination of NH_4^+ and NO_2^- concentrations in the water with Quantofix® (Düren, Germany) test kits for ammonium and nitrate/nitrite. During the experiments, water quality conditions were satisfactory in all systems.

E. virgo

Survival and growth of the mayfly *E. virgo* on floodplain lake sediments were determined in a 10-d 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 [28] and transferred to 20°C in order to terminate the artificial diapause [29]. Sediments were thawed at 4°C 4 d before the start of the experiment.

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 (WU, Mainz, Germany). In addition, the body length of 20 larvae was measured.

The control treatment was fed $1.0.10^7$ μm^3 of small diatoms (*Navicula atomus*:*Nitzscia 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 $0.5.10^7$ μm^3 of diatoms per cm^2 and one drop of *Urtica* suspension. At day 7, the controls were additionally fed $3.0.10^7$ μm^3 *Chlamydomonas monoica* per ml.

At the end of the test, nymphs were collected from the sediment by using a floating technique according to Boivin et al. [30]. Surviving nymphs were counted and body length was measured with an automatic image analyzer using the computer program Research Assistant 3 (RVC, Hilversum, The Netherlands). Growth was calculated by subtracting the average initial length from the individual final length.

The experiment was repeated with addition of food to all treatments, according to the control-feeding regime.

C. riparius

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

For the 10-d bioassay, five replicate 400-ml glass beakers with 75 ml homogenized wet sediment and 300 ml filtered site water were prepared 1 d before the start of the experiment and aerated overnight. Control experiments 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, France) and Tetraphyll® (Tetrawerke, Melle, Germany) (20:1, wt/wt) suspension (250 mg Trouvit–Tertaphyll in 100 ml of Elendt-M7 medium) daily. After 10 d, larvae from five test beakers were collected from the sediment by sieving the sediment over 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.

The first 10 d, the emergence experiment was carried out exactly as the growth test. 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) suspension (250 mg Trouvit–Tertaphyll in 100 ml of Elendt-M7 medium) daily. After 28 d, 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 nonemerged larvae was calculated by subtracting the average initial length from the individual final length.

Both experiments were repeated with addition of food to all treatments, according to the feeding regime in the control.

Data analyses

One-way analysis of variance (ANOVA) tests followed by Scheffé's post hoc test were conducted to test for significant differences between treatments and controls for each endpoint (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 per sediment with a Student's *t* test. Differences were considered significant between the test categories at the 0.05 probability level. Emergence is given as the time (days) at which 50% of the adults had emerged (EmT50). This EmT50 was calculated according to a logistic response model adopted from Haanstra et al. [31].

Correlations between survival, growth, time of emergence, and sediment characteristics were determined with Pearson correlation, using the average survival, growth, and time of 50% emergence and the mean of the repeated analyses for the different sediment parameters. All statistical analyses were conducted using the computer program SPPS® 9.0 for Windows (SPSS, Chicago, IL, USA).

RESULTS

Sediment characteristics

The sediment characteristics of the seven floodplain lake sediments are listed in Table 1, in which the sediments are ranked from relatively clean to contaminated. Concentrations of metals, ΣPAHs, and ΣPCBs were lowest in DeO2. Metal concentrations were highest in DeO3a, DeO3b, and DeO4, and the sum of PAHs and PCBs were highest in, respectively, DeO4 (10.52 mg/kg dry wt) and DeO3b (133.22 μg/kg dry wt). The concentrations of Cd, Zn, ΣPAHs, and ΣPCBs all correlated positively with each other, and Cu was positively correlated with Cd, Zn, and PCBs. Hence, a clear gradient in contaminant concentrations was observed, allowing a ranking of the sediments from relatively clean (DeO2) to heavily contaminated (DeO4).

Sediment organic matter content was lowest in DeO2 (2.7%) and Gen1 (3.9%), while the organic matter content in the other sediments ranged from 8.3 to 11.0%. The lowest total

Table 1. Sediment characteristics: Cd, Cu, Zn, sum of polycyclic aromatic hydrocarbons (ΣPAHs), total P (P), chlorophyll *a* (chl *a*), sum of fatty acids (ΣFAs) and sum of polyunsaturated fatty acids (ΣPUFAs) in mg/kg dry weight, sum of polychlorinated biphenyls (ΣPCBs) in μg/kg dry weight, organic matter content (OM) and CO_2 production (mmolCO_2d/kg dry wt). Sediments are ranked from relatively clean to contaminated

	DeO2	Gen1	Gen3	Och2	DeO3a	DeO3b	DeO4
Cd[a]	0.17	0.41	0.90	1.25	1.41	2.19	1.61
Cu[a]	12	28	45	52	72	82	57
Zn[a]	42	194	386	199	322	507	544
ΣPAHs[a]	0.55	0.94	1.57	2.97	5.76	5.87	10.52
ΣPCBs[a]	4.37	7.17	20.19	33.41	74.34	133.22	109.35
OM	2.7	3.9	9.7	11.0	8.3	9.0	9.5
P[a]	434	1,226	1,137	1,312	1,458	1,703	1,198
Chl *a*	19.31	16.44	76.50	74.05	30.07	43.33	55.40
ΣFAs	0.99	0.89	2.81	3.65	1.23	1.76	3.08
ΣPUFAs	0.44	0.22	1.12	1.34	0.37	0.69	1.23
CO_2	9.46	9.10	23.51	30.75	19.45	20.23	24.33
Vegetation	A[b]	A	M[c]	M	A	A	M

[a] Koelmans and Moermond [15].
[b] A = phytoplankton-dominated lake.
[c] M = macrophyte-dominated lake.

P concentration was also measured in DeO2 (434 mg/kg dry wt) and varied from 1,137 to 1,703 mg/kg dry weight in the other sediments. Macrophytes were present in the lakes DeO4, Gen3, and Och2. Chlorophyll *a* concentrations, FA content, PUFA content, 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* (16.44 mg/kg dry wt) in Gen1 and the highest (76.50 mg/kg dry wt) in Gen3. The labile fraction of the organic matter ranged from 9.10 in Gen1 to 30.75 μmol CO_2/d/kg dry weight in Och2. The lowest FA and PUFA contents were found in Gen 1 (0.87 and 0.22 mg/kg dry wt), and the highest concentrations were found in Och2 (3.59 and 1.34 mg/kg dry wt).

The CO_2 production was positively correlated to OM, chl *a* content, ΣFAs, ΣPUFAs, and macrophyte abundance. The chl *a* positively correlated with OM, ΣFAs, ΣPUFAs, and macrophyte abundance. Organic matter content was positively correlated to ΣFAs and ΣPUFAs. Macrophyte abundance was positively correlated to chl *a*, CO_2 production, ΣFA, and ΣPUFAs. 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 quality parameters, expressing the contaminant levels based on OM did not change these results. However, the relatively clean sediment (DeO2) has the lowest food quantity and quality and the most heavily polluted sediment (DeO4) the highest food quantity and quality.

Survival and growth of E. virgo

Average control survival was 86.3%, and average growth of control nymphs was 220 μm. With increasing contamination levels, a decrease in both survival and growth of the *E. virgo* nymphs was observed (Fig. 2). In the most polluted sediments (DeO3a, DeO3b, and DeO4), significant ($p < 0.01$) lower survival and growth was observed compared to the control. Growth in DeO2 sediment was also significantly lower ($p < 0.01$) than control growth. Survival was negatively correlated with Cu, Zn, PAHs, and PCBs (Table 2), and growth was negatively correlated with PAHs and PCBs.

Additional food did not alter survival or growth of *E. virgo* nymphs, except for growth in DeO2, 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 Cu, Zn, PAHs, and PCBs. No correlations were found with any of the food quality parameters.

10-d survival and growth of C. riparius

Average control survival was 99%, and average growth of control larvae was 7.8 mm. Survival of the midge *C. riparius* was above 90% in the three macrophyte-dominated lakes (Fig. 2). In contrast, significant lower survival ($p < 0.05$) compared to the control was observed in three of the four phytoplankton-dominated lakes (Gen1, DeO3a, and DeO3b). In all sediments, growth was significantly lower than in the control (Fig. 2), except for Gen3, the least polluted macrophyte-dominated lake. Larval growth in the sediments positively correlated with chl *a*, ΣPUFAs, CO_2 production, and the presence of macrophytes.

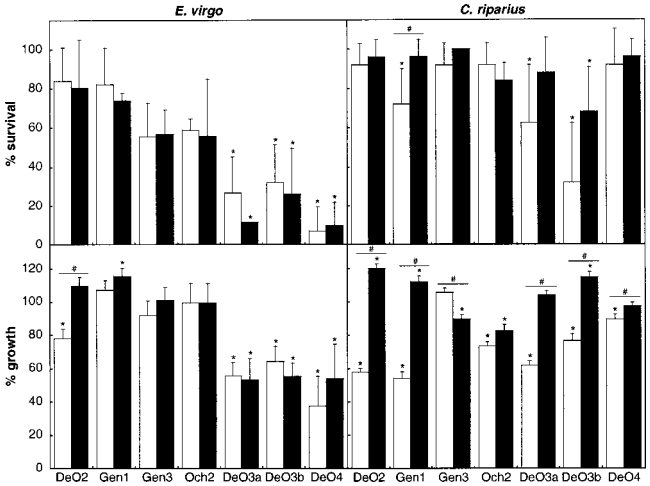

Fig. 2. Survival (%) and growth (%) of *Ephoron virgo* and *Chironomus riparius* larvae after 10 d on sediments in the absence and presence of additional food, given as percentages of the corresponding controls. White bars represent sediments without food, black bars represent sediments with food. Error bars = standard deviation; * = significant difference from control ($p < 0.05$); # indicate significant difference between the absence and presence of additional food ($p < 0.05$).

Table 2. Correlations between growth and survival of *Ephoron virgo* and *Chironomus riparius* after 10 d and growth and EmT50 of *C. riparius* after 28 d with sediment characteristics. Pearson coefficient r^2 and significant level. ΣPAHs = sum of polycyclic aromatic hydrocarbons, ΣPCBs = sum of polychlorinated biphenyls, chl *a* = chlorophyll *a*, ΣFAs = sum of fatty acids, ΣPUFAs = sum of polyunsaturated fatty acids, CO_2 = CO_2 production, M = macrophyte abundance; + = positive correlation with $p < 0.05$; ++ = positive correlation with $p < 0.01$; − = negative correlation with $p < 0.05$; −− = negative correlation with $p < 0.01$

Parameter	Cd	Cu	Zn	ΣPAHs	ΣPCBs	Chl *a*	ΣFAs	ΣPUFAs	CO_2	M
E. virgo										
Survival − food	−	−	−	−−	−−					
Survival + food	−	−	−	−−	−−					
Growth − food				−−	−					
Growth + food	−	−	−	−−	−−					
C. riparius										
Growth − food						+		+	+	+
Growth + food						−−	−	−	−−	−−
28-d growth − food						+	+	+		
EmT50 − food						−−	−	−	−	−−

Addition of food diminished the mortality in Gen1, DeO3a, and DeO3b. As a result, significant lower survival in comparison to the control was found only for DeO3b and, unexpectedly, Och2. In the presence of food, increased growth was observed in all sediments except for Gen3. Growth negatively correlated with chl *a*, ΣFAs, ΣPUFAs, CO_2 production, and the abundance of macrophytes. No correlations were found with any of the contaminant concentrations.

28-d development of C. riparius

Survival exceeded 80% in all control treatments. Average EmT50 in the control was 20.3 d.

In the experiments without additional food, adult emergence was observed only in the sediments from macrophyte dominated lakes (Fig. 3). In the phytoplankton-dominated lake sediments, little (4 and 8% in DeO2 and DeO3a) to no emergence occurred, and 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. Emergence was positively correlated with chl *a*, ΣFAs, ΣPUFAs, CO_2 production, and macrophyte abundance. Growth of nonemerged larvae (not shown) was positively correlated with growth after 10 d on sediments without additional food and with chl *a*, ΣFAs, and ΣPUFAs.

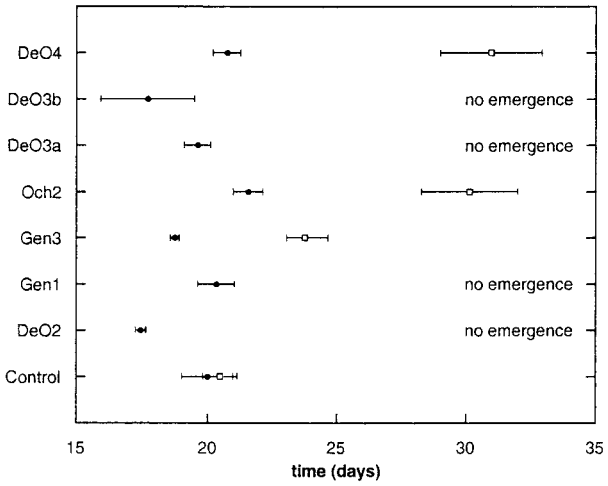

Fig. 3. Emergence of adult *Chinomus riparius* after 28 d of exposure to sediments in absence (□) and presence (●) of additional food expressed as EmT50. Error bars = 95% confidence limits; no emergence = EmT50 could not be calculated because of low or zero emergence.

Addition of food resulted in faster development in all sediments; time of 50% emergence ranged from 17.5 d in DeO2 to 21.5 d in Och2, and all midges emerged within 28 d. The EmT50 value in DeO2 was significantly lower ($p < 0.05$) than in the control, 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.

DISCUSSION

The present study demonstrated that the sensitive mayfly *E. virgo* and the opportunistic midge *C. riparius* responded completely opposite to the varying food and toxicant levels. *Ephoron 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 DeO2 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 DeO2 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 strongly increased. As reported in several studies, mayflies are sensitive to many classes of contaminants [3,17,32,33]. In addition, the species used in the present study, *E. virgo*, is very sensitive to several contaminants in the laboratory [6–8]. Our results clearly demonstrated that it is also a sensitive test organism in sediment toxicity bioassays. This sensitive response of *E. virgo* to sediment-bound toxicants can partly explain their slow recolonization in the River Rhine [34] since the onset of water quality improvement.

In contrast to *E. virgo*, *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 phytoplankton-dominated lake sediments in the absence of additional food. In the presence of additional food, however, survival and growth was acceptable in almost all sediments. Thus, it can be assumed that the reduced survival

and growth was caused not by toxicants but by food deficiency. However, not only food quantity but also food quality may play an important role in the development of *C. riparius* as indicated by the positive correlation between larval growth in the absence of additional food and chl *a*, ΣPUFAs, and CO_2 production. The composition and nutritional value of the organic matter can indeed influence the growth of chironomids [35,36], and lower survival, growth, and reproduction of chironomids in nutritionally poor substrates has been observed in several studies [13,24,25].

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 [37], indicating that the principal food source of *C. riparius* larvae is detritus derived from algal and plant origin [22]. 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 [38].

Food deficiency of *C. riparius* larvae was even more profound in the 28-d emergence test in the absence of additional food, where emergence was observed only in macrophyte-dominated lake sediments, whereas in the presence of additional food, no differences were found between phytoplankton- and macrophyte-dominated lake sediments. In an experiment with natural sediments with different food quantities, Ristola et al. [13] 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 [18,19,22,39].

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 [6,40]. Apparently, in the present set of sediments, the advantages of increased organic enrichment prevail against the potential adverse effects of the toxicants [4,5]. 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 [10]. 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 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.

CONCLUSIONS

The present study demonstrated that *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 determines the persistence of benthic invertebrates and therewith the benthic community composition in complexly polluted ecosystems.

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