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The impact of sediment reworking by opportunistic chironomids on specialised mayflies

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SUMMARY

1. Bioturbation, by definition, changes the structure and properties of sediments, thereby altering the environment of the bioturbator and other benthic species. In addition to the indirect effects of sediment reworking (e.g. changes in water quality), bioturbating species may also directly interfere with other species via competition. This study aims, therefore, to examine both the direct and indirect effects of sediment reworking by an opportunistic detritivore on survival and growth of a specialised mayfly species.
2. Bioturbation was imposed by adding different densities of the midge *Chironomus riparius* to clean and polluted sediments. Changes in water quality and sediment properties, and survival and growth of the mayfly *Ephoron virgo* were assessed.
3. Chironomid density had a strong negative effect on the concentrations of metals, nutrients and particles in the overlying water, but increased the penetration of oxygen into the sediment. Survival and growth of *E. virgo* were strongly reduced in the presence of chironomids. In the polluted sediment, the activity of chironomids enhanced the negative effects of pollution on *E. virgo*. In the clean sediment, inhibition of the mayfly was even more pronounced.
4. This suggests that direct disturbance by *C. riparius* was more important than indirect changes in water quality, and over-ruled the potential positive effects of improved oxygen penetration. The results indicated that the distribution of small insects, such as *E. virgo*, can be limited by bioturbating benthic invertebrates.

Keywords: bioturbation, *Chironomus riparius*, *Ephoron virgo*, sediment quality

Introduction

During the severe water pollution in the River Rhine in the 1960s and 1970s, many contaminants accumulated in the sediments of the embanked floodplains in its lower reaches (Beurskens *et al*., 1993). Although water quality greatly improved in recent decades (Admiraal *et al*., 1993) and recent deposited sediments contain considerably lower concentrations of contaminants, many floodplain lake sediments are still historically polluted with many xenobiotic compounds (Koelmans & Moermond, 2000; De Haas *et al*., 2002). As a consequence many floodplain lake sediments of the River Rhine 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) (Beurskens *et al*., 1993). The benthic communities of these historically polluted floodplain lakes are thus exposed to a diffuse flux of

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sediment-bound toxicants and nutrients, which may alter the benthic community composition (Pinel-Alloul *et al.*, 1996; Canfield *et al.*, 1998). In addition, when contaminants are (temporarily) desorbed from the sediment or transferred to suspended particles due to remobilisation from the sediments to toxicants, the benthic community may be further affected.

One of the processes that may remobilise toxicants is bioturbation. Bioturbation is the reworking of sediment by the feeding and burrowing activity of benthic organisms, which changes the structure and properties of the sediment. These changes include an increase in oxygen penetration depth and sediment oxygen consumption (Hargrave, 1975; Granéli, 1979a; Svensson & Leonardson, 1996), a changed particle size distribution (Iovino & Bradley, 1969; McLachlan & McLachlan, 1976) and increased sediment water content (Cullen, 1973; Rhoads, 1974). These changes may alter sediment–water transfer processes and the bioavailability of various compounds, such as nutrients and toxicants (Granéli, 1979b; Petersen *et al.*, 1995).

A typical example of a sediment reworking species is the midge *Chironomus riparius* (Meigen). Larvae of *C. riparius* live in soft organically rich sediments where they construct burrows in the upper layer of the sediment (Armitage, Cranston & Pinder, 1995). Undulations of their body drive fresh water through the tubes, replenishing oxygen and flushing out metabolites and carbon dioxide (Pinder, 1995). Sediment-dwelling chironomids are also very mobile, leaving the bottom (usually at night) and migrating into the water column (Edgar & Meadows, 1969). Hence, *C. riparius* larvae are assumed to have a high bioturbating capacity and thereby provoke indirect interference through the alteration of the physical environment (Peterson, 1980). The altered sediment properties (Johnson, 1984) and water quality conditions (Pinel-Alloul *et al.*, 1996) influence the distribution and abundance of the benthos (Cummins & Lauff, 1969; Oliver, 1971). In addition to indirect interfering effects on other benthic invertebrates, bioturbators may also exert a more direct influence via interspecific interactions such as competition for space and food (Rasmussen, 1985; Reynoldson *et al.*, 1994; Haden *et al.*, 1999), predation (Kelly, Dick & Montgomery, 2002) or by increased food availability because of re-fractioning of food particles (Van de Bund & Davids, 1993).

One of the species that may be affected by the activities of chironomids is the mayfly *Ephoron virgo* (Oliver), also a sediment inhabiting species. Early-instar nymphs of *E. virgo* live freely on the sediment, feeding on fine particulate organic matter. In later stages they build U-shaped tubes in the sediment and start to filter food, such as detritus and algae, from the water by generating wavelike movements in their burrows with their feathered tracheal gills (Kureck & Fontes, 1996). The nymphs of *E. virgo* are very sensitive to sediment-bound toxicants (De Haas *et al.*, 2002), and only slowly re-colonised the River Rhine when water quality improved after the initiation of the Rhine Action Program in 1987 (Bij de Vaate, Klink & Oosterbroek, 1992).

The aim of this study was to analyse the effects of sediment reworking by an opportunistic detritivore on the specialised mayfly *E. virgo*. To this purpose different densities of the bioturbating midge *C. riparius* were added to sediments. To discriminate between the direct and indirect interference of *C. riparius* on *E. virgo*, experiments were conducted with both a clean and a polluted sediment. The changes in several water quality conditions (turbidity, total phosphorous, and total zinc) and sediment properties (oxygen penetration depth, sediment oxygen consumption, and porosity) were analysed in 7-day bioassays. We evaluated whether survival and growth of the mayfly were affected by direct interference, alteration of the physical environment or a combination of both.

Methods

Experimental design

Two floodplain lakes, one relatively clean and one historically polluted, located in the same flood plain along the River Waal, a part of the River Rhine, were selected for this study. The effect of sediment reworking was tested at four chironomid densities, resulting in eight treatments. All treatments were conducted in presence of the mayfly *E. virgo*. Several treatments were also conducted in the absence of *E. virgo* to assess the contribution of the mayflies to water quality conditions and sediment characteristics. Each treatment included ten test systems; four random test systems were used for oxygen penetration depth measurements, three for sediment oxygen consumption, and three for the *E. virgo* bioassay. Water samples for the measurement of water quality parameters, seven replicates, were taken from the test systems for oxygen penetration depth measurement and the *E. virgo* bioassays. Sediment porosity was measured in sediments from the test systems used for the measurement of the oxygen penetration depth. All experiments were performed for 7 days.

Sample collection, storage and treatment

Approximately 25 L of sediment was collected in November 2002 from one relatively clean and one historically polluted floodplain lake located along the River Waal in the Netherlands, using an Ekman–Birdge grab adjusted to sample the upper 5 cm of the sediment. The sediment was transported to the laboratory, where large debris was picked out by hand. Next the sediment was homogenised, and stored at −20 °C in 500-mL polyethylene bottles within 6 h of sampling in order to eliminate autochthonous organisms. 50 L lake water was collected and filtered (GF/F Whatman®; Whatman International Ltd, Maidstone, U.K.), and stored at 4 °C in the dark, under constant aeration.

Sediment preparation

For each treatment 10 glass jars (150-mL) with 25 mL wet homogenised sediment and 100 mL filtered site water were prepared. In order to restore sediment stratification, the jars were incubated for 7 days in a 20 ± 1 °C climate room with moderate light (approximately 10 μmol m^{-2} s^{-1}) and a 16 : 7 h light : dark regime with 30 min of twilight before and after each light period.

Chironomus riparius

After incubation 0 (control), 5 (low-density; 1667 ind m^{-2}), 10 (medium-density; 3333 ind m^{-2}) or 20 (high-density; 6667 ind m^{-2}) third-instar *C. riparius* (approximately 0.5 cm in length) larvae were introduced into the test systems. The larvae were obtained from a culture maintained in our laboratory, sieved (500-μm) from the sediment, and transferred to the test systems. Remaining larvae were stored at 4 °C. During the experiment evaporated water was replaced with deionised water and emerged adults were replaced with third-instar larvae to maintain the chosen bioturbation capacity. The percentage of midge larvae recovered at the end of the experiments was always higher than 85% of the initial number, including replaced larvae.

Ephoron virgo

To determine the effects of bioturbation by chironomids on survival and growth of the mayfly *E. virgo*, the treatments were stocked with first-instar nymphs (<48 h old, average size 757 ± 41 μm), obtained from field-collected eggs, kept in artificial diapause at 4 °C in our laboratory. Six 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 to terminate the artificial diapause (Greve *et al*., 1999).

Twenty nymphs were randomly transferred into each test system. In addition, the initial body length of 20 larvae was measured 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). At the end of the experiment 75 mL of the overlying water was taken from the test system and stored in 100-mL polyethylene bottles for the analysis of water quality parameters. Next the nymphs were collected from the sediment, counted and their body lengths (from the median ocellus to the end of the abdomen) measured. Growth was determined by subtracting the average initial length from the individual final length. The average growth per treatment was calculated from the mean growth per replicate treatment.

Oxygen measurements

At the end of the experiment, oxygen profiles of the sediment–water interface were measured in four test systems per treatment using a Clark-type oxygen sensor with an internal reference and guard cathode (Unisense OX100; Unisense, Aarhus, Denmark). The sensor was connected to a high-sensitivity picoammeter (Unisense PA2000; Unisense) and driven into the sediment using a micromanipulator (Unisense MM33; Unisense). The oxygen sensor was calibrated using a pH/Oxi 340i oxygen meter (WTW, Weilheim, Germany) equipped with a CellOx 325 electrode (WTW). The oxygen concentration in the overlying water ranged from 9.35 to 9.85 mg L^{-1}. The sediment oxygen profiles were used to determine the oxygen penetration depth. Following the measurements of oxygen penetration depth 75 mL of the overlying water was taken from the test system and stored in 100-mL polyethylene bottles for the analysis of water quality parameters. The remaining water was removed and sediment porosity was measured.

Three test systems per treatment were used for sediment oxygen consumption measurements. The initial oxygen concentration was measured using a Clark-type oxygen sensor. After this measurement the test systems were covered with a glass lid to prevent access of air. After 24 h the lid was removed and the oxygen concentration was again measured. The oxygen consumption was measured as the reduction in oxygen concentration in the known volume of the overlying water.

To measure the contribution of oxygen respiration by *C. riparius* larvae to total oxygen consumption, groups of 0, 10 and 20 larvae were incubated in 30-mL glass jars with 20 mL of oxygen-saturated Elendt-M7 medium (six replicates). Measurements were performed as described for sediment oxygen consumption measurements.

Water quality characteristics

Water quality characteristics were measured from seven replicates. Turbidity of the overlying water was measured using a Turb 350IR turbidity meter (WTW). Total phosphorus (total P) in the overlying water was determined according to Murphy & Riley (1962) within 2 days of sampling. For zinc analyses two samples of 2 mL overlying water were acidified with 40 µl 69–70% nitric acid (Baker, Philipsburg, NJ, U.S.A.) and stored at −20 °C until analysis. The samples were analysed for Zn by air–acetylene Flame Atomic Absorption Spectrometry (Perkin-Elmer 1100B; Norwalk, CT, U.S.A.). Quality control of the metal analysis was carried out by analysing blanks and reference material (NIST:SRM 1643; National Institute of Standards and Technology, Gaithersburg, MD, U.S.A.).

Sediment characteristics

Sediment porosity was measured in four replicate samples. The remaining 25 mL of overlying water was removed and the wet sediment was homogenised and stored in preweighed 50-mL polyethylene bottles at −20 °C. After freeze-drying, sediment porosity was calculated from weight loss on drying.

The organic matter content of the sediment was measured as loss-on-ignition by combustion of 2 g dry sediment at 550 °C for 6 h (Luczak, Janquin & Kupka, 1997) in triplicate. Chlorophyll *a* and phaeophytin were measured according to Lorenzen (1967) in triplicate from 1 g dry sediment. 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 chlorophyll *a* is already partly degraded into phaeophytin. Total phosphorus in the sediment was determined according to Murphy & Riley (1962).

Previous analysis of contaminant levels in these and other floodplain lake sediments showed that Cd, Cu, Zn, sum of PAHs, and sum of PCBS were all highly correlated (De Haas *et al.*, 2002). Therefore, in this study only Cd, Cu, and Zn were analysed as indicators of contamination levels in the tested sediments. For the metal analysis approximately 5 mg dry sediment (triplicate) was weighed and placed in a 3 mL Teflon digestion vessel and 50 µl 70% nitric acid (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, U.S.A.). The vessels were heated to 175 °C in 15 min and maintained at 175 °C for another 30 min. The samples were diluted to exactly 2 mL with deionised water and analysed by air–acetylene Flame Atomic Absorption Spectrometry (Perkin-Elmer 1100B).

Statistical analyses

One-way analyses of variance (ANOVA) tests followed by Scheffé's *post hoc* tests were conducted to test for significant differences among treatments for both survival and growth of *E. virgo*, and the different water and sediment parameters. To assess the contribution of sediment and chironomid density in accounting for the observed variation in survival, growth and water and sediment parameters, two-way ANOVAS were performed with sediment and chironomid density as the independent variables. 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, U.S.A.).

Results

Floodplain lake characteristics

Water quality and sediments characteristics of the relatively clean and historically polluted floodplain lakes are listed in Table 1. Water quality conditions in both lakes, according to Dutch water quality guidelines (CIW, 2000), are not currently considered as polluted. The contaminant concentrations in the clean sediment were below sediment quality criteria (CIW, 2000), metal concentrations in the polluted sediment were slightly below sediment quality criteria, but both PAHs and PCBs exceeded sediment quality criteria. Food quantity and quality for detritivores were higher in the polluted sediment than in the clean sediment. The organic matter content was higher in the polluted sediment (7.8% as opposed to 1.2%), as were total phosphorus concentrations (1820 as opposed to 596 mg kg^{-1} dw) and chlorophyll *a* concentrations (16.3 as opposed to 7.3 mg kg^{-1} dw).

Table 1 Initial water quality conditions and sediment characteristics of the floodplain lakes used in this study

	Clean	Polluted
Water		
Turbidity (NTU)	1.58 (0.16)	2.89 (0.30)
Total P (μg L^{-1})	3.79 (0.21)	3.54 (0.34)
Total Zn (μg L^{-1})	<10.14	<10.14
Sediment		
Cd (mg kg^{-1} dw)	0.20 (0.05)	2.05 (0.15)
Cu (mg kg^{-1} dw)	12 (1.7)	45 (1.3)
Zn (mg kg^{-1} dw)	39 (2.8)	245 (21.2)
∑PAHs (mg kg^{-1} dw)*	0.55 (0.13)	5.9 (1.9)
∑PCBs (μg kg^{-1} dw)*	4.4 (1.8)	133 (5.9)
Porosity (%)	21.6 (0.47)	41.3 (0.93)
OM (%)	1.2 (0.04)	7.8 (1.03)
Chl *a* (mg kg^{-1} dw)	7.3 (0.94)	16.3 (1.09)
Total P (mg kg^{-1} dw)	596 (37.1)	1820 (34.0)

*Previously measured concentrations (from De Haas *et al.*, 2002). Clean and polluted refers to the quality of the sediment deposit; ∑PAHs = sum of polycylic aromatic hydrocarbons; ∑PCBs = sum of polychlorinated biphenyls; Total *P* = total phosphorus; OM = organic matter content; b.d. = below detection limit; standard deviations are given in parentheses.

Overlying water

Turbidity, total phosphorus, and total zinc in the overlying water were lower in the clean control than in the polluted control, but only total phosphorus was significantly different ($P < 0.001$) (Fig. 1a–c). With increasing chironomid density an increase of turbidity, total phosphorus, and total zinc were observed in both the clean and the polluted sediment. Significantly higher turbidity compared with controls was observed at all chironomid densities in both sediments ($P < 0.001$). For total phosphorus, significant increases compared with control treatments were observed at medium and high chironomid densities for both sediments ($P < 0.001$), and significantly higher zinc concentrations compared with control concentrations were observed at the high chironomid density of both sediments ($P < 0.001$).

The two-way ANOVA showed that for turbidity only chironomid density accounted for the observed variation ($P < 0.001$), and the interaction term between sediment and chironomid density was not significant (Table 2). This indicates that turbidity of the overlying water is dependent on chironomid density, but independent of the degree of pollution of the sediment. The two-way ANOVAs for total phosphorus and zinc indicated that both sediment and chironomid density were significant in explaining the observed variation ($P < 0.001$). However, the statistical interaction term between sediment and chironomid density was not significant (Table 2). This indicates that both the type of sediment and chironomid density affected total phosphorus and zinc concentrations in the overlying water, but that the effect of chironomid density is independent of sediment type.

Sediment

The oxygen penetration depth in the clean control was significantly greater than in the polluted control (3.08 and 1.99 mm respectively, $P < 0.001$). A rise in chironomid density resulted in an increase in oxygen penetration depth in both the clean and the polluted sediment (Fig. 1d). The oxygen penetration depth in the polluted sediment at the highest chironomid density was significantly different from the control ($P < 0.001$), whereas the oxygen penetration depth in

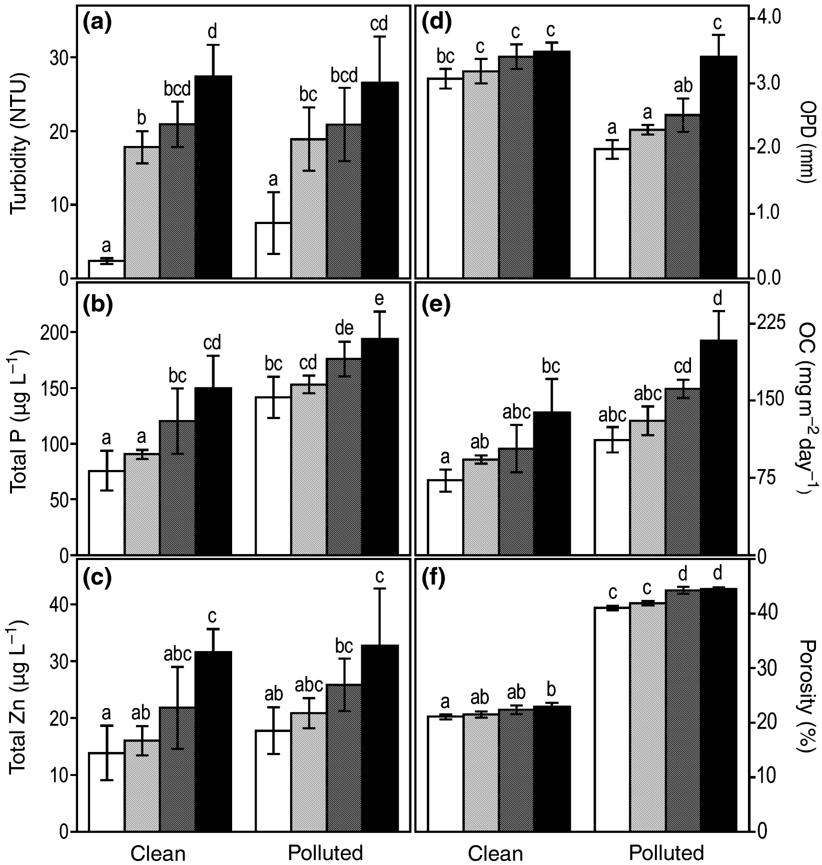

Fig. 1 Water quality conditions and sediment properties of clean and polluted sediment treatments after 7 days exposure to different chironomid densities (no mayflies present). □, control; ▩, low chironomid density; ▩, medium chironomid density; and ■, high chironomid density. A, turbidity of the overlying water in NTU; B, total phosphorus (total P) of the overlying water in μg L^{-1}; C, total Zn of the overlying water in μg L^{-1}; D, oxygen penetration depth (OPD) into the sediment in mm; E, oxygen consumption (OC) of the sediment in mg m^{-2} day^{-1}; and F, porosity of the sediment in percentage. Error bars = standard deviations; bars sharing the same letter are not significantly different ($P < 0.05$).

Table 2 Two-way ANOVA (*P*-values) for water quality characteristics and sediment properties, with the type of sediment and chironomid density as independent variables

Parameter	Sediment	Density	Sediment·density
Turbidity	0.216	<0.001	0.206
Total phosphorus	<0.001	<0.001	0.096
Total zinc	0.012	<0.001	0.504
OPD	<0.001	<0.001	<0.001
OC	<0.001	<0.001	0.353
Porosity	<0.001	<0.001	0.006

OPD = oxygen penetration depth; OC = oxygen consumption.

the clean sediment was only slightly deeper at the highest chironomid density.

The sediment oxygen consumption of the polluted control was higher than that of the clean control (109.7 and 71.0 mg m^{-2} day^{-1} respectively), but not significantly different (Fig. 1e). Sediment oxygen consumption increased with increasing chironomid density in both the clean and the polluted sediment and a significantly higher oxygen consumption compared with the control was observed at the high chironomid densities in both sediments ($P < 0.05$). *Chironomus riparius* larvae consumed 2.75 μg O_2 $larvae^{-1}$ day^{-1} (regression coefficient, $R^2 = 0.835$, $P < 0.01$) (E.M. De Haas, unpublished data), which is 19.1 and 27.7% of the oxygen consumption measured in polluted and clean sediment, respectively.

Sediment porosity of the clean sediment was much lower than that of the polluted sediment, and increased with increasing chironomid density in both sediments (Fig. 1f). Significantly higher sediment porosity compared with the clean control was observed at the high chironomid density ($P < 0.05$). Porosities of the polluted sediments at medium and high chironomid densities were significantly higher than that of the polluted control ($P < 0.001$).

The two-way ANOVA verified that both sediment type and chironomid density accounted for the observed variation in changed sediment properties ($P < 0.001$) (Table 2). Their interaction terms were significant in explaining the observed variation ($P < 0.01$) for both oxygen penetration depth and sediment porosity, but not for sediment oxygen

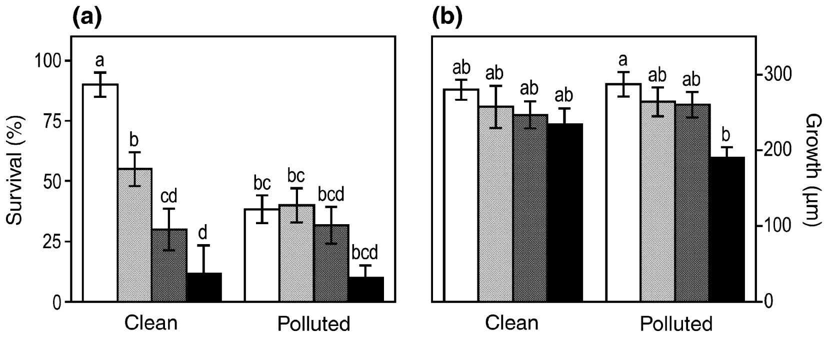

Fig. 2 Survival and growth of *Ephoron virgo* nymphs after 7 days of exposure to clean and polluted sediment containing different chironomid densities. □, control; ▨, low chironomid density; ▩, medium chironomid density; and ■, high chironomid density. A, survival (%); and B, growth (µm). Error bars represent standard errors; bars sharing the same letter are not significantly different ($P < 0.05$).

consumption. This indicates that both sediment type and chironomid density affected all three measurements. However, the effect of chironomid density was dependent on sediment type for both oxygen penetration depth and sediment porosity, but independent for sediment oxygen consumption.

Ephoron virgo

Survival in the clean control (90%) was significantly ($P < 0.001$) higher than in the polluted control (38.3%). Survival declined with increasing chironomid density (Fig. 2). In clean sediment, significantly lower survival compared with the control was observed at all chironomid densities ($P < 0.01$). The low survival in the polluted sediment was significantly further affected only at the high chironomid density ($P < 0.01$). The two-way ANOVA showed that only chironomid density was significant in explaining the observed variation of survival ($P < 0.001$) (Table 3).

Growth of the mayflies was not significantly different between the clean and the polluted control (276 and 289 µm, respectively). Growth decreased slightly with increasing chironomid density on clean sediment although not significantly (Fig. 2). Significantly lower growth compared with control growth was found on polluted sediment with the highest chironomid density ($P < 0.05$). A two-way ANOVA demonstrated that only chironomid density accounted for the observed variation in growth ($P < 0.001$) (Table 3).

Table 3 Two-way ANOVA for survival and growth of *Ephoron virgo*, with the type of sediment and chironomid density as independent variables

Parameter	Variable	*P*-value
Survival	Sediment	0.261
	Density	0.006
	Sediment·Density	0.698
Growth	Sediment	0.649
	Density	0.002
	Sediment·density	0.502

Discussion

Effects of sediment reworking on water quality conditions and sediment properties

Our results have demonstrated a marked effect of *C. riparius* larvae on the benthic environment, since significant effects of chironomid density on all measured water quality parameters and sediment properties were observed. The increase in turbidity, total phosphorus and total zinc in the overlying water in the presence of chironomids in our study supports observations from other studies (Granéli, 1979b; Lee & Swartz, 1980; Krantzberg, 1985; Hansen, Mouridsen & Kristensen, 1998). Increasing chironomid density resulted in a deeper oxygen penetration depth and an increase in sediment oxygen consumption, since chironomid burrows effectively increase the total area for sediment oxygen uptake (Lee & Swartz, 1980). A stimulating effect of chironomid larvae on oxygen penetration depth and sediment oxygen consumption, by drawing in currents of oxygen rich water for respiration through their burrows, is also in agreement with other studies (Hargrave, 1975; Granéli, 1979a; Svensson & Leonardson, 1996).

Sediment type had a significant influence on the impact of bioturbation on water quality conditions (except turbidity) and sediment properties. Total phosphorus and total zinc concentrations in the overlying water of the polluted sediment were higher than in the overlying water of the clean sediment, because the polluted sediment contained higher phosphorus and zinc concentrations (1820 and

245 mg kg^{-1}, respectively) compared with clean sediment (596 and 42 mg kg^{-1} dw, respectively). Oxygen penetration depth in the clean sediment in the absence of chironomids was much higher than in the polluted sediment because the clean sediment contained less organic matter (1.2%) than the polluted sediment (7.8%). Sediment oxygen penetration depth is highly dependent on the input of labile organic matter (Kristensen & Hansen, 1995). Although the oxygen penetration depth and oxygen consumption were not related in this study, the penetration of oxygen into sediment is controlled by the balance between the downward transport and consumption processes of all benthic organisms (Kristensen & Hansen, 1995).

Interacting effects of chironomid density and sediment type were observed for oxygen penetration depth and sediment porosity. Although significant effects of the type of sediment on changing water quality conditions and sediment properties were observed, the pattern in changes was comparable for both sediments, and only the intensity of the changes differed between clean and polluted sediments. Hence, the effect of chironomid density was more important than the effect of sediment type.

Effects of sediment reworking on E. virgo

The changes in water quality parameters and sediment properties brought about by sediment reworking coincided with reduced survival and growth of *E. virgo*. This suggests that changes in physicochemical conditions may have contributed to the decreased performance of *E. virgo*. However, the effect of direct disturbance by chironomids on survival and growth of *E. virgo* may also play a major role, since both survival and growth of *E. virgo* decreased with increasing chironomid density. This raises the question of the relative contribution of altered water quality and sediment properties as opposed to direct disturbance by the chironomids to decreased survival and growth of the mayfly nymphs.

The increased particle and nutrient release because of sediment reworking observed in this study is not likely to inhibit mayfly survival and growth. In a study on the effects of pulp mill effluent on survival and growth of the mayfly *Baetis tricaudatus* (Dodds), higher survival and growth were observed when the mayflies were exposed to 1 and 10% effluent, which contained elevated nutrient concentrations compared with control river water (Lowell, Culp & Wrona, 1995). Similarly, the zinc concentrations in the overlying water alone (<35.4 μg L^{-1}) cannot explain the decreased survival and growth of *E. virgo*, since in a study of the effects of waterborne zinc on nymphs of *E. virgo*, a 10-d LC50 of 1840 μg L^{-1} was observed (Van der Geest *et al*., 2001). Although only Zn was analysed in the overlying water in this experiment other toxicants, such as metals and organic compounds, may have also been released in the overlying water as observed by Zoumis *et al.* (2001). Furthermore, the joint effects of all toxicants released from the sediment by bioturbation of chironomids in conjunction with oxygen deficiency could have contributed to the reduced performance of *E. virgo* (Lowell & Culp, 1999; Van der Geest *et al*., 2002). Van der Geest *et al.* (2002) demonstrated that anoxic and hypoxic conditions had detrimental effects on the survival of early *E. virgo* nymphs, but that at oxygen concentrations of 50% or higher, no effects on survival were observed after 4 days of exposure. However, oxygen depletion in combination with toxicant exposure, copper or diazinon, demonstrated that the adverse effects of copper for *E. virgo* nymphs were more severe at lowered oxygen concentrations, although such an effect was not observed for diazinon. Thus, in the polluted sediment the combined effect of oxygen and toxicants is a plausible cause of the observed increased mortality. Although increased reworking may have improved the survival of *E. virgo* via increasing oxygen penetration into the sediment, the simultaneous liberation of toxicants might have counteracted this. In summary, there are evident risks of low water quality for *E. virgo* nymphs in polluted sediments, but it is unlikely that such conditions are also present in the clean sediment. Hence, other processes must be responsible for the observed decreased mayfly survival.

Increasing densities of *C. riparius* larvae had a significant negative effect on survival of *E. virgo* nymphs in clean sediment. In the polluted sediment, however, only a slight negative effect was observed when chironomid density was increased, but survival was already poor when chironomids were absent, as observed in a previous study (De Haas *et al*., 2002).

During the experiments, it was observed that chironomid larvae in the clean sediment left their burrows more frequently than those in the polluted sediment. Since larvae of *C. riparius* mainly feed on detritus and organic matter present in the sediment, and the amount of food available in the clean sediment was lower than in the polluted sediment, the larvae in the clean sediment had to actively search for food. Additionally, chironomid emergence was only observed in the polluted sediment, indicating higher food availability in this type of sediment De Haas *et al.* (2002). An increase in time spent foraging outside their burrows when food is scarce was also observed in a study of the effects of food availability on the activity of *C. tentans* (Fabricius) larvae (Macchiusi & Baker, 1992) and with increasing chironomid densities, intraspecific competition intensifies among chironomid larvae (Rasmussen, 1985). It is therefore possible that, during foraging, chironomids in our study directly disturbed mayfly nymphs with negative consequences for their growth and survival. Interference competition by the amphipod *Gammarus lacustris* (Sars) on the net-spinning caddisfly *Ceratopsyche oslari* (Banks) had a comparable detrimental effect on net-building success because of destruction of the nets by the swimming and feeding activities of *G. lacustris* (Haden *et al.*, 1999). Kelly *et al.* (2002) observed that *Gammarus* attack, capture, and consume live *B. rhodani* mayfly nymphs, and nymphal body parts were found when *Gammarus* was present. Predation of the detritivorous *C. riparius* larvae on *E. virgo* nymphs is, however, unlikely, and no evidence of attack and consumption has been obtained. *Ephoron virgo* nymphs are too large for *C. riparius* to consume; the maximum particle size that can be ingested by third or fourth-instar *C. riparius* larvae is 60 and 100 μm, respectively (Vos *et al.*, 2002), whereas the average minimum and maximum width of first-instar *E. virgo* nymphs were 72.4 and 140 μm, respectively.

In contrast to survival, growth of *E. virgo* nymphs was only slightly reduced by increasing chironomid density. This decrease may be because of either direct competition for food or interference by chironomids in the feeding activity of the mayflies (Reynoldson *et al.*, 1994).

Our results may have some wider implications for the benthic fauna, sediment reworking and lake management. Firstly, this study shows that the return of the specialised mayfly *E. virgo* to the River Rhine, and associated waters, may be possible only in recently deposited clean sediments and that local old deposits are still toxic and unfit to support this species. Secondly, by demonstrating a strong impact of bioturbation on overlying water quality, we have shown a potential role for bioturbation in mobilisation of pollutants. Thirdly, our results indicate that the distribution of small specialised insect species, such as *E. virgo*, may be limited by bioturbating benthic invertebrates, especially in polluted ecosystems.

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