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

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Chapter III

INTERACTING EFFECTS OF TOXICANTS AND ORGANIC MATTER ON THE MIDGE *CHIRONOMUS RIPARIUS* IN POLLUTED RIVER WATER

Abstract

Toxicants and organic matter in river water have contrasting impacts on macrofauna. Through manipulations of both factors, their interactive effects on organisms was evaluated. This way, an attempt was made to clarify the presence or absence of pollution "tolerant" and "sensitive" species in rivers affected by mixed sources of pollution. Under controlled conditions, larval growth of the "tolerant" midge *Chironomus riparius* was measured in different types of river water containing varying levels of particles (obtained by selective filtration) and toxicants (either complex mixtures or 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 is hypothesized that the extent to which beneficial effects of organic compounds on organisms take place is species specific.

Introduction

In many rivers pollution has caused a strong reduction in biodiversity. This ecological degradation has prompted measures to reduce pollution and, as a result, the water quality of some rivers, like the Rhine, has improved (Van Urk *et al.*, 1989; Bij de Vaate and Oosterbroek, 1992; Admiraal *et al.*, 1993; Hendriks, 1994). In other rivers, like the Meuse, levels of dominant toxicants (like cadmium) have dropped, but low concentrations of hundreds of toxicants still coincide with organic waste (RIWA, 1996). In such polluted rivers, sensitive taxa have been observed to be replaced by tolerant taxa (Clements and Kiffney, 1994). Indeed, "sensitive" species are absent and "pollution tolerant" species are flourishing in the River Meuse (Ketelaars and Frantzen, 1995). Question is whether these "tolerant" species are indeed tolerant of high toxicant levels, or whether they take advantage of the high nutrient levels in the river water (Welch, 1980; Lanno *et al.*, 1989; Barreiro Lozano and Pratt, 1994; Dubé and Culp, 1996; Stuijfzand *et al.*, 1998), and/or profit from the absence of other species. The first two possibilities can be tested against the latter by performing experiments under controlled conditions. However, in many rivers, both toxicants and organic enrichment are closely interrelated (Thomas and Meybeck, 1992; Renoldi *et al.*, 1997), making it difficult to distinguish between the separate effects of these factors. In this study, an attempt was made to discriminate between the effects of toxicants and the effects of organic enrichment on macrofauna in river water, using the midge *Chironomus riparius* (Meigen) as a test species. Larval growth was measured in different types of river water containing varying levels of food (obtained by selective filtration) and toxicants (either complex mixtures; River Meuse or metal dominated; River Dommel). From these experimental observations a reconstruction of the (interacting) effects of food and toxicants can be made. Using this reconstruction, an attempt can then be made to clarify the presence or absence of pollution "tolerant" and "sensitive" species in rivers affected by mixed sources of pollution.

Materials and Methods

Site description

The Meuse is a large European river flowing from France through Belgium and The Netherlands (Fig. 3.1). The middle and lower parts of the river are contaminated by industrial and municipal waste water (Admiraal *et al.*, 1993). One of the major sources of pollution is located near the city of Liège, just upstream of the border between Belgium and The Netherlands. Experiments were performed with water sampled at Eijsden (EIJ), which is situated along the Meuse at the Dutch/Belgian border (50°47'00" N, 5°42'50" E). At this site, the water quality is affected by a wide variety of pollutants, as is indicated by chemical analysis (RIWA, 1996) and macroinvertebrate communities are characterized by a low biodiversity (Ketelaars and Frantzen, 1995).

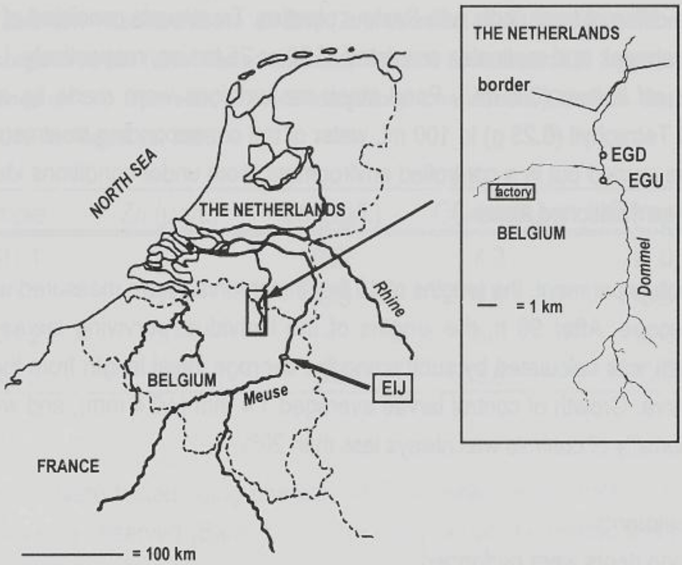

Fig. 3.1: Map of the River Meuse and its tributary the Dommel. EIJ: Eijsden; EGU: Eindergatloop Upstream; EGD: Eindergatloop Downstream.

The River Dommel is a tributary of the River Meuse (Fig. 3.1). At the sampling site Eindergatloop upstream (EGU; 51°14'00" N, 5°25'30" E), the water is organically enriched by the upstream input of municipal waste water. A few hundred meters downstream of this site, water from a small inlet (Eindergatloop) flows into the Dommel. This water contains high levels of metals, caused by runoff from soil surrounding a zinc factory (Postma, 1995). Downstream of the junction between the Dommel and the Eindergatloop (EGD; 51°14'20" N, 5°25'23" E), organic enrichment coincides with high metal levels.

Chironomus riparius

Midges (*Chironomus riparius*) were cultured in the laboratory, in glass aquaria with cages placed on top. The sediment was collected at a reference site, the 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 climate room was 20°C ±1°C, and a 16 h light : 7 h dark regime was applied, with 30 minutes twilight before and after a light period. In culture the life cycle of *C. riparius* is completed in three weeks.

Experimental set-up

At the start of each experiment, glass jars (180 mL) were supplied with 100 mL treatment water. Newly hatched, first instar larvae from at least 3 egg ropes were distributed randomly into the different treatments with Pasteur pipettes. Treatments consisted of 1 or 3 replicates per treatment, and replicates consisted of 50 or 25 larvae, respectively. Larvae were given food *ad libitum* (0.6 mL). Food stock suspensions were made by adding Trouvit (5 g) and Tetraphyll (0.25 g) to 100 mL water of the corresponding treatment. The experiments were carried out in a controlled environment room under conditions identical to the midge culture mentioned above.

At the start of each experiment, the lengths of 10 first instar larvae were measured using a binocular microscope. After 96 h, the lengths of the individual surviving larvae were measured. Growth was calculated by subtracting the average initial length from the final length of each larva. Growth of control larvae averaged 1.4 mm (±0.4 mm), and was 1.0 mm minimum. Mortality of controls was always less than 20%.

Experimental treatments

Two types of experiments were performed:

I. Experiments with water from the River Dommel (metal polluted, organically enriched). On September 19, 1995 and August 20, 1996, water was sampled at the locations Eindergatloop Upstream (EGU) and Eindergatloop Downstream (EGD). The river water was filtered (1.2 μm; glass fibre Whatman GF/C). Zinc, cadmium and organic and inorganic carbon levels were measured in filtered river water, using flame-AAS, furnace-AAS and a carbon analyser (Table 3.1). DSW was used for controls.

II. Experiments with fractions of Meuse water (complex mixture of organic and inorganic contaminants, organically enriched):
   a. Water from Eijsden (sampled on October 10 and November 14, 1995 and June 24 and August 12, 1997) was not filtered (EIJ tot) or filtered (EIJ 1.2) using glass fibre Whatman GF/C filters. DSW was used for controls.
   b. Water from Eijsden (sampled on January 16 and January 30, 1996) was fractionated through filters of different pore sizes: 0.2 μm (cellulose-acetate), 0.7 μm (glass fibre Whatman GF/F), 1.2 μm (glass fiber Whatman GF/C), 10 μm (nylon mesh). Also, untreated Eijsden water (tot) was tested. DSW was used for controls.
   c. Water from Eijsden (sampled on November 28 and December 5, 1995) was centrifuged. One treatment consisted of the supernatant (EIJ sup), while the pellet was added to DSW (DSW pell). One treatment consisted of untreated Eijsden water (EIJ tot). As a control, Eijsden water was centrifuged, and the pellet was resuspended in this water afterwards (EIJ pell). In addition, filtered Eijsden water

(1.2 μm; Whatman GF/C) was tested (EIJ 1.2), in order to be able to compare the results with previous experiments. DSW was used for controls.

Table 3.1: Concentrations of zinc (Zn), cadmium (Cd), organic carbon (OC) and inorganic carbon (IC) in filtered site water. <: below detection limit (0.1 μg/L). Sites: EGU: Eindergatloop Upstream; EGD: Eindergatloop Downstream. Dates: 1: September 19, 1995; 2: August 20, 1996.

Sample	Zn (μg/L)	Cd (μg/L)	OC (mg/L)	IC (mg/L)
EGU 1	64	0.2	4.3	11.0
EGD 1	863	30.9	4.6	13.8
EGU 2	31	<	5.7	13.0
EGD 2	228	5.8	5.6	20.0

Statistics

The results were tested using one-way ANOVA, when appropriate. If inhomogeneity of variances was observed (Bartlett's test: $p<0.05$), a non-parametric test (Kruskall Wallis) was used.

Results

In organically rich Dommel water containing low toxicant levels (EGU), larvae showed enhanced growth ($p<0.05$) compared to controls (Fig. 3.2, light shaded bars); midges incubated in EGU 1 water even grew 44% more than controls. Downstream from the point source of metals, larvae grew to a size equal to (EGD 2) or slightly but significantly ($p<0.05$) less (EGD 1) than controls. Mortality in all treatments was low; even in river water containing the highest metal concentrations (EGD 1), 96% of the larvae survived.

Growth data from the bioassays were compared to laboratory derived toxicity data for similar zinc and cadmium levels, calculated from Postma *et al.* (1995). The expected zinc and cadmium growth inhibition (Fig. 3.2, dark shaded bars) was determined by calculating a dose-response relationship for both metals according to Haanstra *et al.* (1985). The larvae used by Postma *et al.* (1995) originated from the same laboratory culture as the ones used in the present study, and the experimental set-up is similar to that from Postma *et al.* (1995). It is clear that the response of *C. riparius* to a toxicant in polluted river water is different from the response to similar toxicant levels in reference water. In EGD 1 water growth inhibition was only 15% while, based on the results of Postma *et al.* (1995), an inhibition of 93% was expected (when assuming concentration additivity of zinc and cadmium).

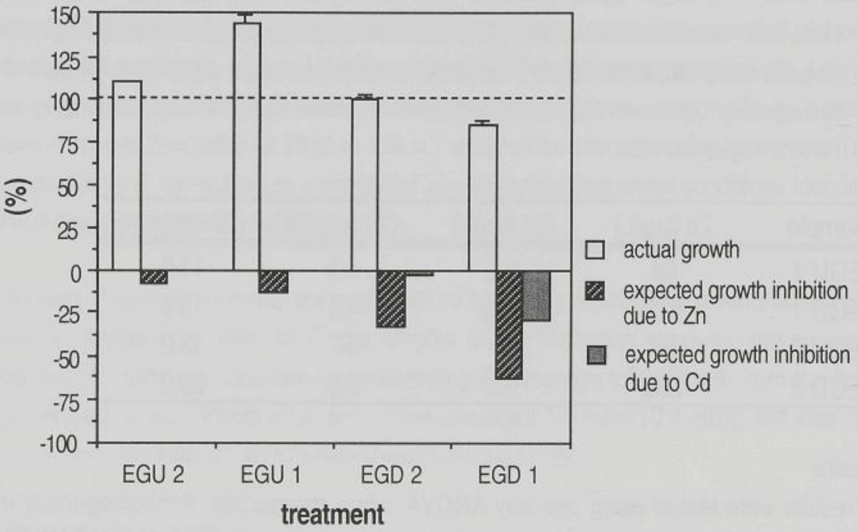

Fig. 3.2: Growth (with standard error) of *C. riparius* after 96h exposure to Dommel water from Eindergatloop Upstream (EGU) and Eindergatloop Downstream (EGD). Numbers following site code refer to sampling dates. 1: September 19, 1995; 2: August 20, 1996. Growth is presented as percentage of controls. Expected growth inhibition due to zinc and cadmium are calculated from growth toxicity data of Postma *et al.* (1995).

The role of particulate matter in river water was evaluated by exposing *C. riparius* to Meuse water in the absence or presence of particles. In all four experiments midges performed better in Meuse water containing particles than in filtered river water (Kruskal Wallis, $p<0.05$), in which, on two of four sampling dates, midge larvae grew even less than in control water (DSW) (Fig. 3.3). To study the role of particulate organic matter in river water in more detail, *C. riparius* was exposed to different size fractions of suspended matter. Midge growth increased with increasing filter pore size and thus with increasing levels of suspended matter (Fig. 3.4). On January 16 1996, larvae subjected to Meuse water filtered through a 10 μm mesh and to unfiltered water grew significantly more than controls ($p<0.05$).

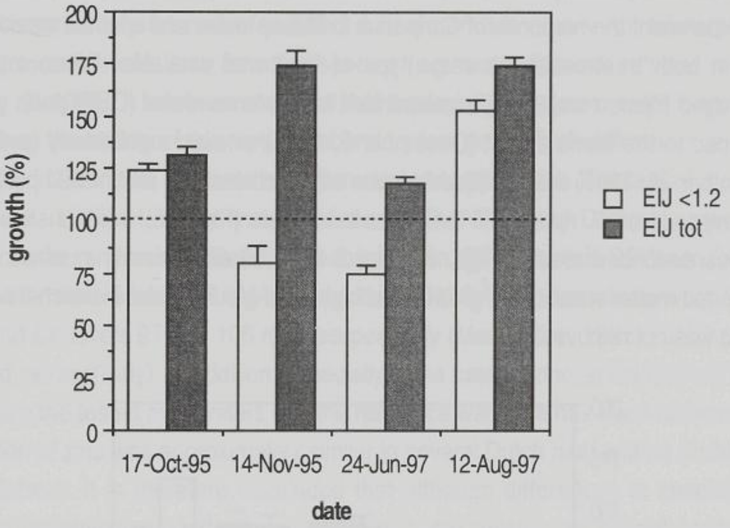

Fig. 3.3: Growth (with standard error) of *C. riparius* after 96h exposure to Meuse water from Eijsden. River water was filtered (EIJ <1.2) or not filtered (tot). Growth is presented as percentage of corresponding controls.

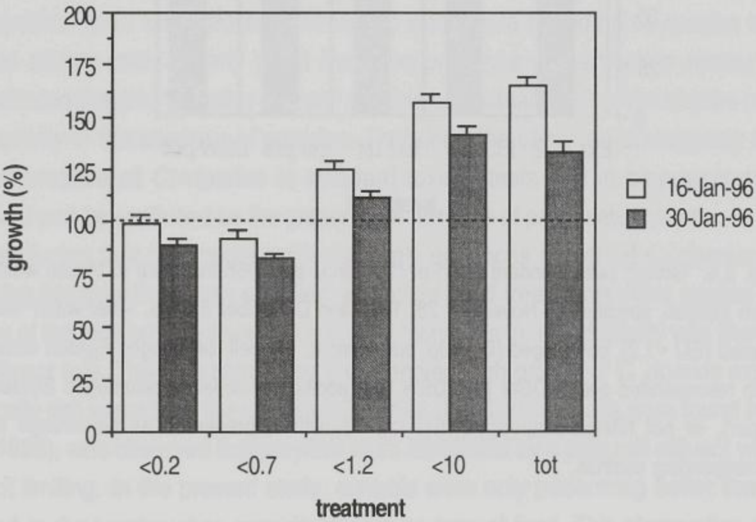

Fig. 3.4: Growth (with standard error) of *C. riparius* after 96h exposure to Meuse water from Eijsden. River water was filtered over different pore sizes, or not filtered (tot). Growth is presented as percentage of corresponding controls.

In the previous experiments, controls differed from other treatments in medium (standard water vs. Meuse water) and food (standard food vs. standard food plus particles). In the next experiment the response of *C. riparius* to Meuse water and controls was determined, while in both treatments the same type of food was available. When a pellet from centrifuged Eijsden water was resuspended in reference water (DSW pell), growth was enhanced to the same extent (December 5, 1995) or even significantly ($p<0.05$) more (November 28, 1995) than in Eijsden water with particles (EIJ tot and EIJ pell) (Fig. 3.5). The response of *C. riparius* to the other treatments (Fig. 3.5) was consistent with the previous described results (Figs. 3.3 and 3.4); in all experiments in which the role of suspended matter was tested, growth was highest in Meuse water in which the particulate fraction was not removed.

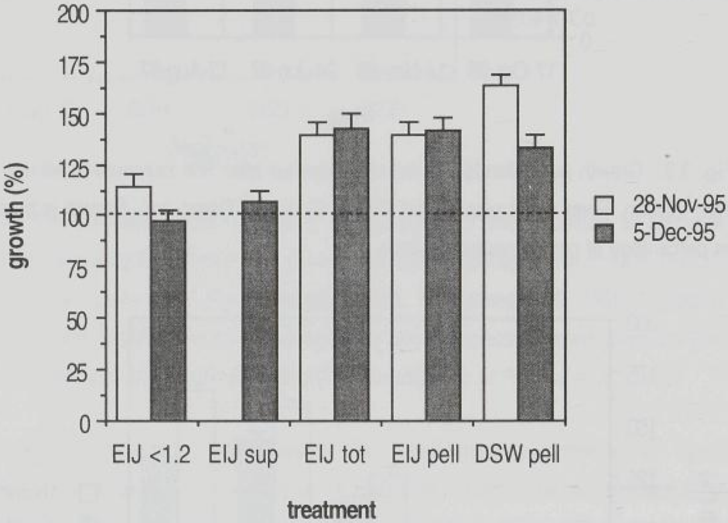

Fig. 3.5: Growth (with standard error) of *C. riparius* after 96h exposure to Meuse water from Eijsden, sampled on November 28, 1995 and December 5,1995. River water was filtered (EIJ <1.2), centrifuged (EIJ sup: supernatant; EIJ pell: centrifuged Eijsden water with resuspended pellet; DSW pell: DSW with addition of pellet of centrifuged Eijsden water), or not filtered/centrifuged (EIJ tot). Growth is presented as percentage of corresponding controls.

Discussion

The results of the Dommel bioassays suggest that the succes of the "pollution tolerant" midge *C. riparius* is due to tolerance to high toxicant (in this case metals) concentrations.

However, this assumption proved to be untrue when comparing the sensitivity of the midge in Dommel water to its sensitivity to zinc and cadmium in laboratory toxicity tests. Although *C. riparius* is extremely tolerant of polluted river water, it is clearly not tolerant of the measured toxicant levels. Also for other species (e.g. water flea and fathead minnow; Diamond *et al.*, 1997), it has been observed that the toxicity of a given metal in site water was lower than the toxicity of this metal in laboratory test water. Such differences in effects of a metal in different test solutions are often related to speciation (Pantani *et al.*, 1995; Landrum *et al.*, 1996). However, in spite of the fact that DSW and Dommel water differ, both types of water show similarities in ionic composition; Ca^{2+} levels in DSW and Dommel (at EGD) water were 55 and 46 mg/L, respectively. HCO^{3-} concentrations were 85 and 75 mg/L, and Cl^{-} levels 97 and 106 mg/L respectively (Maas *et al.*, 1993; Tubbing *et al.*, unpublished, respectively). In addition, especially in the case of zinc, speciation will hardly differ between the tested river waters and the reference waters; it has been observed that the speciation of zinc was approximately similar in several Dutch hard waters (Tubbing *et al.*, unpublished). It is therefore concluded that although differences in speciation or composition between both waters play a role to some extent, this cannot explain the observed large difference between effects of metals in river water and reference water. Alternatively, food (in this case components of particulate organic matter) may be a significant factor determining the performance of *C. riparius* in site water.

The experiments in which fractions of Meuse water were tested on *C. riparius* indicated that the midge could clearly profit from the presence of particulate matter. It was demonstrated that this "pollution tolerant" species can actually be inhibited by the prevailing water quality in the absence of particles. Therefore, the observed discrepancy between the performance of *C. riparius* in standard toxicity tests and in bioassays can for a significant part be explained by the presence or absence of particulate matter.
The conclusion that particulate matter strongly enhances growth of *C. riparius* may be surprising because food was supplied *ad libitum* in all treatments. This implies that the quantity of the food provided was not a limiting factor, but that food quality was likely to play a significant role. This is illustrated by the observed high growth of *C. riparius* exposed in organically rich water from the upstream Dommel site. Similar results were found by Lowell *et al.* (1995), who observed that mayflies were stimulated by a pulp mill effluent while food was not limiting. In the present study, controls were only performing better than larvae exposed to river water when provided the same type of food. This observation supports the idea that toxicants in the dissolved fraction in Meuse water induce negative effects on the midge, but this was obscured in untreated river water due to the positive effects of particulate organic matter.

The observed compensating effects can, however, not exclusively be attributed to

particulate organic matter. Growth was not (significantly) inhibited when midges were exposed to Meuse water without particles, implying that also in the dissolved fraction of the river water, there are factors which can compensate for possible negative effects of the present toxicants. Lowell *et al.* (1995) suggested that besides compounds like nutrients and hormonal substances, changes in palatability may cause stimulating effects on macrofauna. It has been observed that growth of the chironomids *C. riparius* (Rasmussen, 1985) and *Paratendipes albimanus* (Ward and Cummins, 1979) was increased after enrichment of detritus by microflora. It is possible that microflora present in Eijsden water, which were absent in reference water, also positively affected midge growth.
Although the the precise nature of the food stimulus in eutrophic river water remains unknown, it seems evident that its effect substantially enhances (on average >30%) growth of *C. riparius* (compared to growth in oligotrophic DSW). This positive effect occurs in eutrophic rivers with varying levels of pollution, as shown by the (over)compensation of toxic effects by food (organic enrichment).

In river water toxicants are partly bound to particles (Thomas and Meybeck, 1992; Renoldi *et al.*, 1997). Therefore, in the present study, increasing particle levels should have coincided with an increasing amount of toxicants. Nevertheless, negative effects of toxicants bound to these particles were insignificant compared to the positive nutritional effects on *C. riparius*. Comparable observations were made when *Daphnia magna* was exposed to metals in untreated Meuse water (De Ruiter and Hendriks, 1996), while in contrast another study demonstrated that for *D. magna*, contaminants were more toxic when bound to particles than free in the aqueous phase in laboratory test water (Taylor *et al.*, 1998). For *C. riparius* (and possibly to a lesser extent for *D. magna*), it seems that a stimulus caused by food is superimposed on toxic effects induced by dissolved and sorbed compounds. It is concluded from the present observations, that particulate matter in river water has great potential to reduce inhibiting effects of pollution on *C. riparius*, not (only) by reducing the bioavailability of toxicants, but by serving as a supplementary, superior food source.

Nalepa and Landrum (1988) stated that species considered to be tolerant of pollution (in the ecological sense of being found in polluted areas) are not necessarily most tolerant of toxicants. As indicated in the present study, the success of a "pollution tolerant" test species is not explained by tolerance of this species to toxicants per se, but by its ability to take advantage of organic enrichment. While the sensitivity to different toxicants is known to be strongly species specific (e.g. Phipps *et al.*, 1995; Masnado *et al.*, 1995 vs. McKinney and Wade, 1996), the present study suggests that organic compounds present in river water have strong stimulating effects that are also likely to depend on species specific capacities. The combined effects of toxicants and food may therefore partly explain large discrepancies between the distribution of species in the field, and the outcome of laboratory toxicity tests.

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