Variables determining the response of invertebrate species to toxicants, A case study on the River Meuse.

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Chapter II

BIOASSAYS USING THE MIDGE *CHIRONOMUS RIPARIUS* AND THE ZEBRA MUSSEL *DREISSENA POLYMORPHA* FOR EVALUATION OF RIVER WATER QUALITY

Abstract

To evaluate if the water quality of the River Meuse affects macrofauna species, the impact of water from this river on two representative species was tested under controlled conditions. Short-term bioassays with reference populations of the midge *Chironomus riparius* and the zebra mussel *Dreissena polymorpha* were performed simultaneously, using growth and filtration rate as sublethal parameters, respectively. Filtration rates of mussels seemed to be slightly inhibited by Meuse water in 1994 and 1995, although this was only significant in the first year. Apparently, even though this species is inhabiting the River Meuse, the water quality still causes sublethal effects. In contrast to the mussel, midges were less sensitive to Meuse water in laboratory experiments; growth inhibition was never observed, while in some experiments growth was even enhanced. In the period of testing, it was not possible to relate effects on macrofauna species in laboratory tests to individual substances in polluted river systems. Since the response of the test species was marginal, it is recommended to include more sensitive species as tested in the present study, especially in view of a general improvement of the water quality in the River Meuse.

Introduction

The River Meuse has a large catchment area stretching from France through Belgium and The Netherlands (Fig. 2.1). Despite being a source of drinking water for millions of people in The Netherlands and Belgium, the river is polluted with hundreds of toxicants and organic waste. Industrial, agricultural and domestic wastes, in combination with modified habitats (due to canalization and construction of weirs) have had enormous impact on the species composition of the macrofauna community in the River Meuse (Admiraal *et al.*, 1993). Typical riverine species have disappeared during the past decades (Klink, 1985), and the species that presently inhabit this river, are considered to be pollution insensitive (Ketelaars and Frantzen 1995).

Species composition of benthic communities has often been related to water quality (e.g. Maltby *et al.*, 1995; Battegazzore and Renoldi 1995; Wilson 1994; Ketelaars and Frantzen 1995; Bij de Vaate 1995). However, it remains unknown if the presence or absence of species is caused by the toxicant levels in the river, or by other factors, like modified habitats. E.g., Bournaud *et al.* (1996) found that the disappearance of Ephemeroptera and Trichoptera from the River Rhône was mainly due to water regulation, and only partly caused by contamination of the river. The aim of this study was to evaluate if macrofauna species are affected by the water quality of the River Meuse. To this purpose, the influence of water quality was separated from the influence of habitat modification, by testing the effects of river water on macrofauna species in laboratory bioassays.

Two benthic macroinvertebrates that inhabit the catchment of the River Meuse have been selected: the zebra mussel *Dreissena polymorpha* and the midge *Chironomus riparius*. In 1990 and 1991, bioassays performed with the zebra mussel *Dreissena polymorpha* showed that filtration rates were severely reduced after exposure to Meuse water (Kraak *et al.*, 1994a). In 1992 and 1993, this mussel species became much more abundant in the River Meuse than in previous years (Ketelaars and Frantzen, 1995). To evaluate if zebra mussels are still affected by the poor water quality, reference populations were tested under controlled conditions. Since *C. riparius*, in contrast to *D. polymorpha*, occurs even at heavily polluted sites (Postma *et al.*, 1995; Evrard 1994), it was expected that the midge is more tolerant to contaminated conditions, as occur in the Meuse. Effects of Meuse water on filtration rates (mussel) and growth (midge) have been determined simultaneously in different seasons, since the water discharge, and hence the concentrations of contaminants, are known to be highly variable. Also, contamination of the River Meuse is known to be highest in the middle reaches (RIWA, 1996), and therefore, waters from upstream and downstream locations were compared. Attempts will be made to relate effects on midge growth to water quality parameters, measured in regular chemical monitoring by a Dutch governmental institute (Institute for Inland Water Management and Waste Water Treatment).

Materials and Methods

Site description and sample dates

Bioassays were conducted with water sampled at different locations along the River Meuse. In general, the river water is relatively clean upstream, in France (Pouilly-sur-Meuse: 49°32'43" N, 5°06'25" E) and Belgium (Hastière: 50°13'00" N, 4°49'50" E). Just before the border between Belgium and The Netherlands, at Liège (50°43'10" N, 5°41'50" E), the river water becomes severely polluted. The water quality remains poor when passing the border at Eijsden (50°47'00" N, 5°42'50" E), but improves gradually downstream (Grave, 51°45'43" N, 5°44'91" E) (Ketelaars and Frantzen 1995). A map of the catchment of the Meuse, including the sample sites, is presented in Fig. 2.1. Water samples were taken from the following locations along the River Meuse: Pouilly-sur-Meuse (PO), Hastière (HA), Liège (LI), Eijsden (EIJ), Grave (GR).

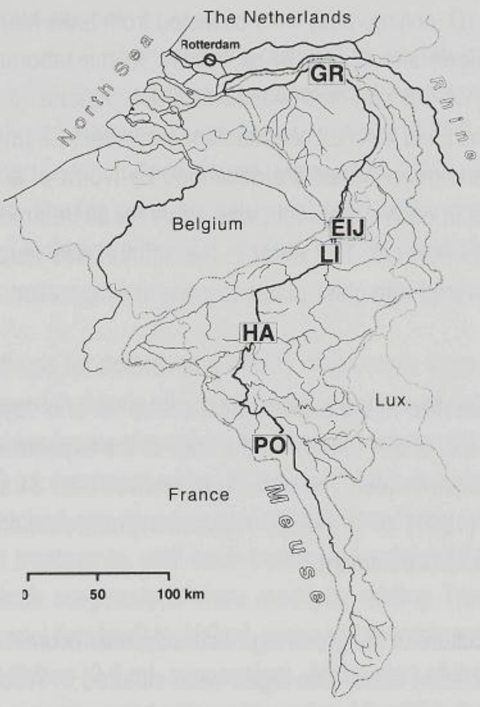

Fig. 2.1: The catchment of the River Meuse, including the experimental sites where water was collected for laboratory assays on mussels and midges. GR = Grave, EIJ = Eijsden, LI = Liège, HA = Hastière, PO = Pouilly-sur-Meuse.

The experiments with mussels were carried out in weeks 15, 19 and 28 in 1994 and weeks 14, 15, 17, 18, 19, 28 and 34 in 1995. In these experiments, water from Eijsden was always tested. Meuse water from Grave was tested in weeks 14, 18, 19 and 28 of 1995. The experiment with water from Pouilly-sur-Meuse, Hastière, Liège and Eijsden was sampled in week 17 of 1995.
Experiments with midges were carried out in weeks 14, 15, 16, 17, 18, 19, 34, 37, 41, 42 of 1995. In these experiments, water from Eijsden was always tested. Meuse water from Grave was tested in weeks 14, 15, 18, 19, 34, 37 and 41 of 1995. The experiment with water from Pouilly-sur-Meuse, Hastière, Liège and Eijsden was sampled in week 17 of 1995.

Immediately after transfer to the lab, the river and lake water were filtered (1.2 μm). The water was kept in vessels in a dark room, at 4°C, until further use (2 days maximum). The pH of all samples taken in 1994 and 1995 varied between 7.5 and 8.4.

Dreissena polymorpha

Experiments using zebra mussels were performed according to Kraak *et al.* (1994b), with slight modification. Zebra mussels (*D. polymorpha*) were collected from Lake Markermeer (The Netherlands), a relatively clean site (Kraak *et al.*, 1991). In the laboratory, the mussels were sorted by length (1.5-2.5 cm) and placed in glass aquaria (6 l), each aquarium containing 25 mussels and 3 l of filtered Lake Markermeer water (1.2 μm). Water quality characteristics of Lake Markermeer water are described by Ivorra *et al.* (1995). The average length of the mussels in each experiment was equal for all treatments, and ranged from 1.75 (± 0.2) to 2.11 (± 0.1) cm. The water in the aquaria was aerated and kept at 15°C. The aquaria were covered with glass plates to prevent evaporation. A 16 : 8 h light : dark regime was applied.

The animals were allowed to acclimatize to the experimental set-up for one day, as has been proven to be sufficient by Kraak *et al.* (1994b). At the start of the experiment, each aquarium was supplied with 3 l treatment water. The water was renewed after 24 and 48 h. Each treatment consisted of 2 (1994) or 3 (1995) replicates per treatment. Lake Markermeer water was used as a control treatment.

After 48 h, 60 mL of a continuous culture of the green alga *Scenedesmus acuminatus* was added to the water to measure filtration rates. The algae were cultured in Woods Hole medium. The density of the algal cells in the aquaria was approximately 20,000 cells/mL. The algal concentration decreased due to the filtration activity of the mussels. When the mussels started filtering, 5 min after addition of the algae, three water samples were taken (5 mL). This was repeated 10 and 20 minutes after the first sampling. The concentration of algae in the water was measured using a Coulter Counter. The filtration rate was calculated from the declining number of algae, using Coughlan's (1969) formula:

$$m = \frac{M}{nt} \ln \frac{C_0}{C_t}$$

in which

m = volume of water filtered by *D. polymorpha* (mL/mussel/h)

M = volume of water in the aquaria (3 l)

n = number of mussels in each aquarium (25)

t = duration of filtration measurement (h)

C_0 = concentration of algae at the start of the filtration measurement

C_t = concentration of algae after t hours

Filtration rates of controls were always above 50 mL/mussel/h. The average filtration rates of controls was 95 (± 36) mL/mussel/h, which indicates that the mussels were in good condition (Kraak *et al.*, 1994b).

Chironomus riparius

Midge larvae (*C.riparius*) were cultured in the laboratory, in glass aquaria with cages placed on top. The sediment was collected at a reference site (Oostvaarders Plassen) and the overlying water was Dutch Standard Water (DSW), a standardized synthetic analogue of common Dutch surface waters (Maas *et al.*, 1993). The water was aerated, and oxygen saturated. The temperature in the controlled environment room was 20°C ±1°C, and a 16 h light : 7 h dark regime was applied, with 30 minutes twilight before and after each light period.

The methods for determining effects of toxicants were derived from Postma *et al.* (1995), who showed that toxic effects on growth can be well determined by measuring length of first instar midge larvae. An experimental treatment consisted of a glass jar (180 mL), supplied with 100 mL treatment water. At the start of the experiment, first instar larvae from at least three hatched egg ropes were distributed randomly with glass Pasteur pipettes over the different treatments, until each treatment contained 50 newly hatched first instar larvae. Food stock suspensions were made by adding Trouvit (5 g) and Tetraphyll (0.25 g) (commercial fish food) to 100 mL water of the corresponding treatment. Larvae were given food *ad libitum* (0.6 mL suspension). At the start of the experiment, the lengths of 10 first instars were measured using a binocular. After 96 h, length was measured of the surviving larvae. Growth of the individual larvae was calculated by subtracting the final length of each larva from the average initial length. The average initial length varied from 0.96 (± 0.07) to 1.20 mm (± 0.05). Growth of control larvae was always between 1.4 (± 0.21) and 1.7 (±

0.52) mm, and mortality of the controls was always less than 10%. DSW and Lake Markermeer water were used as control treatments. The experiments were carried out in a controlled environment room under identical conditions as the laboratory culture of the larvae.

It was calculated if growth data of *C. riparius* were correlated to water quality parameters, measured at Eijsden. At this site, the river water is frequently analyzed for 197 chemical and physical water quality parameters by the Institute for Inland Water Management and Waste Water Treatment (RIZA). Besides standard parameters, the selection of the measured variables is based on the (frequency of) appearance of elevated concentrations of a certain compound in the River Meuse. Some parameters are measured more frequently than others; measurements are performed daily, weekly or every four weeks. Consequently, not all parameters were determined on the sampling dates for the bioassays. It was tested if there was a correlation between the measured parameters and growth of *C. riparius*, if there were at least 5 paired observations. In total, 46 water quality parameters could be tested for correlation with growth: As, Cd, Cr, Cu, Fe, Ni, Pb, Zn, Ca, Cl, K, Mg, Na, atrazine, chloridazon, choline-esterase inhibitors, lindane, simazine, chloroform, di-isopropyl ether, ethylene dichloride, methyl chloroform, methylene blue active compounds, tetrachloroethene, trichloroethene, volatile and non volatile halogens, chlorophyll a, KjN, NH_4, NO_2, NO_3, organic carbon, P, phaeophytine, PO_4, SiO_2, SO_4, suspended matter, ash residue, clarity, conductivity, O_2, pH, temperature, water discharge.

Statistics

Filtration rates of zebra mussels and growth of midge larvae are presented as percentages of the corresponding controls, in order to make comparison of data over time possible. The results were tested using one- or two-way ANOVA, when appropriate. When two treatments were compared, a student's t-test was applied. Correlations between chemical parameters and growth data were tested using Spearman's correlation test.

Results

Filtration rates of mussels exposed to water from Eijsden were lower in 1994 than in 1995 (Fig. 2.2). In 1994, filtration rates were on average 48% inhibited, and in weeks 15 and 28, filtration rates were significantly ($p<0.05$) lower than those of the controls. In 1995, filtration rates of mussels exposed to Eijsden water were not significantly lower than those of controls, although filtration rates on average were inhibited by 25%. When comparing the effects of river water from the two Dutch locations along the River Meuse, it seems that mussels filtered more algae in Grave water than in Eijsden water, although this is not significant (Fig. 2.3).

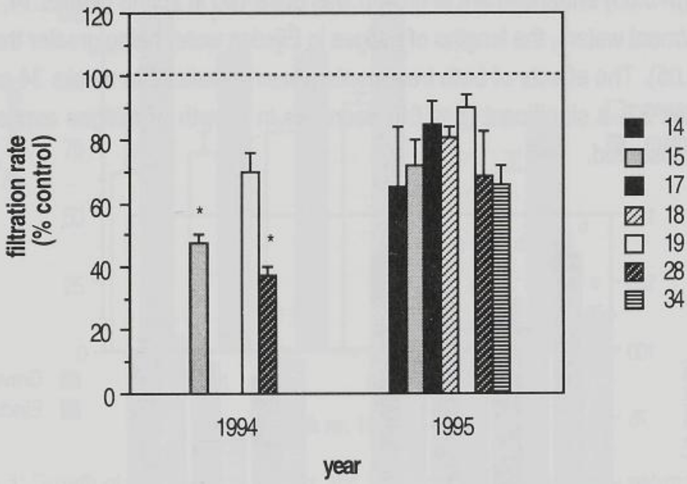

Fig. 2.2: Filtration rates (with standard error) of *Dreissena polymorpha* after 48 h exposure to water from the River Meuse (at Eijsden). Experiments were performed in weeks 14, 15, 18, 19 and 28, in 1994 and 1995. Filtration rates are presented as percentages of the corresponding controls. "*" denotes a significant difference from controls ($p<0.05$).

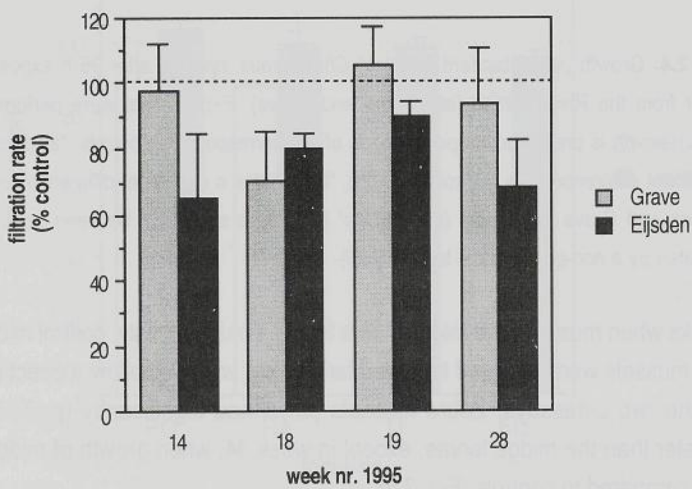

Fig. 2.3: Filtration rates (with standard error) of *Dreissena polymorpha* after 48 h exposure to water from the River Meuse (at Eijsden and Grave). Experiments were performed in 1995. Filtration rates are presented as percentages of the corresponding controls.

Midges were not affected (week nr. 34, 41), or even grew significantly ($p<0.05$) more than controls (week 14, 15, 18, 19) in Meuse water from Eijsden and Grave (Fig. 2.4). A significant ($p<0.05$) enhancement of growth was observed in spring (weeks 14, 15, 18, 19) in both treatment waters, the lengths of midges in Eijsden water being greater than in Grave water ($p<0.05$). The effects of both treatments were neutralized in weeks 34 and 41 (Fig. 2.4). In week 37, a significant ($p<0.05$) decrease in growth of midges exposed to river water was observed.

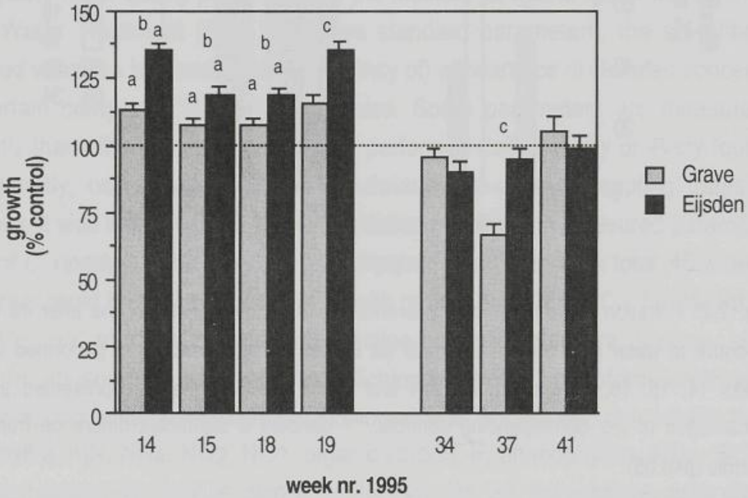

Fig. 2.4: Growth (with standard error) of *Chironomus riparius* after 96 h exposure to water from the River Meuse (at Eijsden and Grave). Experiments were performed in 1995. Growth is presented as percentages of the corresponding controls. "a" denotes a significant difference from controls ($p<0.05$), "b" denotes a significant difference between Eijsden and Grave treatments ($p<0.05$), "c" denotes a significant between treatments, indicated by a non-parametrical test ($p<0.05$).

In the weeks when mussels and midges were tested simultaneously, control midges as well as control mussels were exposed to Lake Markermeer water, to allow a direct comparison between the two bioassays. Zebra mussels performed significantly ($p<0.05$) worse in Eijsden water than the midge larvae, except in week 34, when growth of midges was not enhanced compared to controls (Fig. 2.5).

When comparing effects of Meuse water from different locations on the zebra mussel and the midge larvae, sublethal effects did not differ significantly between the two species, except in the case of exposure to river water from Liège, in which the mussels performed significantly ($p<0.05$) worse than the midge (Fig. 2.6).

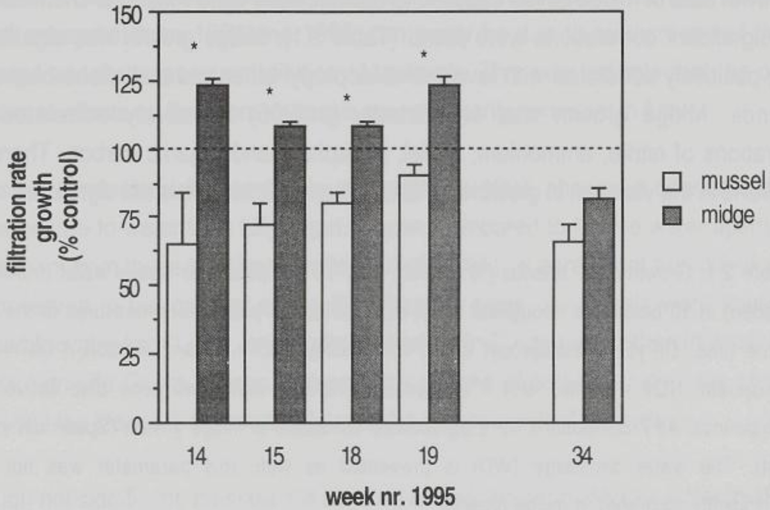

Fig. 2.5: Growth of *Chironomus riparius* and filtration rates of *Dreissena polymorpha* (with standard error) after exposure to Eijsden water. The two species were tested simultaneously, at several weeks in 1995. Growth and filtration rates are presented as percentages of the controls (in Lake Markermeer water). "*" denotes a significant difference between species ($p<0.05$).

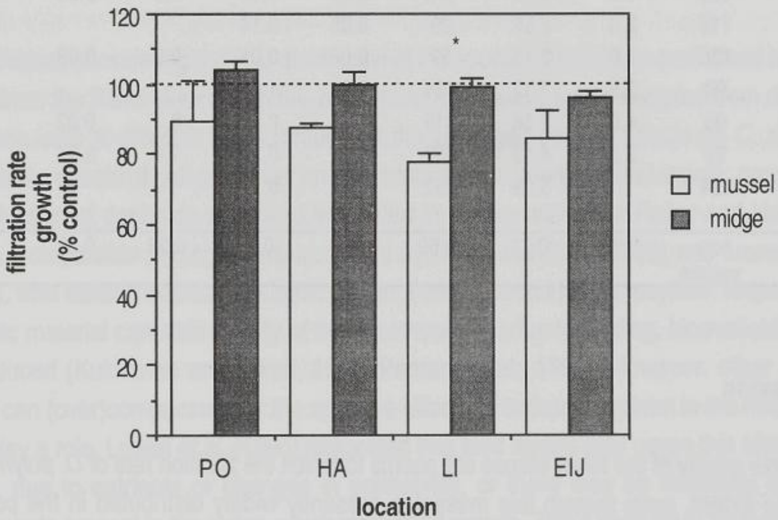

Fig. 2.6: Growth of *Chironomus riparius* and filtration rates of *Dreissena polymorpha* (with standard error) after exposure to water from different sites along the River Meuse. Water sampled on 28/4/95. PO = Pouilly (F), HA = Hastière (B), LI = Liège (B), EIJ = Eijsden (NL). Growth and filtration rates are presented as percentages of the controls (in Lake Markermeer water). "*" denotes a significant difference between species ($p<0.05$).

When growth data of midge larvae exposed to Eijsden water were related to chemical data, several significant correlations were found (Table 2.1). Midge growth was significantly ($p<0.05$) positively correlated with levels of di-isopropyl ether and methylene-blue-active compounds. Midge growth was significantly ($p<0.05$) negatively correlated with concentrations of nitrite, ammonium, nickel, phosphate and organic carbon. There is a positive trend in the variation in growth and water discharge, but this is not significant.

Table 2.1: Growth of *C. riparius* (% control), after 96 h exposure to Meuse water (from Eijsden) in 10 bioassays throughout 1995, and 7 chemical parameters measured at the same time. DiPyEr = diisopropyl ether, Ni = nickel, OC = organic carbon, PO4 = phosphate, NO2 = nitrite, NH4 = ammonium, MBAC = methyl ethylene blue active compounds. All 7 compounds were significantly correlated to midge growth (Spearman's test). The water discharge (WD) is presented as well, this parameter was not significantly correlated to midge growth.

week	growth (%)	Ni (µg/L)	OC (mg/L)	PO4 (mg/L)	NO2 (mg/L)	NH4 (mg/L)	DiPyEr (µg/L)	MBAC (mg/L)	WD (m3/s)
14	135	2.0	2.31	76	0.05	0.10			543
15	119	2.0	2.08	11	0.03	0.01	1.5	0.03	319
16	124	2.0	2.16	100	0.06	0.16			357
17	106	1.0	2.74	174	0.06	0.16	0.5	0.03	434
18	119	2.0	2.59	99	0.05	0.14			363
19	136	1.0	2.15	59	0.04	0.01	9.9	0.08	202
34	90	2.5	3.12	436	0.21	1.06			25
37	95	4.0	3.56	319	0.17	0.71	0.3	0.02	56
41	99	2.3	4.13	157	0.09	0.25	1.4	0.03	87
42	124		3.09	252	0.12	0.72			45
	corr. values	-0.73	-0.71	-0.69	-0.66	-0.63	0.90	0.89	ns

Discussion

The water quality of the River Meuse still seems to inhibit the filtration rate of *D. polymorpha* to some extent, even though this mussel is presently widely distributed in the polluted reaches of the river (Ketelaars and Frantzen, 1995). However, it seems that the water quality of the River Meuse has improved compared to the early nineties (Kraak *et al.*, 1994a). Trend analysis performed on chemical data of the Meuse, obtained between 1977 and 1994, showed that concentrations of metals have decreased in recent years, but most other groups of contaminants showed no apparent trend over the years (Baggelaar and

Baggelaar, 1995). The observed improvement of the water quality, as determined by the mussel assay between 1994 and 1995, can partly be due to an increase of the water discharge and the consequent dilution of toxicants. This may indicate that the observed decrease in effects on the zebra mussel is not necessarily persistent in future.

Bioassays conducted with planktonic microalgae in 1993, showed a decrease in growth after exposure to water from Liège and Eijsden, compared to Meuse water upstream and downstream from these locations (Tubbing *et al.*, 1995). In agreement with these results, it was observed in the present study, that filtration rates of mussels were lowest when exposed to water from Liège, and performed worse in Eijsden water than in water from the downstream site Grave. Hence, although the degree of pollution may have changed, it seems that the Meuse is still most polluted in the middle reach of the river.

Although not significant, mussels still seemed to be inhibited by Meuse water in 1995. The set-up of the filtration bioassays only allows duplicates or triplicate treatments, causing a low statistical power. Therefore, considering the improvement of water quality, the bioassays with zebra mussels may not be sensitive enough to monitor acute toxicity of Meuse water in the future, or a more sensitive test design should be selected. On the other hand, if the water quality improvement is reversing (e.g. in a year with less rainfall), this bioassay may be suitable for appliance.

As expected, considering the field distribution of the species, the midges performed better in water from the River Meuse than the zebra mussels. Meuse water, sampled from different locations, and at different times throughout the year, had positive effects on *C. riparius*. Stimulating effects of polluted water on macrofauna have been reported before; growth and reproduction of daphnids were also enhanced in Meuse water (De Ruiter and Hendriks, 1996). Comparable findings were observed by Dubé and Culp (1996) and Lowell *et al.* (1995), who studied effects of pulpmill effluents on chironomids and mayflies, respectively. Organic material can alter toxicity of many compounds; due to binding, bioavailability can be reduced (Kukkonen and Oikari, 1991; Pantani *et al.*, 1995). However, other factors which can (over)compensate for the negative effects of toxicants present in the river water may play a role. Lowell *et al.* (1995) suggested that food quality may cause this stimulating effect, due to nutrients or changes in palatability, or there may be hormonal or other compounds present in the water, which may affect growth. There seemed to be a seasonal trend in response of midges to the river water. The enhanced effects on growth in springtime may be influenced by the dilution of toxicants due to a high water discharge and/or by improvements in food quality in this time of year. Although the river water was filtered in this study, it is possible that small particles passed through the filter, and midges could have been able to profit from this potential food source. This will be studied in future experiments. It is concluded that the hypertrophic midge *C. riparius* is not a suitable species to assess adverse effects of pollution on macrofauna species, especially if the presence of toxicants coincides with organic enrichment.

In general, there were more stimulating factors for *C. riparius* in Eijsden water than in Grave water, overcompensating for the higher toxicant levels at Eijsden. The opposite effect is observed when the zebra mussel was exposed to Meuse water; mussels generally performed better in Grave water than in water from Eijsden. It is possible that the factors that enhance midge growth have no or less positive effects on filtration rates of zebra mussels. However, the different response of the species to the two site waters could also be explained by a species-specific sensitivity to complex mixtures. When the mussel *Anodonta imbecilis* was exposed to effluent of a pulp mill, it was found to be more sensitive than the daphnid *Ceriodaphnia dubia* (McKinney and Wade, 1996). In contrast, in another study, when these species were subjected to a synthetic mine effluent, *C. dubia* was more sensitive than *A. imbecilis* (Masnado *et al.*, 1995). The present observations on an arthropod and a bivalve at two sites are compatible with those of McKinney and Wade (1996) and Masnado *et al.* (1995), assuming that the River Meuse carries a different pollutant load at the two sites tested.

Although 7 out of 46 measured parameters were significantly correlated with the growth of *C. riparius*, it seems that not any of them are individually causally related to midge growth. For instance, it is not likely that phosphate and organic carbon would inhibit growth, and that di-isopropyl ether and methylene-blue-active compounds would enhance growth; one would expect the opposite effect. Also, levels of nickel are so low (1-4 μg/L), that it is unlikely that they would affect the midge larvae. Powlesland and George (1986) found that growth of *C. riparius* was not affected under 2.5 mg Ni/L. Levels of nitrite were also too low to expect a negative effect (30-210 μg/L); Neumann *et al.* (1994) found that *Chironomus piger* was not affected by nitrite concentrations under 1 mg/L. Ammonium concentrations (10-1060 μg/L) were also neglectable (Williams *et al.*, 1986; Schubauer-Berigan *et al.* 1995). Therefore, it is concluded that the observed differences in midge growth were apparently caused by multiple compounds, or by single compounds which were not measured.

Even though many parameters were measured in the Meuse, these were not predictive for the (negative or positive) effect of the river water on macrofauna. Hendriks *et al.* (1994) monitored the effect of concentrated water from the River Rhine on bacteria, and more than 89% of the observed toxicity could not be attributed to identified compounds. Bioassays conducted with phytoplankton in 1993, indicated that growth rates were (slightly) inhibited by organic compounds in concentrated river water (Van Dijk *et al.*, 1995), however, it remained unknown which (groups of) organic compounds were toxic, and if inorganic compounds were toxic as well. These findings suggest that it seems impossible to relate effects on aquatic macrofauna to individual substances in rivers with a complex pollution, unless a specific toxicant is present in exceptionally high concentrations. To assess the contribution of contaminants to the degradation of aquatic ecosystems, it is necessary to use ecotoxicological tests (bioassays) in addition to routine chemical measurements. This also holds for the River Meuse, where hundreds of compounds are present in the river water. However, in order to be effective, these (short-term) bioassays may need to include more sensitive species as tested in the present study. In current studies, sensitive riverine insects are being tested for application.

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