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

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## SUMMARY

The aim of this study was to identify which key factors of water quality determine the response of macrofauna species to polluted river water. It was evaluated if toxic barriers exist for indigenous species to re-establish in rivers in the process of sanitation, and which variables determine the response of "pollution tolerant" species to deteriorated conditions. Three macrofauna test species that are supposed to differ in pollution tolerance were selected: the caddisfly *Hydropsyche angustipennis* ("relatively sensitive"), the zebra mussel *Dreissena polymorpha* ("intermediately tolerant") and the midge *Chironomus riparius* ("tolerant"). The River Meuse has hardly shown signs of ecological recovery in recent years and therefore this river was used in a case study.

During 1994 and 1995, it was evaluated whether the water of the Meuse directly affects macrofauna species (*Chapter II*). To this purpose, the "intermediately tolerant" zebra mussel *D. polymorpha* and the "tolerant" midge *C. riparius* were kept in Meuse water under controlled conditions. Effects of Meuse water on filtration rates (mussel) and growth (midge) were determined simultaneously. Water was sampled along different sites and in different seasons, as the pollution level is highly variable along the stream and throughout the year. Filtration rates of mussels seemed to be only slightly inhibited by the water quality of the Meuse, while in previous years (1990 and 1991) filtration rates were strongly decreased. The better performance of *D. polymorpha* in 1994 and 1995 may be an indication of improvement of the water quality of the Meuse. In contrast to the zebra mussel, midges were less sensitive to Meuse water; growth inhibition never occurred, and enhanced growth was observed frequently. The results indicated that the classification of the midge as a pollution tolerant species and the zebra mussel as an intermediately tolerant species holds when water quality alone is considered. In *Chapters III* and *IV* it was evaluated which factors determine the success of species in polluted rivers.

One of the questions in this study was whether "pollution tolerant" species are tolerant of toxicant levels, or whether they take advantage of the high nutrient levels in the river water. In many rivers, both toxicants and organic enrichment are closely interrelated, making it difficult to distinguish between the effects of the separate factors. In *Chapter III*, an attempt was made to discern the effects of toxicants and the effects of organic enrichment on macrofauna in river water, using the midge *C. riparius* as a test species. Larval growth of the "tolerant" midge was measured in different types of river water containing varying levels of particles (obtained by selective filtration) and toxicants (either complex mixtures or

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metals). Exposure of first instar larvae to water from the polluted Rivers Meuse and Dommel showed that growth was less inhibited by toxicant levels in river water than expected based on laboratory toxicity tests. Factors present in polluted river water stimulated growth of midges to such an extent that inhibiting effects of high toxicant concentrations were neutralized, and at low toxicant levels, were overcompensated for. It was indicated that particulate matter has great potential to reduce inhibiting effects of toxicants on *C. riparius*, not (only) by reducing the bioavailability of toxicants, but by serving as a supplementary, superior food source. The success of the "pollution tolerant" midge was not explained by tolerance of this species to toxicants, but by its ability to take advantage of coinciding organic enrichment. It was suggested that the extent to which beneficial effects of organic compounds take place are likely to be species specific.

Following the results of *Chapter III*, it was hypothesized in *Chapter IV* that, in addition to species specific sensitivities to toxicants, the persistence of species in polluted rivers depends on species specific capacities to modify or compensate for negative effects of toxicants. The response to organic compounds present in site water, like humic acids (HA), may be essential in ranking pollution tolerant and pollution sensitive invertebrates. The zebra mussel *D. polymorpha* and the midge *C. riparius* were exposed to metal polluted water from the River Dommel. Additionally, the responses of both species to metals in the presence and absence of organic matter (HA) were compared in laboratory tests. In spite of a lower tolerance of *C. riparius* to metals in laboratory studies, the midge was the most tolerant of the two test species to metal polluted site water. The results indicated that the sensitivities of the two test species determined in laboratory tests were inversely related to their sensitivities to polluted river water. In accordance with these results, midge larvae were protected from Cu toxicity by HA, while metal toxicity was not reduced or even amplified by HA for the zebra mussel. Thus, the presence of HA in site water may partly account for discrepancies between responses of species to field bioassays and laboratory toxicity tests. It was suggested that these differences in responses to metals in site water are strongly influenced by species specific interactions with organic compounds (like HA). It is concluded that the response to organic compounds present in site water largely determines whether a species is classified as "pollution tolerant" or "pollution sensitive".

Following the indicated water quality improvement (*Chapter II*), it was determined in 1996 whether ecotoxicological barriers exist in the River Meuse for characteristic riverine insect species (*Chapter V*). To this purpose, caddisflies of the genus *Hydropsyche* were incubated in cages in the Meuse and their survival and development were monitored. A comparison was made with incubations in the River Rhine in which populations of some caddisfly species have re-established. Survival of caddisflies in the River Rhine was fairly high, while there was almost no survival in the River Meuse in three out of five field

experiments. The incubations of *Hydropsyche* in the River Meuse provide evidence that even adequate structural habitat would be insufficient for the re-establishment of *Hydropsyche* species. Chemical factors (like pesticides, but also unidentified compounds) as well as physical factors (like oxygen and current velocity) are likely to be limiting in the River Meuse for *Hydropsyche* species.

According to the results in the preceding chapters and the high incidence of chemical spills in recent years, it was suggested that insecticides may be an important barrier for insect life in the Meuse. There are several variables that determine the impact of an insecticide on invertebrates. In *Chapter VI*, aquatic insects were subjected to the frequently occurring insecticide diazinon, taking the variables taxon, developmental stage and exposure time into account. Effects of diazinon on the caddisfly *H. angustipennis* and the midge *C. riparius* were determined in the laboratory during different exposure times, using mortality, activity and growth as endpoints. Last instars of both species displayed a clear behavioural response at concentrations much lower than those affecting survival. Even a small increase in exposure time strongly decreased survival of midges and caddisflies (1.4 - 8.4 times). Within the spectrum of tested insects reported in literature, *H. angustipennis* is the second most sensitive, and *C. riparius* the most tolerant species. However, the ranking of species is strongly dependent on the developmental stage; differences between species are often smaller than differences between instars of one species. The great difference in sensitivities between young and old larvae implicate that the impact of a pesticide strongly depends on the seasonal timing. Incidents in spring and summer will have a maximal impact on insect communities, since young larvae are especially abundant in these seasons. In addition to the negative effect of diazinon at low concentrations, recovery of typical riverine insects like *H. angustipennis* will be slow, considering their relatively long life cycle. Consequently, even if the prevailing water quality of disturbed rivers improves, frequently occurring incidents will prevent the return of riverine insects like caddisflies.

Multiple factors determine the response of species to complex water pollution. Therefore, species sensitivity to pollution is likely to be different in the field than in toxicity tests where individual compounds are tested. The present study indicated that organic enrichment can act as a stressor for one species, but may benefit another species under the same conditions. Riverine species like caddisflies appear more vulnerable to multiple stressors than generalistic species. Also, the developmental stage of an organism and the length of its life cycle determine the effect of pollution on populations in the field. Hence, not only are characteristic riverine species likely to be more affected by deteriorated water quality than generalistic species, the river species are also at risk because of their long life cycles. In conclusion, there are many species specific factors that alter the response of an

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invertebrate species to deteriorated water quality, making it difficult to classify a species as tolerant or sensitive to toxicants or other stress factors. The present study has identified several of these factors, which helps to further clarify and predict distributions of macrofauna species in rivers affected by complex pollution.

The following recommendations to river management were proposed:

- River water quality should be assessed using characteristic riverine species, so that indigenous species are sufficiently protected, and environmental risks are not under- or overestimated.
- Characteristic river insects like the caddisfly *Hydropsyche* are essential for monitoring ecological rehabilitation of the River Meuse, and therefore, these species should be added to the selection of AMOEBA species.
- Investments made to improve the water quality of the River Meuse, should focus on the prevention of incidents, in addition to the efforts to improve the "overall" water quality.
- Because of the interaction of organic enrichment and toxicants a coordinated reduction of both groups of pollutants is recommended.
- The prevention of unnaturally strong water discharge fluctuations due to water abstractions (by hydro-electric power stations) should be included in environmental plans.