In situ bioassays with *Chironomus riparius*: Laboratory-field comparisons of sediment toxicity and effects during wintering

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A field bioassay was developed to study the in situ effects of sediment contamination on Chironomus riparius *larvae. The survival, development rate and increase in* Chironomus *larvae biomass was compared between laboratory bioassays with the field cages. The incidence of mentum deformities was compared not only between laboratory and field bioassays but also with observations on field populations of* Chironomus *larvae. Survival in the field bioassays was slightly higher than the laboratory, except at locations with known contamination of the surface water. The influence of surface water quality in field bioassays was demonstrated in translocation experiments, in which clean sediment was placed in a polluted site, and vice versa. In a field bioassay carried out in the autumn, an inverse relationship between the rate of development and the initial larval density in the field cages was apparent. In addition, in the field bioassays with* C. riparius *considerable seasonal variation in the survival and incidence of mentum deformities was found. Field bioassays performed during the winter season indicate that low temperatures can interact with or add to the effects of sediment contamination on chironomid populations.*

Keywords: survival, mentum deformities, *Chironomus*, biomass

Introduction

In the past decades several bioassays have been developed for the assessment of sediment toxicity. Historically, sediment quality assessment of sediment toxicity has been laboratory oriented, with testing confined to laboratory-grown species under controlled conditions. Although bioassays are useful tools for risk assessment, it is also widely accepted that the extrapolation of effects observed in the laboratory to the complexity of the field situation is problematic (Munawar et al., 1991). Interactions between physical, chemical and biological stressors which are specific for the exposure site can result in effects occurring in the field that are different from those observed in laboratory bioassays. Since field bioassays are designed to expose test organisms to the actual field situation, these tests may bridge the gap between laboratory and field observations. In some cases, field bioassays may even replace investigations of field populations, e.g. in cases where no organisms can be collected from polluted sediments.

Several in situ bioassays for testing responses on field sediments have been developed, e.g. for phytoplankton (Munawar and Munawar, 1987), the fresh water amphipods *Hyalella azteca* (Shaw and Manning, 1996; Chappie and Burton, 1997) and *Gammarus pulex* (Schultz and Liess, 1999; Crane and Maltby, 1991), the marine amphipod *Corophium volutator* (Kater et al., 2000), the water fleas *Daphnia magna* (Pereira et al., 1999) and *Ceriodaphnia dubia* (Sasson-Brickson and Burton, 1991; Ireland et al., 1996), the midge *Chironomus tentans* (Chappie and Burton, 1997; Sibley et al., 1999), the mayfly *Caenis* sp. (Shaw and Manning, 1996), the caddisfly *Limnephilus lunatus* (Schultz and

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Liess, 1999) and the oligochaete *Lumbriculus variegatus* (Sibley et al., 1999).

At our institute a field bioassay was developed for *Chironomus riparius*. *C. riparius* is easy to culture in the laboratory and is, together with *C. tentans*, commonly used for sediment toxicity testing (Den Besten et al., 1995; Watts and Pascoe, 1996; Giesy et al., 1988). A recent study demonstrated that *C. riparius* is more physically robust than *C. tentans*, resulting in less variable effect data (Watts and Pascoe, 2000). For the in situ bioassay with *C. riparius* special cages were developed to ensure an optimal exchange between the test organisms and the environment. The present paper describes a study on the effects of sediment pollution on *C. riparius* in laboratory and field bioassays and in chironomid populations in the field. The main question was to investigate whether sediment toxicity is different under field conditions compared to the laboratory. In addition, the effect of larval density on the rate of development of *C. riparius* in field bioassays was studied. Special attention was given to seasonal variation in the effects on chironomids. Field bioassays carried out during the winter season were combined with monitoring of the success of emergence.

Methods

Sampling sediment and chironomids at field sites

Sediment samples were taken using an Ekman grab (0.025 m^2). Several grabs of the top layer of the sediment (0–10 cm) were pooled and stored in glass bottles or PVC buckets at 4°C until use for chemical analysis or bioassays. Storage time was no longer than four weeks. For studies on field populations of midge larvae, sediment was sieved on a 500 μm mesh sieve. Midge larvae were sorted out and preserved in 70% ethanol. In the laboratory, third or fourth instar *Chironomus* larvae were selected for analysis of mentum deformities (see below).

Laboratory sediment bioassay with *C. riparius*

For the laboratory bioassay with *Chironomus riparius* the standard method described by Van de Guchte et al. (1993) and Den Besten et al. (1995) was modified in order to have larger numbers of larvae in the tests. In addition, the standard temperature was lowered from 20°C to 15°C in order to make conditions more comparable to the situation in the field. Sediment-water systems were made in aquaria (surface area 12 × 20 cm) by mixing 200 ml of sediment with 800 ml of Dutch Standard Water (DSW, see Maas et al., 1993) for 24 h at 15°C, after which the sediment was allowed to settle for three days. Egg packets from a laboratory culture of *Chironomus riparius* were kept in small open vials in the overlying water of the sediment-water systems until hatching. 250 larvae were then taken out of the vials and distributed in the aquaria and kept at a temperature of 15°C. The aquaria were aerated continuously and three times per week the larvae were fed with a food suspension (Trouvit: during first two weeks 3 × 0.01 g dw per aquarium per week; from third week onwards 3 × 0.3 g dw per aquarium per week). In addition, the sediment-water systems were checked twice a week to meet validity criteria for the following parameters: pH, oxygen concentration, nitrite concentration, ammonium concentration and conductivity. After 5.5 weeks the number of living second, third and fourth instar larvae was counted, after which the larvae were preserved in 70% ethanol for analysis of mentum deformities (see below). The sensitivity of the test organisms was checked at the start of each series of tests by determining the acute 96h toxicity of potassium dichromate using second instar larvae.

Field bioassay with *C. riparius*

For the in situ bioassay with *C. riparius* a double sided field cage was developed as shown in Figure 1. The inner cage consists of a polyethylene container (total volume approx. 1.1 L) with a lower compartment that is filled with sediment (volume of 400 ml) and an upper compartment that contains two openings covered with a gauze with mesh size 150 μm (total window area of 200 cm^2 for exchange of water). The inner cage is closed by fastening a polyethylene disc on top of the container. This container is enclosed by a second container that is made of stainless steel gauze of a mesh size of 500 μm and that is closed also by a polyethylene disc. In the laboratory, sediment previously collected at a test location (<4 weeks old) was transferred to the lower compartment of the inner cage. At the start of the experiment 70 second instar larvae from the laboratory culture of *C. riparius* were placed on top of the sediment in the field cage and allowed to settle. If necessary, the field cages were then kept in temperature rooms in the laboratory for acclimation to ambient surface water temperature (rate of temperature change: 2°C per day; for water temperatures at the field sites, see Results). At the field location, the cages were attached to a pole (drilled in the sediment) just above

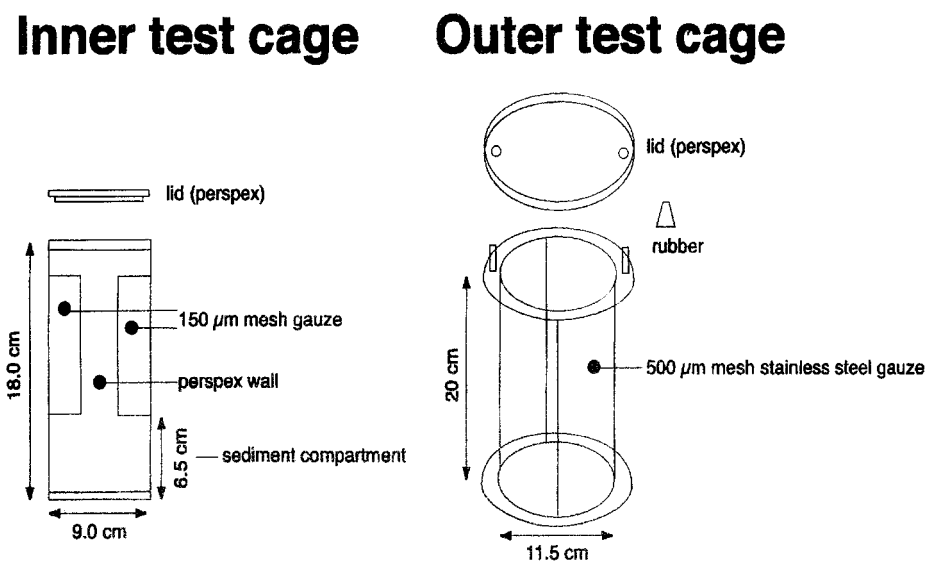

Figure 1. Diagram of the field cage used for in situ bioassays with *C. riparius*.

the sediment, ensuring that with fluctuating water levels the cages would remain submerged. If necessary, extra cages were set out in the field in order to check the rate of development of the larvae (and aiming at 50% or more fourth instar larvae at the reference site). After four weeks (or a longer period in colder seasons) the cages were taken out of the water and transferred to the laboratory. The sediment was sieved on a 500 μm mesh sieve, after which the midge larvae were counted and preserved for mentum analysis (see below).

Determination of larval biomass

Of each of the preserved larvae the length was measured using a microscope. The biomass of the midge larvae was calculated using the length of the larvae and relations between length and biomass that have been published by Smit et al. (1993).

Analysis of mentum deformities

Mentum deformities of field larvae or larvae from laboratory or field bioassays were investigated according to the method described by Warwick (1988). The following deformities were screened: Köhn gaps, tooth asymmetry, tooth deletion (other than breakage, including tooth surface reduction of >50%), tooth displacement, tooth addition and tooth splitting. Mentum fractures were identified using the characteristic irregular tooth margin induced by the rupture of the mentum and were discarded.

Physico-chemical analysis of sediment

Physico-chemical analyses were carried out as described in Den Besten et al. (1995) and Reinhold-Dudok van Heel and Den Besten (1999). Contaminant levels were normalised according to the approach described by CUWVO (1990), in order to compensate for differences in sorption characteristics between sediments (standard sediment was defined as having a 25% particle fraction <2 μm and 10% organic matter on a dry weight basis; for details see Den Besten, 2003 elsewhere in this issue). Normalised contaminant levels were compared with the Dutch sediment quality criteria. The final sediment classification ranged from class 0 (not contaminated) to class 4 (highly contaminated) based on the highest classification of individual contaminants (Ministry of Transport, Public Works and Water Management, 1994).

Test sites

Field bioassays were carried out in the following locations: Oostvaardersplassen (OVP), Ketelmeer (KET), Brabantsche Biesbosch (BRB), Delfland (DEL) and Dommel (DOM). Based on the levels of about 40 different contaminants, sediment from the Oostvaardersplassen and Delfland is indicated to be relatively clean (class 1), whereas sediment from Ketelmeer, Brabantsche Biesbosch and Dommel can be regarded as strongly polluted (class 3/4). With regard

to the quality of surface water, Oostvaardersplassen is relatively clean. Surface water of Ketelmeer (receiving water from the river Rhine) may contain elevated levels of contaminants due to resuspension of polluted sediments; this may be the case even more in the Brabantsche Biesbosch (receiving water with elevated levels of metals and PAHs from the river Meuse). Surface water from Delfland is known to contain high levels of herbicides; surface water quality at the selected site in the Dommel is influenced by upstream sediment contamination with heavy metals (discharged until 1992 by a zinc factory) and other pollutants from a sewage treatment plant. In some experiments sediment from the shallow site OVP (water depth <0.5 m) was translocated to the deeper location Vossemeer (water depth 0.5–2 m) which is close to location KET but has a much better water quality (limited influence of Rhine water) and therefore is regarded as another reference site. The geographic position of the field locations is shown in Figure 2.

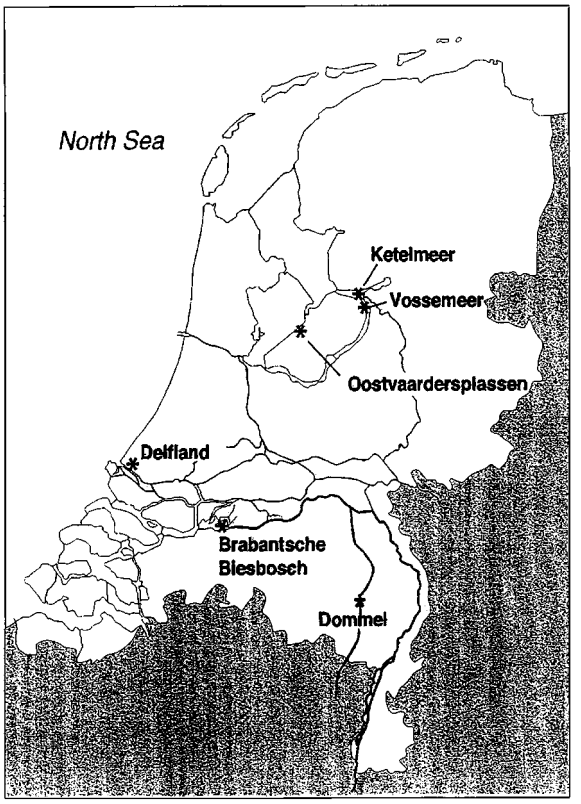

Figure 2. Locations in the Netherlands selected for the field studies.

Description of the experiments

For a laboratory-field comparison, laboratory bioassays were carried out in June 1994, while field bioassays were carried out twice, in April–May (duration 35 days) and in May–June 1994 (duration 28 days). In addition, in April, May and June 1994 chironomids were sampled from the field.

In July–August 1993, September-October 1993 and November 1993-March 1994 field bioassays were carried out at the sites OVP en KET, in order to compare the effects between three different seasons. In addtion, in May 1998 laboratory bioassays were carried out with sediment from the polluted site DOM at 10, 15 and 20°C.

In 1998 a translocation experiment was carried in which sediment from the reference site OVP was translocated in field cages to the contaminated site BRB while sediment from BRB was translocated to OVP. In addition controls were carried out to test survival and incidence of mentum deformities of *Chironomus riparius* in the sediments at the original locations.

In order to investigate the influence of larval density on survival and biomass, in October-November 1998 field bioassays were started with variable numbers of larvae (between 30 and 90 per cage). Sediment from the reference site OVP was transferred to field cages and placed for 5.5 weeks in the relatively unpolluted location Vossemeer (see Figure 2).

Finally, field bioassays were carried out in order to study the wintering of *Chironomus* larvae. After acclimation, in December 1998, 10 field cages with sediment from the reference site OVP were placed at a depth between 0.5 and 1 m in the location Vossemeer and 10 field cages with sediment from location BRB were placed in location BRB. The initial larval density was 70 individuals per cage. In January, March and April 1999 survival and mentum deformities were determined and in addition, in April 1999 field cages were transferred to the laboratory in order to monitor the emergence of chironomids. The inner compartments of the latter cages (see Figure 1) were transported to the laboratory at the end of the winter period and kept there as sediment-water systems in fly cages, in order to collect adult midges. The larvae were slowly acclimatised to a higher temperature by increasing the temperature from 12 to 20°C by 1°C per day; during this acclimatisation and the following weeks the larvae were fed with a Trouvit suspension (once a week 0.1 g dw of Trouvit). The emergence of *Chironomus* larvae from the field cages that had been transferred

to the laboratory was checked daily by counting adult midges after which they were preserved in ethanol.

Statistics

Results are presented as means ± SD. Differences were tested by Student's T-test. P = 0.05 was used as the standard significance level. Regression analysis was performed with Excel 7.0.

Results

Laboratory-field comparison

The water temperature during the first series of field bioassays (April–May 1994; duration 35 days) was between 7 and 18°C (increasing rapidly in time during the bioassay), and between 16 and 20°C during the second series of field bioassays (May–June 1994; duration 28 days). Only for the first series it was necessary to acclimatise the field cages at the start of the experiment (from 15°C to 7°C in four days). The acclimatised larvae were still in their second instar stage at the start of the experiment. The two series of field bioassays produced consistent results, except at location DOM. Survival in the field bioassays (data of the two series taken together) was slightly higher than in the laboratory bioassays, except in the case of location DEL where the percentage survival in the field bioassay was reduced to zero (Figure 3). In the locations BRB and DOM the survival in the laboratory bioassay was significantly lower than in the reference site OVP. In comparison with the reference site, survival in the field bioassays was significantly lower at the locations BRB and DEL. At location DOM, a significantly lower survival (mean percentage of 26%) was found after exposure in April–May, whereas no effect was observed in the field bioassay of May–June. In the laboratory bioassays more than 60% of the larvae were in the fourth instar stage at the end of the test. Except for location DEL, at the end of the field bioassays 50% or more of the larvae were in the fourth instar stage. The incidence of mentum deformities measured in these experiments was generally low, but in comparison to the reference site significant differences were found in the laboratory bioassays for the locations KET and DOM. In comparison to the reference site, in the field bioassay at location BRB

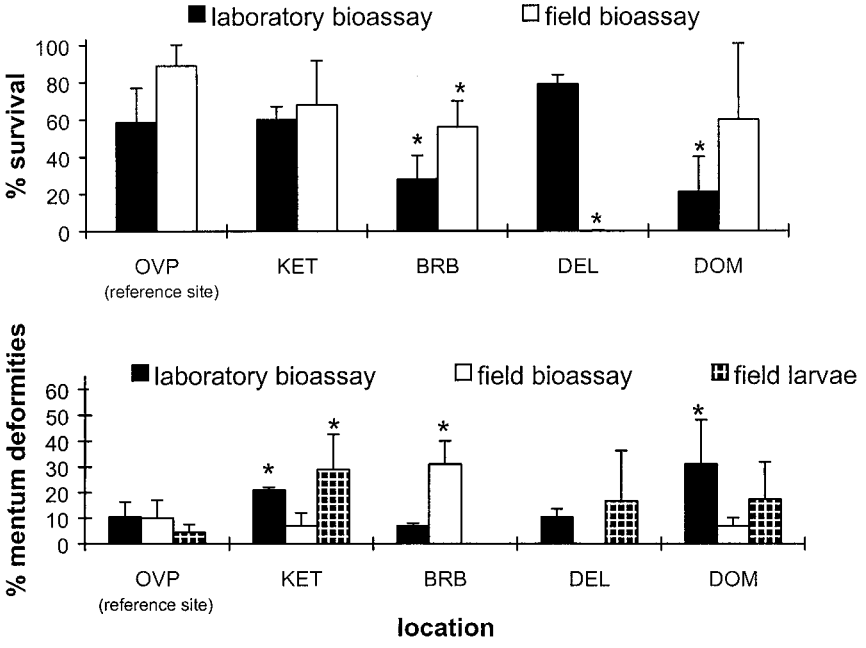

Figure 3. Upper part: comparison of survival of *C. riparius* after four to five weeks exposure in laboratory bioassays or field bioassays to contaminated sediments. Bars represent mean percentages ± SD (for laboratory bioassays n = 3; for field bioassays n = 6). Lower part: mentum deformities in *C. riparius* larvae after 4 weeks exposure in laboratory bioassays (n = 3), field bioassays (n = 6), or in *Chironomus* larvae from field populations (n = 3). *Significantly different from reference (OVP; $p < 0.05$). Each single observation of mentum deformities represents screening of 50 larvae (if survival permitted this).

a significantly higher incidence of deformities was found. The field population of *Chironomus* larvae at location KET was found to have a significantly higher incidence of mentum deformities than in OVP sediment (Figure 3). The incidence of mentum deformities measured in field populations of *Chironomus* at the locations KET and DEL was more comparable to the percentages observed in the laboratory bioassays than to effects observed in the field bioassays (Figure 3).

Variation in bioassay responses in different seasons or at different temperatures

Figure 4 shows a comparison of field bioassays at the sites OVP en KET that were carried out in three different seasons. The duration of the field bioassays in summer, autumn and winter were, respectively, 4, 6 and 16 weeks with temperature ranges of respectively 17–20°C, 8–15°C and 2–8°C. Survival and incidence of mentum deformities observed in the field bioassays carried out in summer with sediment from OVP and KET were comparable to the corresponding observations for OVP and KET in the laboratory-field comparison that was carried out in 1994 (compare results shown in Figure 3 with those shown in Figure 4). For example, survival at site KET was 59% in the field bioassay carried out in July–August 1993 and 68% in April–June 1994. In autumn and winter significant differences were found between the degree of effects at the polluted site and the reference site, but in addition, mortality and incidence of mentum deformities were significantly higher in winter compared to summer and autumn. The mean survival in the field bioassay at site KET was in the winter season as low as 7%, compared to 41% at the reference site. The mean percentage of mentum deformities at site KET was 34%, which is significantly higher than the percentage observed in summer (12%) and autumn (11%). In laboratory bioassays with metal-contaminated sediment from river Dommel (site DOM) the effects of temperature as modifying factor were studied. Survival of *Chironomus* larvae in laboratory bioassays with polluted sediment from location DOM was somewhat lower at 10°C compared to the higher temperatures and corresponded to a strongly increased incidence of mentum deformities (Table 1).

Translocation experiment

The results of the translocaton experiment are shown in Table 2. After translocation, survival of *C. riparius*

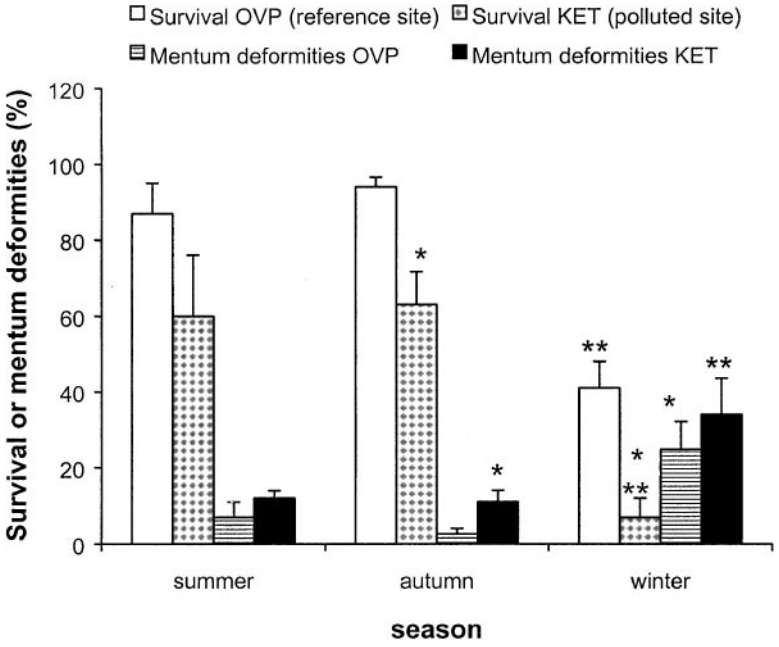

Figure 4. Survival and frequency of mentum deformities of *C. riparius* in field bioassays carried out in Summer, Autumn and Winter. Exposure times were 4, 6 and 16 weeks, respectively. Bars represent mean percentages ± SD (n = 3). *Significantly different from reference ($p < 0.05$); **Results in winter significantly different ($p < 0.05$) from those found in summer and autumn.

Table 1. Effect of temperature on survival and mentum deformities of *Chironomus riparius*, in a laboratory exposure experiment to contaminated sediment from river Dommel (site DOM).[1]

Temperature/ Sediment	Survival (%)	Mentum Deformities (%)
10°C/DOM	65.4 ± 6.1	56.7 ± 4.2*
15°C/DOM	76.0 ± 6.1	13.0 ± 4.6
20°C/DOM	70.0 ± 3.0	16.3 ± 3.2
15°C/OVP (laboratory control)	80.8 ± 9.2	10.5 ± 5.8

[1] Values are means ± SD (n = 3).
*Significantly different from percentage mentum deformities in DOM sediment at 15 and 20°C ($p < 0.05$).

in OVP sediment at site BRB was slightly lower while the incidence of mentum deformities had increased. These differences were not significant, however. By contrast, translocation of BRB sediment to the OVP reference site resulted in a significantly higher survival and a significantly lower percentage of mentum deformities.

Effect of larval density on rate of development and growth

Figure 5 shows the effect of larval density on survival, development and biomass in reference sediment that had been translocated to the relatively unpolluted location Vossemeer (for map, see Figure 2). The field bioassay was carried out for 5.5 weeks, in October-November 1998, during which the water temperature dropped from 14 to 7°C. While the survival at the end of the experiment was around 50% and independent of the initial larval density, a significant effect of larval density was observed on the proportion of larvae that had reached the fourth instar stage (Figure 5): with increasing larval density the percentage of larvae that reached the fourth instar stage dropped from 23 to 2%, and the percentage of third instars increased accordingly. The trend of increasing numbers of third instar larvae was found to be accompanied with a slightly decreasing larval biomass. Also for the fourth instar larvae a trend of decreasing mean larval biomass with increasing initial larval density was found, except for the cage with an initial larval density of 90 per cage, where a relatively high larval biomass was measured (Figure 5).

Survival and emergence of *Chironomus* larvae after wintering in field bioassays

During the experiment (December–April), the temperature in the surface water at the two field locations Vossemeer and BRB varied between 1 and 12°C, with lowest temperatures in the first two months. Already in January, six weeks after the start of the experiment, at both sites more than 90% of the larvae were in the third instar stage (not shown). In March about 50% of the larvae were in the fourth instar stage, while in April the percentage had increased to 95% or more (not shown; no differences found in rate of development between the two sites). In sediment from OVP (kept in Vossemeer) 62% of the larvae survived during the experiment (the four months winter period; Figure 6). A significantly lower survival rate (31%) was found in the BRB location (Figure 6). At the end of the field exposure (April), the mean biomasses of the fourth instar larvae in OVP and BRB sediment were, respectively, 0.9 and 1.0 mg per individual (not shown). For both locations, the success of emergence was estimated in the laboratory during a six weeks period following the end of the field bioassay in April 1999. No differences between the two sites were observed in the time until emergence (ranging between 3 and 6 weeks incubation

Table 2. Translocation experiment with the *Chironomus riparius* field bioassay.[1]

Condition	Survival (%)	Mentum Deformities (%)
Reference sediment from OVP; field bioassay at the same site	64.9 ± 11.5	12.9 ± 8.4
Reference sediment from OVP translocated to field bioassay at site BRB	48.1 ± 5.5	28.0 ± 15.8
Polluted sediment from BRB; field bioassay at the same site	39.9 ± 10.3	48.5 ± 10.9
Polluted sediment from BRB translocated to field bioassay at reference site OVP	76.7 ± 6.8*	15.3 ± 8.9*

[1] Values are means ± SD (n = 3).
*Siginificantly different from non-translocated situation ($p < 0.05$).

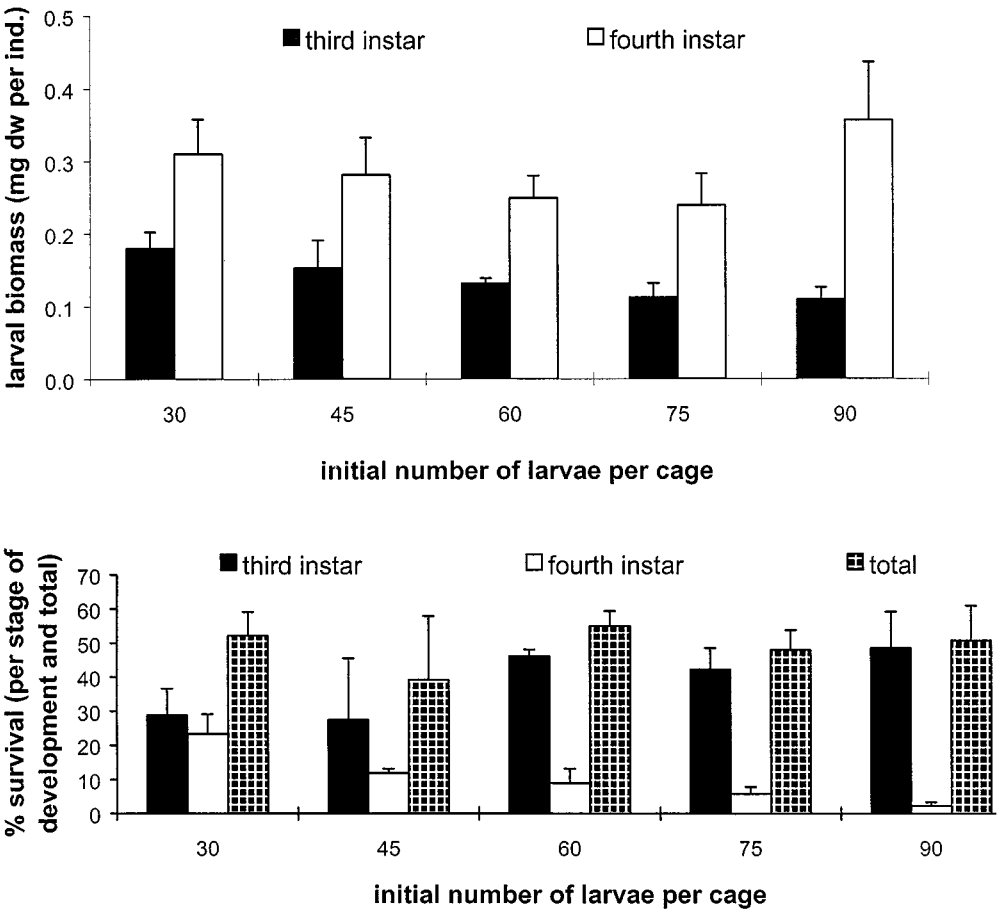

Figure 5. Effect of larval density on survival, rate of development and larval biomass of *C. riparius* after 5.5 weeks incubation in a field bioassay with reference sediment. Bars represent mean ± SD (n = 3). r^2 of the analysis of the regression between initial larval density and mean percentage of third and fourth instars at the end of the bioassay was 0.75 and 0.90, respectively; r^2 for the relation between initial larval density and mean biomass of third and fourth instars (for the latter excluding biomass found at highest initial density) was 0.94 and 0.96, respectively. Regression coefficients were significant at $p < 0.05$.

in the laboratory; not shown). The percentage of emerging larvae was much lower than the survival rates found at the end of the exposure in the field: for OVP sediment 12% vs 62%, and for BRB 8% vs 31% (all expressed as percentage of the number of larvae at the start of the field bioassay). Still, the success of emergence in BRB sediment was significantly lower than in OVP sediment (Figure 6). Figure 6 also shows the incidence of mentum deformities measured after different exposure times in the field bioassays. In sediment from BRB, considerably higher percentages of mentum deformities were found than in OVP sediment. The highest incidence of mentum deformities (57% in BRB sediment in January) was already found after six weeks of exposure.

Discussion

In a number of studies reported in literature, stronger effects in field bioassays in comparison with laboratory tests were found, e.g. with fresh water zooplankton species tested on moderately polluted sites (Sasson-Brickson and Burton, 1991) and with the marine amphipod *Corophium volutator* (Kater et al., 2001). In the laboratory-field comparison of the present study with *C. riparius*, effects in laboratory bioassays were for a number of sites somewhat stronger than under field conditions. Also in the laboratory bioassay with reference sediment from Oostvaarderplassen (OVP) a relatively low survival was found (about 60%), while 80% or more is considered normal in the standard

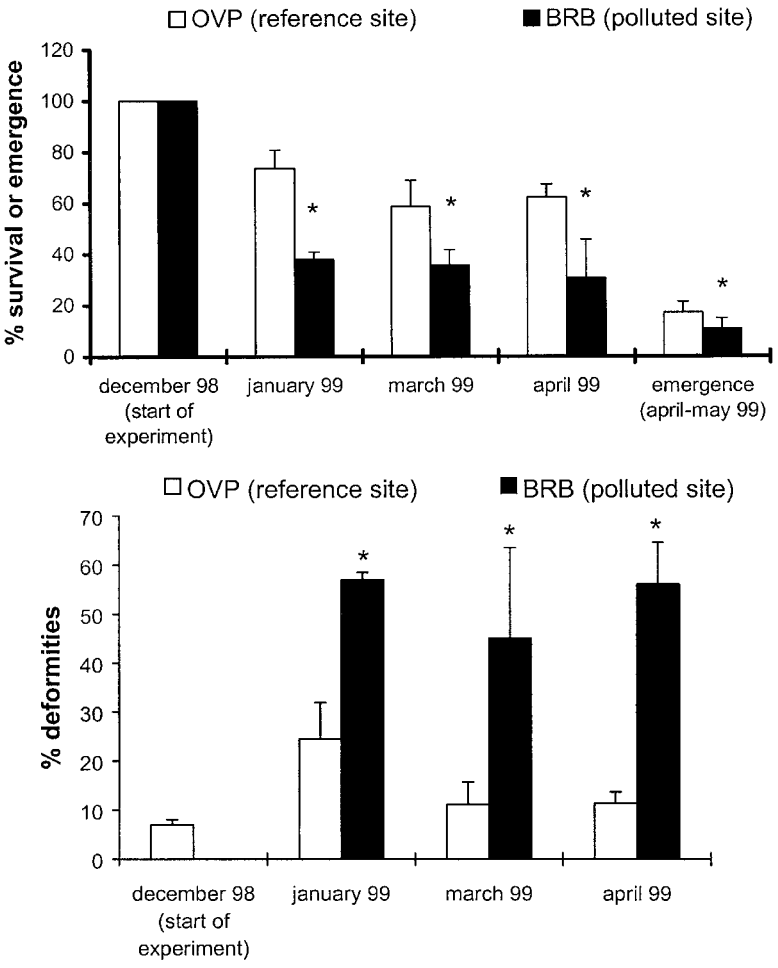

Figure 6. Upper part: comparison of survival of *C. riparius* in field bioassays during winter using reference sediment and contaminated sediment, from December 1998 until April 1999, and the following success of emergence in April–May 1999. Lower part: mentum deformities in *C. riparius* in field bioassays during winter. Bars represent mean ± SD (n = 3). *Significantly different from reference ($p < 0.05$).

Chironomus bioassays with sediment from this area (Maas et al., 1993). The latter could be confirmed in a laboratory bioassay with OVP sediment carried out later (as control in the temperature effect experiment), in which 81% survival was found.

Many factors can influence the toxicity of contaminants in the sediment. It has been reported that sediment toxicity can be modified during sediment sampling and the manipulations necessary for laboratory bioassays (Sasson-Brickson and Burton, 1991; Pereira et al., 1999). For example, changes in the redox conditions during the necessary homogenisation of sediment for laboratory bioassays could cause an increase of the toxicity caused by metals. This could have modified sediment toxicity especially in the laboratory bioassays with sediment from the known metal-polluted sites in Brabantsche Biesbosch and Dommel.

The strongest differences between the responses of laboratory and field bioassays were observed at locations where water quality might have been an important additional factor in the observed toxicity. In the delta of the river Rhine (represented in the present study by site KET) there are several areas where the water quality has improved much faster than sediment quality (Den Besten et al., 2000). In those cases, it is unlikely that the water quality should enhance effects on *C. riparius* in field bioassays. However, there can also be sites where for chironomids pollutants in the surface water form a second route of exposure, besides exposure via sediment pore water or sediment particles. This is the

most likely explanation for the 100% mortality in the field bioassay in the Delfland location (DEL) and the increased incidence of mentum deformities observed in the Brabantsche Biesbosch (BRB), being two locations with known pollution of the surface water (see description of test sites). The influence of surface water quality in the Brabantsche Biesbosch site was also demonstrated by the lower survival of midge larvae in OVP sediment that had been translocated to the polluted site BRB, and vice versa, by the higher survival in BRB sediment after translocation to the clean reference site.

Finally, in the present study considerable seasonal variation was observed in the degree of effects on midge larvae. The above-mentioned comparison of sediment toxicity in field and laboratory bioassays was carried out in April–June. In relation to the much stronger effects in sediment from site KET observed in the winter season (mean values: 7% survival compared to 59% and 68% in the field bioassays performed in July–August 1993 and April–June 1994, respectively, and 60% in the laboratory bioassay carried out at 15°C in June 1994), it can be concluded that effects under field conditions can also be much stronger than in the laboratory. The differences in effects cannot be simply explained by the longer exposure time (in winter season 2.7–4 times longer than in autumn or summer respectively), especially not when taking into consideration that also contaminant uptake will be slower at lower temperatures (Heugens et al., 2001). Therefore, it seems most likely that for *C. riparius* low temperatures (<8°C) are an additional stress factor (see below).

With sediment from river Dommel (DOM), only in the laboratory bioassay induction of mentum deformities was observed. The difference with the low percentage of mentum deformities in the field bioassay corresponds with the higher survival of chironomids in the field bioassay with sediment from site DOM, compared to the laboratory bioassay. The incidence of mentum deformities observed in larvae collected from the field site in the Dommel used in the present study is slightly higher than values reported by other investigators for the frequency of mentum gaps in *Chironomus riparius* collected in the metal-polluted upstream parts of the river Dommel (Groenendijk et al., 1998). Interestingly, also adaptation to metals has been demonstrated in populations of *Chironomus riparius* from the river Dommel (Postma, 1995; Postma and Groenendijk, 1999).

In one of the experiments an inverse relation between the rate of development (percentage of larvae in fourth instar stage) and the initial larval density in the field cage was demonstrated. These field bioassays were carried out in the autumn, during which water temperatures were dropping and populations of (benthic) algae might have collapsed. Therefore, it cannot be excluded that the rate of larval development might have been down regulated by a low food availability. With increasing initial larval density, the percentage of third instar larvae increased while a decreasing larval biomass for this stage was observed. In the case of the trend of the decreasing percentage of fourth instars, the larval biomass showed a similar trend up to 75 larvae per cage, but a relatively high biomass was found in the cages with an initial 90 larvae per cage. Although the latter observation seems difficults to explain (and should probably be discounted), the general conclusion from this experiment is that the rate of larval development can be reduced at higher larval densities when conditions, such as food availability, are less favourable.

As already mentioned, the results from the experiments described in the present paper indicate that low temperatures are an additional stress factor that interacts with stress caused by contaminants. In relation to this point it is interesting that in the study on the wintering of *C. riparius* in field cages, the highest percentage of deformities was found already after six weeks of exposure. This is another indication that exposure time (or exposure intensity) seems not to be the main factor that could explain the degree of effect in the field. The survival of chironomids in the reference sediment during winter was lower than in the two series of field bioassays carried out in April–June 1994 (cf: around 50% compared to nearly 90%, respectively). Possibly, the larvae that were placed in the field cages at the start of the winter experiment in December could not reach the point of an arrest of development that is typical for the diapause of chironomids in cold climates (Armitage et al., 1995). *C. riparius* has been reported to winter in the fourth instar stage (Groenendijk et al., 1998), but recently also diapause phenomena in the third instar stage have been reported for this species (Goddeeris et al., 2001). On the other hand, it is also possible that the results reflect a normal winter mortality, as has been shown to occur in field populations of *C. riparius* by others (Postma et al., 1995; Groenendijk et al., 1998). The decrease in the percentage of mentum deformities in larvae kept in reference sediment indicates that the mortality is higher among larvae with these abnormalities. Although the incidence of mentum deformities in the OVP reference sediment was significantly lower than in the polluted

BRB sediment, only a small difference was observed in the success of emergence. Other experiments have suggested that the timing of emergence depends strongly on the biomass development of the larvae (Naber and Den Besten, 1997). Despite the higher larval densities in the reference sediment, compared to the sediment from the Brabantsche Biesbosch, only a minor difference in the mean fourth instar larval biomass was found at the end of the field bioassay (in April: 0.9 compared to 1.0 mg). Since these larval biomasses are quite high (compare with values in Figure 5) it is not likely that the low success of emergence is due to insufficient feeding during the last part of the experiment (in the laboratory).

In conclusion, it was shown in the present study that different factors can enhance effects on *Chironomus riparius* in field bioassays compared to those measured in the laboratory bioassays. One factor is the presence of contaminants in surface water, e.g. near effluents. Secondly and thirdly, low food availability and low temperatures can be additional stress factors. These factors may interact strongly with the effects caused by sediment contamination.

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