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### Persistence of benthic invertebrates in polluted sediments.

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# CHAPTER 5

## THE IMPACT OF SEDIMENT REWORKING BY OPPORTUNISTIC CHIRONOMIDS ON SPECIALIZED MAYFLIES

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*Submitted*

Bioturbation, the reworking of sediment by the feeding and burrowing activity of benthic organisms, is known to change the structure and properties of the sediment and consequently the flux of gasses, nutrients, and toxicants between sediment and water. Thus, bioturbators alter their own environment, and also that of other benthic species. Besides these indirect effects, benthic species may interact via competition for space and food, and by predation. This study aims, therefore, to examine the effects of sediment reworking by an opportunistic detritivore on survival and growth of a specialized mayfly species. To this purpose sediment reworking was imposed, by adding different numbers 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. 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. The survival and growth of *E. virgo* was strongly reduced in the presence of chironomids. In the polluted sediment the activity of chironomids enhanced the negative effects of pollution on *E. virgo*, but in the clean sediment inhibition of the mayfly was even more pronounced. This suggests that the direct disturbance by *C. riparius* was more important than the decreased water quality, and overruled the potential positive effects of improved oxygen penetration. This study implies that the distribution of specialized insects, like *E. virgo*, can be limited by bioturbating benthic invertebrates.

## INTRODUCTION

Bioturbation, the reworking of sediment by the feeding and burrowing activity of benthic organisms, changes the structure and properties of the sediment. These changes include an increase in oxygen penetration depth and oxygen consumption of the sediment (HARGRAVE 1975, GRANÉLI 1979b, SVENSSON & LEONARDSON 1996), a changed particle size distribution (IOVINO & BRADLEY 1969, MCLACHLAN & MCLACHLAN 1976) and increased sediment water content (CULLEN 1973, RHOADS 1974). This induces changes in the sediment-water transfer and the bioavailability of various compounds, such as nutrients and toxicants (GRANÉLI 1979a, PETERSEN et al. 1995).

Changes in sediment properties and nutrient and toxicant fluxes may alter the environment not only for the bioturbators themselves, but also for other benthic invertebrates (RHOADS & YOUNG 1970). These changed 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). Besides these indirect effects of bioturbators on other benthic invertebrates, interspecific interactions may also occur by competition for space and food (RASMUSSEN 1985, REYNOLDSON et al. 1994, HADEN et al. 1999), by predation (KELLY et al. 2002), or by increased food availability due to re-fractioning of food particles (VAN DE BUND & DAVIDS 1993).

Larvae of *Chironomus riparius* live in soft organic enriched sediments and construct burrows in the upper layer of the sediment (ARMITAGE et al. 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 may be mobile under less favorable conditions, leaving their tubes and undergoing migration (EDGAR & MEADOWS 1969). Hence, *C. riparius* larvae are assumed to have a high bioturbating capacity and to provoke interspecific competition. One of the species that may be affected by these activities is the mayfly *Ephoron virgo*, 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 burrow U-shaped tubes in the sediment and start to filter food, such as detritus and algae, from the water by generating wavelike movements 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 since water quality improved (BIJ DE VAATE et al. 1992). In spite of this water quality improvement, certain floodplain lake sediments of the River Rhine still contain high concentrations of xenobiotic compounds (KOELMANS &

MOERMOND 2000, DE HAAS et al. 2002). Therefore, floodplain lake sediments of the River Rhine act nowadays 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 sixties and seventies of the past century (BEURSKENS et al. 1993). The benthic communities of some of these floodplain lakes are thus exposed to a diffuse flux of sediment-bound toxicants and nutrients, while other lakes have newly deposited, cleaner sediments. The sediments of these different lakes are therefore suitable to study the combined effects of sediment reworking and historical pollution.

The aim of this study was to analyse the direct and indirect effects of sediment reworking by an opportunistic detritivore on the specialized mayfly *E. virgo*. To this purpose different numbers of the bioturbating midge *C. riparius* were added to clean and polluted sediment. Changes in water quality conditions and sediment properties, and the response of the mayfly *E. virgo* were assessed. Survival and growth of the mayfly were related to chironomid density, type of sediment, and water- and sediment characteristics.

## **MATERIALS AND METHODS**

### **Sample collection, storage and treatment**

About 25 L sediment was collected in September 2002 from a relatively clean and a historically polluted floodplain lake located along the River Waal, a branch of the River Rhine, the Netherlands, using an Ekman-Birdge grab, which was adjusted to sample the upper 5 cm of the sediment. The sediment was transported to the laboratory, where 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. 50 L lake water was collected in jerry cans. The water was filtered (GF/F, Whatman®, Maidstone, England), and stored at 4°C in the dark, under constant aeration.

### **Experimental design**

A clean and polluted sediment were selected for this study and the effects of sediment reworking were tested at four chironomid densities, resulting in eight treatments. Per treatment ten replicates were prepared, four for oxygen penetration depth measurements, three for sediment oxygen consumption measurements, and three for the *E. virgo* bioassays. Samples for the measurements of water quality parameters, seven replicates, were carefully taken from test systems from 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 after taking the water samples. All experiments were performed for 7 days. For each treatment ten replicate glass jars (150-ml) with 25 ml wet homogenized sediment and 100 ml filtered site water were prepared. In order to restore sediment stratification, the jars were incubated for seven days in a  $20 \pm 1^\circ\text{C}$  climate room with moderate light ( $\sim 10 \mu\text{molm}^2/\text{s}$ ) and a 16:7 hour light:dark regime with 30 min of twilight ( $\sim 5 \mu\text{molm}^2/\text{s}$ ) before and after each light period. During the experiment evaporated water was replaced with deionized water.

### ***Chironomus riparius***

After incubation of the test systems 0 (control), 5 (low-density;  $1667 \text{ ind/m}^2$ ), 10 (medium-density;  $3333 \text{ ind/m}^2$ ) or 20 (high-density;  $6667 \text{ ind/m}^2$ ) third instar *Chironomus riparius* ( $\sim 0.5 \text{ cm}$ ) larvae were introduced into the test systems. The larvae were obtained from a culture maintained in our laboratory, sieved ( $500\text{-}\mu\text{m}$ ) from the sediment, sorted, and transferred to the test systems. Remaining larvae were stored at  $4^\circ\text{C}$ . Emerged adults were replaced with third instar larvae to maintain the chosen bioturbation capacity. The amount of recovered midges at the end of the experiments was always higher than 85% of the initial density.

### ***Ephoron virgo***

To determine the effects of bioturbation by chironomids on survival and growth of the mayfly *Ephoron virgo*, three of the ten replicates of each treatment were stocked with first instar nymphs ( $< 48 \text{ h}$  old, average size  $757 \pm 41 \mu\text{m}$ ). These were obtained from field-collected eggs, kept in artificial diapause at  $4^\circ\text{C}$  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  $20^\circ\text{C}$  in order 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 twenty larvae was measured using a Leica<sup>®</sup> MZ 8 Microscope equipped with a Leica<sup>®</sup> 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 test 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 body length was

measured. Growth was calculated by subtracting the average initial length from the individual final length.

### **Oxygen measurements**

At the end of the test, oxygen profiles of the sediment-water interface were measured in four of ten replicates per treatment using a Clark-type oxygen sensor with an internal reference and guard cathode (Unisense OX100, Aarhus, Denmark). The sensor was connected to a high-sensitivity picoammeter (Unisense PA2000, Aarhus, Denmark). The electrode was driven into the sediment using a micromanipulator (Unisense MM33, Aarhus, Denmark). The oxygen sensor was calibrated using a pH/Oxi 340i oxygen meter (WTW, Weilheim, Germany) equipped with a CelloX 325 electrode (WTW, Weilheim, Germany). The oxygen concentration in the overlying water ranged from 9.35 to 9.85 mg/L. From the sediment oxygen profiles the oxygen penetration depth was calculated. Following the measurements of oxygen penetration depth (OPD) 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 of the ten replicates per treatment were used for sediment oxygen consumption measurements. The initial oxygen concentration was measured using a Clark-type oxygen sensor. After this the test systems were covered with a glass lid to prevent access of air and after 24 h the oxygen concentration was again measured. The oxygen consumption was measured from the fall in oxygen concentration in the known volume of the overlying water.

To measure the contribution of oxygen respiration by *C. riparius* larvae on the 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**

Turbidity of the overlying water was measured using a Turb 350IR turbidity meter (WTW, Weilheim, Germany). Total phosphorus (total P) in the overlying water was determined using an ammonium molybdate spectrometric method (MURPHY & RILEY 1962) within 2 days after sampling. For zinc analyses two samples of 2 ml overlying water were acidified with 40  $\mu$ l 69-70% nitric acid (Baker, Philipsburg, USA), and stored at -20°C until analysis. The samples were analyzed by air-acetylene Flame Atomic Absorption Spectrometry (Perkin-Elmer 1100B). Quality control of the metal analysis was carried out by analyzing blanks and reference material (NIST:SRM 1643, National Institute of

Standards and Technology, Gaithersburg, MD, USA). The measured values deviated less than 10% from certified values. Blanks were below detection limit ( $\sim 10 \mu\text{g/L}$ ).

### **Sediment characteristics**

Sediment porosity was measured in quadruplicate. The remaining 25 ml of overlying water was removed and the wet sediment was homogenized and stored in pre-weighed 50-ml polyethylene bottles at  $-20^\circ\text{C}$ . After freeze-drying sediment porosity was calculated from weight loss on drying.

The OM content of the sediment was measured as loss-on-ignition by combustion of 2 g dry sediment at  $550^\circ\text{C}$  for 6h (LUCZAK et al. 1997) in triplicate. Chlorophyll *a* (chl *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. Chl *a* and phaeophytin contents were summed, because in sediments chl *a* is already partly degraded into phaeophytin. Total phosphorus in the sediment was determined using an ammoniummolybdate spectrometric method (MURPHY & RILEY 1962).

For sediment metal analysis (Cd, Cu, and Zn)  $\sim 5$  mg dry sediment (triplicate) was weighed, placed in a 3 ml Teflon digestion vessel, and  $50 \mu\text{l}$  70%  $\text{HNO}_3$  Ultrex<sup>®</sup> (J.T. Baker<sup>®</sup>, Phillipsburg, NJ, USA) was added. Every 30 samples a blank (no sediment) and a reference (NIST:SRM 2704) was digested for quality control. The vessels were sealed and placed in a lined digestion vessel assembly and digested using a CEM<sup>®</sup> MD-2000 microwave system (CEM laboratories, Matthews, NC, USA). The vessels were heated to  $175^\circ\text{C}$  within 15 min and maintained at  $175^\circ\text{C}$  for another 45 min. The samples were diluted to exactly 2 ml with deionized water and analyzed by air-acetylene Flame Atomic Absorption Spectrometry (Perkin-Elmer 1100B). Standards and Technology, Gaithersburg, MD, USA). The measured values deviated less than 10% from certified values. Blanks were below detection limit ( $\sim 10 \mu\text{g/L}$ )

### **Statistical analyses**

In order to assess the contribution of sediment and chironomid density to explain the observed variation in survival, growth, and water and sediment parameters a Two-way ANOVA was performed with the General Linear Model Univariate procedure using a full factorial model 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<sup>®</sup> 10.0 for Windows (SPSS, Chicago, IL, USA).

## RESULTS

### Floodplain lake characteristics

The characteristics of the water and sediments of both floodplain lakes are listed in Table 5.1. Water quality conditions in both lakes were in agreement with Dutch water quality guidelines (CIW 2000) and thus not considered as polluted.

The concentrations of Cd, Cu, and Zn in the clean sediment were lower than the sediment quality criteria (CIW 2000), but contaminant concentrations in the polluted sediment were much higher.

Also food quantity and quality was higher in the polluted sediment than in the clean sediment. The organic matter content (OM) was higher in the polluted sediment than in the clean sediment (7.8% and 1.2% respectively), total phosphorus concentrations were higher in the polluted sediment compared to clean sediment (1820 and 596 mg/kg dw, respectively), and also the chl *a* concentrations were higher in the polluted sediment than in the clean sediment (16.3 and 7.3 mg/kg dw, respectively).

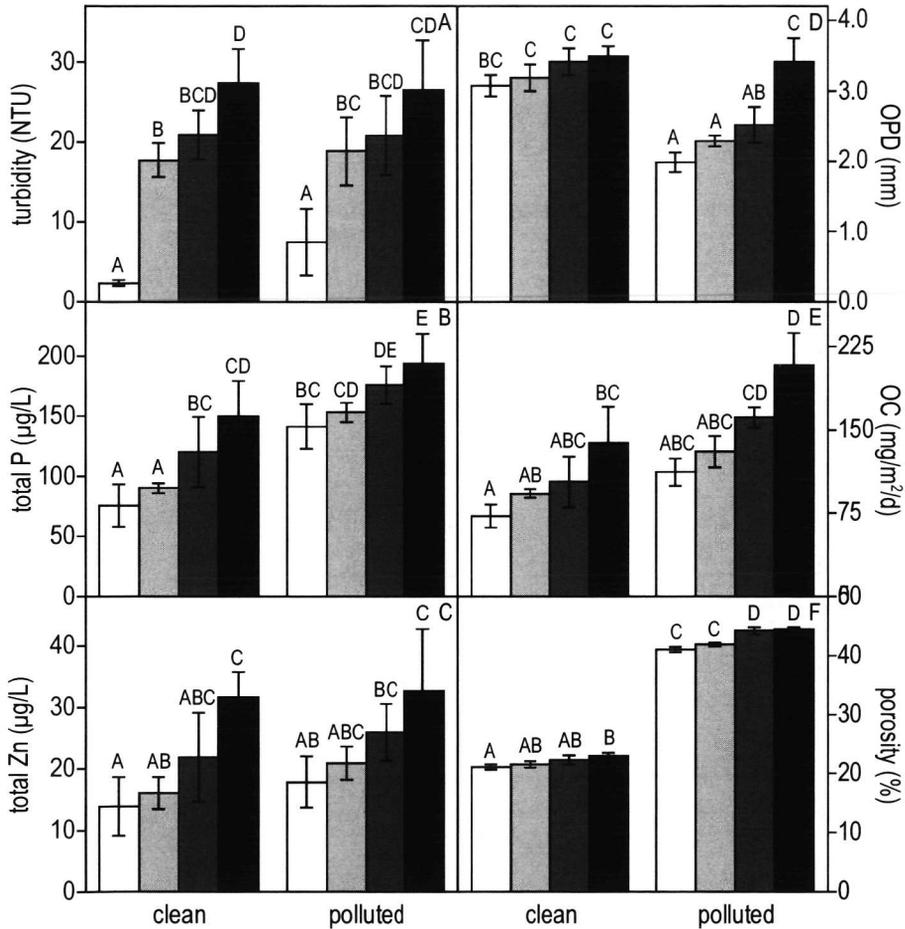
**Table 5.1.** Water quality and sediment characteristics of the floodplain lakes used in this study. Clean and polluted refers to the quality of the sediment deposit; total P = total phosphorus; OM = organic matter content; b.d. = below detection limit (~ 10 µg/L).

	clean	polluted
<b>water</b>		
turbidity (NTU)	1.58	2.89
total P (µg/L)	3.79	3.54
total Zn (µg/L)	b.d.	b.d.
<b>sediment</b>		
Cd (mg/kg)	0.20	2.05
Cu (mg/kg)	12	45
Zn (mg/kg)	39	245
porosity (%)	21.6	41.3
OM (%)	1.2	7.8
chl <i>a</i> (mg/kg)	7.3	16.3
total P (mg/kg)	596	1820

### Overlying water

Turbidity, total phosphorus, and total zinc in the overlying water was lower in the clean control than in the polluted control, but only total phosphorus was significantly different ( $P < 0.001$ ) (Figure 5.1<sub>A-C</sub>). With increasing chironomid density an increase of turbidity, total phosphorus, and total zinc was observed in both the clean and the polluted sediment. Significant higher turbidity compared

to controls was observed at all chironomid densities in both sediments ( $P < 0.001$ ). For total phosphorus significant increases compared to control treatments were observed at medium and high chironomid densities for both sediments ( $P < 0.001$ ), and significantly higher zinc concentrations compared to control concentrations were observed at the high chironomid density of both



**Figure 5.1.** 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; C = total Zn of the overlying water in µg/L; D = oxygen penetration depth (OPD) into the sediment in mm; E = oxygen consumption (OC) of the sediment in mg/m²/d; and F = porosity of the sediment in percentage. Error bars = standard deviations; bars sharing the same letter are not significantly different ( $P < 0.05$ ).

sediments ( $P < 0.001$ ). Turbidity, total phosphorus, and total zinc were not significantly different between treatments with and without *Ephoron virgo* (not shown). Thus, the presence of *E. virgo* in the test systems had no influence on water quality conditions as expected.

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 5.2). This indicates that turbidity of the overlying water is dependent on chironomid density, but independent on the degree of pollution of the sediment. The Two-way ANOVA for total phosphorus and zinc corroborated 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 in explaining the observed variation in total phosphorus and zinc concentrations (Table 5.2). This indicates that both the type of sediment and chironomid density affected the total phosphorus and zinc concentrations in the overlying water, but that the effect of chironomid density is independent on the type of sediment.

**Table 5.2.** Two-way ANOVA ( $P$  values) for water quality characteristics and sediment properties, with the type of sediment and chironomid density as the independent variables.

parameter	sediment	density	sediment density
turbidity	0.216	0.000	0.206
total phosphorus	0.000	0.000	0.096
total zinc	0.012	0.000	0.504
OPD	0.000	0.000	0.000
OC	0.000	0.000	0.353
porosity	0.000	0.000	0.006

### Sediment

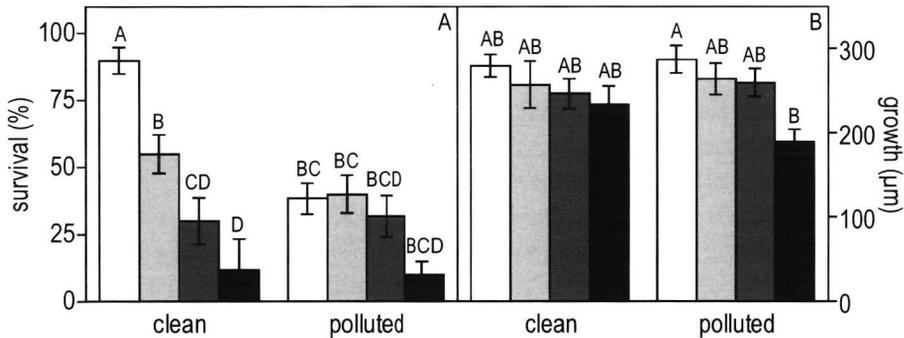
The oxygen penetration depth (OPD) in the clean control was significantly deeper than in the polluted control (3.08 and 1.99 mm, respectively,  $P < 0.001$ ). With increasing chironomid density an increase in OPD was observed in both the clean and the polluted sediment (Figure 5.1D); however, the trend seen in clean sediment is not significant. The OPD at the high chironomid density of the polluted sediment was significantly deeper than in the control ( $P < 0.001$ ), but not significantly different from the OPD in the clean sediment.

The oxygen consumption (OC) of the polluted control was higher than the OC of the clean control (109.7 and 71.0 mg/m<sup>2</sup>d, respectively), but not significantly different (Figure 5.1E). OC increased with increasing chironomid density in both the clean and the polluted sediment and a significant higher OC compared to

control OC was observed at the high chironomid densities of both sediments ( $P < 0.05$ ). *Chironomus riparius* larvae consumed  $2.75 \mu\text{g O}_2/\text{larvae}/\text{d}$  ( $R = 0.835$ ,  $P < 0.01$ ) (independent measurements, results not shown), which is 19.1% and 27.7% of the OC 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 (Figure 5.1F). Significant higher sediment porosity compared to the clean control was observed at the high chironomid density ( $P < 0.05$ ). Porosity of the polluted sediment at medium and high chironomid densities was significantly higher than sediment porosity of the polluted control ( $P < 0.001$ ).

The Two-way ANOVA verified that both type of sediment and chironomid density accounted for the observed variation in changed sediment properties ( $P < 0.001$ ) (Table 5.2). For both OPD and sediment porosity the interaction term was significant in explaining the observed variation ( $P < 0.01$ ) but not for OC. This indicates that both the type of sediment and chironomid density affected OPD, OC, and sediment porosity. The effect of density is dependent on the type of sediment for both OPD and sediment porosity, but independent for OC.



**Figure 5.2.** 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 ( $P < 0.05$ ).

### *Ephoron virgo*

Survival in the clean control (90%) was significantly ( $P < 0.001$ ) higher than in the polluted control (38.3%) and survival in both sediments declined with increasing chironomid density (Figure 5.2A). In clean sediment, significant lower survival compared to 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 ( $P < 0.001$ ) both sediment and the interaction term were not significant in explaining the observed variation in survival (Table 5.3).

Growth of the mayflies was not significantly different between the clean and the polluted control (276 and 289  $\mu\text{m}$  respectively). Growth decreased slightly, but not significantly, with increasing chironomid density on clean sediment (Figure 5.2B). On polluted sediment significant lower growth compared to control growth was found at the high chironomid density ( $P < 0.05$ ). A Two-way ANOVA demonstrated that only chironomid density accounted for the observed variation of growth ( $P < 0.01$ ) (Table 5.3).

**Table 5.3.** Two-way ANOVA for survival and growth of *Ephoron virgo*, with the type of sediment and chironomid density as the independent variables.

parameter	sediment	density	sediment density
survival	0.261	0.006	0.698
growth	0.649	0.002	0.502

## DISCUSSION

### Effects of sediment reworking on water quality and sediment properties

Our results clearly demonstrated that *Chironomus riparius* larvae strongly altered the benthic environment, since a significant effect of chironomid density on all measured water quality parameters and sediment properties was observed. The increase of turbidity, total phosphorus, and total zinc in the overlying water in the presence of chironomids in our study, is in concurrence with observations from other studies (GRANÉLI 1979a, LEE & SWARTZ 1980, KRANTZBERG 1985, HANSEN et al. 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 in agreement with other studies (HARGRAVE 1975, GRANÉLI 1979b, SVENSSON & LEONARDSON 1996).

The type of sediment influenced the impact of bioturbation on water quality conditions and sediment properties, since a significant effect on water quality, except for turbidity, and sediment properties was observed for the type of sediment. 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, respectively) compared to clean sediment (596 and 39 mg/kg dw, respectively).

The 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 sediments is often controlled by the balance between the downward transport and consumption processes of all benthic organisms (KRISTENSEN & HANSEN 1995). Coherent to the oxygen penetration depth, the oxygen consumption in the polluted sediment was much higher than in the clean sediment.

Interacting effects of chironomid density and the type of sediment 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 sediment. Hence, the effect of chironomid density was more important than the effect of sediment type.

#### **Effects of sediment reworking on *Ephoron virgo***

The changes in water quality parameters and sediment properties brought about by the sediment reworking coincided with the decrease in survival and growth of *Ephoron virgo*. This suggests that these changes 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 about the contribution of the altered water quality and sediment properties and the direct disturbance by the chironomids to the decreased survival and growth of the mayfly nymphs.

The increased particle and nutrient release due to 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*, higher survival and growth were observed when the mayflies were exposed to 1% and 10% effluent, which contained elevated nutrient concentrations compared to control river water (LOWELL et al. 1995). Also the zinc concentrations in the overlying water alone (< 35.4 µg/L) can not explain

the decreasing survival and growth of *E. virgo*, since in a study on the effects of waterborne zinc to nymphs of *E. virgo* a 10-day LC50 of 1840 µg/L was observed (VAN DER GEEST et al. 2001). However, the joint effects of all toxicants released from the sediment by bioturbation of chironomids in conjunction with oxygen deficiency could have contributed to the decreasing performance of *E. virgo* (LOWELL & CULP 1999, VAN DER GEEST et al. 2002). 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* by increased oxygen penetration in 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 on polluted sediments, but it is unlikely that such conditions are also present in the clean sediment. Hence, other processes are responsible for the observed decreased mayfly survival.

*C. riparius* larvae had a significant negative effect on survival of *E. virgo* nymphs in clean sediment with increasing densities. In the polluted sediment, however, only a slight negative effect was observed when the chironomid density increased, but survival was already poor when chironomids were absent, as observed in a previous study (DE HAAS et al. 2002). Since larvae of *C. riparius* are mainly feeding on detritus and organic matter present in the sediment and the amount of food available on the clean sediment was lower than in the polluted sediment the larvae in the clean sediment had to actively search for food. During the experiments it was observed that the chironomid larvae in the clean sediment left their burrows more frequently than the larvae in the polluted sediment. In agreement, an increase in the time spent on foraging outside their burrows when a low amount of food was available, was observed in a study on the effects of food availability on the activity of *Chironomus tentans* larvae (MACCHIUSI & BAKER 1992). It is therefore possible that the chironomids in our study directly disturbed the mayfly nymphs during their foraging. Indeed, with increasing chironomid densities the intraspecific competition between the chironomid larvae intensifies (RASMUSSEN 1985), and the amount of time spent on foraging of the chironomids outside their burrows could have increased with increasing chironomid densities. Consequently survival and growth of *E. virgo* could have decreased by increased interference with *C. riparius* larvae. Interference competition by the amphipod *Gammarus lacustris* on the net-spinning caddisfly *Ceratopsyche oslari* had a comparable detrimental effect on the net-building success by *C. oslari*, due to destruction of the nets by the swimming and feeding activities of *G. lacustris* (HADEN et al. 1999). KELLY et al. (2002), observed that *Gammarus* sp. attack, capture, and consume live *Baetis*

*rhodani* mayfly nymphs, and nymphal body parts were found when *Gammarus* sp. was present. Predation of the detritivorous *C. riparius* larvae on *E. virgo* nymphs is, however, not likely, and no evidence of attack and consumption has been observed. *E. virgo* nymphs are too large for *C. riparius* to be consumed, the maximum particle size that can be ingested by third and fourth instar *C. riparius* larvae is 60 and 100  $\mu\text{m}$  respectively (VOS et al. 2002). The average minimum and maximum width of first instar *E. virgo* nymphs were 72.4 and 140  $\mu\text{m}$  respectively. In contrast to survival, growth of *E. virgo* nymphs was only slightly affected by increasing chironomid density. This decrease can be due to either direct competition for food or interference by chironomids in the feeding activity of the mayflies (REYNOLDS et al. 1994).

In summary, the available evidence indicated that the direct effect of sediment reworking by *C. riparius* is strongly reducing the survival of young *E. virgo* nymphs, and that water quality modified by sediment reworking plays a distinct strong role in the polluted sediment. Our observations may have some implications for the views on the benthic fauna and sediment reworking in lakes. Firstly, this study shows that the return of the specialized mayfly *E. virgo* in the River Rhine, and associated waters, is possible only in recently deposited clean sediments and that local old deposits are still toxic and unfit to support this species. Secondly, by showing a strong impact of bioturbation on the water quality of the overlying water, we showed a potential role of bioturbation in mobilization of pollutants. Thirdly, this study implies that the distribution of small specialized insect species, like *E. virgo*, may be limited by bioturbating benthic invertebrates.

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