Insects in polluted rivers: an experimental analysis

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In large European rivers, the number of aquatic insect species has been strongly reduced during the last century, and therefore they could play a key role in assessing the ecological status of recovering aquatic communities. However, river insects are rarely used as ecotoxicological test organisms, which makes an effective use of distribution data very limited. This study aimed therefore to develop ecotoxicological test schemes with riverine insects as sensitive tools for eco(toxico)logical management of large rivers. To this purpose, three representative river insects (the caddisflies *Hydropsyche angustipennis* and *Cyrnus trimaculatus* and the mayfly *Ephoron virgo*) were selected and laboratory cultures and standardized ecotoxicity tests were developed. To gain insight in the sensitivity of these species and to discern potential key factors limiting the distribution of these species, the effects of different stress factors (copper, diazinon and low oxygen) were determined in experiments with increasing complexity and environmental relevance: from acute single compound tests to behavioral, mixture toxicity and multi stress experiments. For copper and diazinon, it was demonstrated that the sensitivity of these species is very high in comparison with aquatic insects traditionally used in ecotoxicity tests and adverse effects were demonstrated at ecologically relevant concentrations. The joint toxicity of copper and diazinon was significantly higher compared to the toxicity of the single compounds. Finally we demonstrated that nymphs of the mayfly *E. virgo* kept under low oxygen conditions were significantly more sensitive to toxicants. Our results demonstrated that mixture toxicity and multi stress may prevent the revitalization of benthic communities and underline the importance of using indigenous species in defining water quality conditions for ecological rehabilitation.

Keywords: aquatic ecotoxicology, polluted rivers, aquatic insects, Trichoptera, Ephemeroptera

RIVERINE ECOSYSTEMS

Rivers are the most important freshwater resource for man (Chapman, 1992). Large rivers and their flood plains have several economically important functions like draining the land and carrying away the water, providing shipping routes, connecting coastal ports with our main cities, and providing hydroelectric power. The water is also used for industrial applications, irrigation of agricultural land and as a main source for drinking water. But also in terms of biodiversity, large rivers are of main importance, despite the intensive anthropogenic impact. Large rivers and their flood plains are complex ecosystems consisting of numerous habitats and biotic communities (Chapman, 1992). They form living areas and migration routes for many terrestrial and aquatic organisms, including birds, amphibians, mammals and insects. The high level of spatio-temporal heterogeneity makes riverine floodplains among the most species-rich environments known (Ward *et al*., 1999).

A pristine and resilient riverine ecosystem is, amongst others, characterized by a high biodiversity of especially the macro-faunal community (Ward, 1992). Macro-invertebrates play a key role in the dynamic riverine food web as an important link in the turnover of organic material (Jonsson & Malmqvist, 2000) and provide a food source for many fish and bird species. However, *'More than one-half of the world's major rivers are being seriously depleted and polluted, degrading and poisoning the surrounding ecosystems'*, says the World Commission on Water for the 21st Century. These threats resulted in impoverished ecosystems with a low biodiversity in which opportunistic species are dominant (for example Nijboer & Verdonschot, 1997). This study, however, focused on water quality issues as a potential cause of an impoverished riverine community, and therefore no attention will be paid to habitat deterioration. One should in keep in mind, however, that in order to establish and maintain an ecosystem with natural characteristics, all requirements must be met, including a suitable habitat.

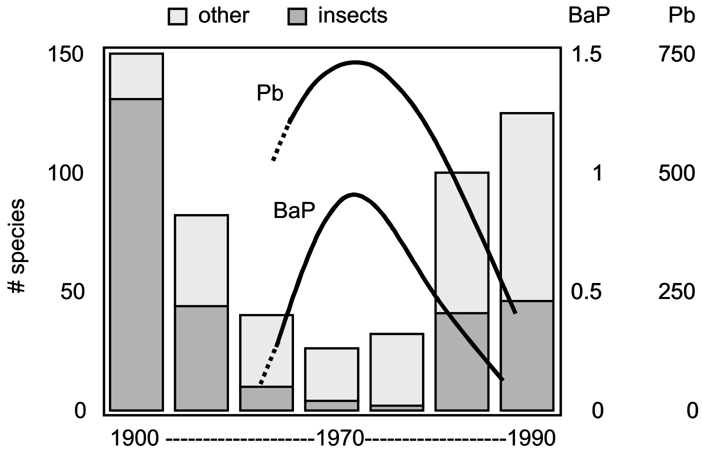


Figure 1. Changes in number of macro-fauna species (Landesamt für Wasser und Abfall Nordrhein-Westfalen, 1988) and in concentrations of two model contaminants, a metal (Pb) and a PAH (BaP), in the river Rhine during the previous century. The toxicant concentrations are measured in sediment core samples (in mg/kg) and plotted against the estimated year of deposition (from: Beurskens *et al.,* 1994).

The chemical characteristics of the river water (including the presence of toxicants) determine to a great extend the presence or absence of species as well as the physiological performance of individual organisms in that river (for example Leland & Fend, 1998; Chapman, 1992). In some large European rivers, the climax of pollution, caused by discharges of many industrial, agricultural and domestic activities, was reached in the late 1960s, early 1970s. The river Rhine, for example, was excessively polluted at that time (Beurskens *et al.,* 1994; Tittizer *et al.,* 1994). This was indeed clearly reflected in the changes in species richness during the previous century: an analysis of the development of the benthic macro-fauna in the 20th century reveals a drastic decline in the number of species from the mid-1950s to the early 1970s (Tittizer *et al.,* 1994). In Fig. 1, the changes in biodiversity in the river Rhine during the previous century are presented, clearly coinciding with the changes in concentrations of two selected model contaminants (a metal and a PAH).

Nowadays the basic water quality of the river Rhine has improved to such an extent that no direct adverse effects can be revealed in standard bioassays (Hendriks *et al.,* 1994). For other large European rivers, like for example the rivers Meuse, Elbe and Oder, rehabilitation is, however, until now not as successful as for the river Rhine (Van Dijk *et al.,* 1994), in spite of water quality improvements. Having solved the problems associated with high concentrations of a relatively small number of key toxicants (as in the 1960s-1970s), the question remains, therefore, if the present diffuse pollution with a wide range of toxicants in low concentrations (Hendriks *et al.,* 1994; Heemken *et al.,* 2000) hamper further ecological recovery of riverine communities. Many different pollutants still enter the large rivers from point sources and effluent discharges from domestic and industrial sources, from diffuse sources and through atmospheric deposition (Chapman, 1992). Adverse effects of this 'cocktail of chemicals' (mixture toxicity) on riverine biota are difficult to determine and are therefore at this moment hardly investigated. This is even more complicated by the fact that besides toxicants, several other variables influence the distribution of aquatic organisms: most large European rivers are nowadays, for example, affected by eutrophication (Van Dijk *et al.,* 1994). Also lowered oxygen concentration, often as a result of this eutrophication, and changes in salinity and temperature are observed. These different environmental stressors can interact in a variety of ways, which are until now hardly explored. It is, however, most likely that such interactions influence the response of aquatic organisms to polluted river water and insight in the joint effects of multiple stressors is therefore required.

Riverine insects and water quality

In the process of rehabilitation of the large European rivers measures were taken to allow the number of aquatic species to increase. The contribution of aquatic insects to the benthic community in the river Rhine at this moment is, however, much lower than at the beginning of the previous century (Fig. 1). Based on these observations, we argue that riverine insects, which are representative for pristine riverine ecosystems, could play a key role in assessing the ecological status of aquatic communities and in indicating ecological recovery. This is also supported by the Dutch approach to indicate ecosystem quality: in the so-called AMOEBE, several representative plant and animals species have been chosen, the presence and abundance of which (relative to a defined target situation) should indicate a intact ecosystem. For the large rivers two midges (Diptera), one caddisfly (Trichoptera) and one mayfly (Ephemeroptera) are incorporated (Van Dijk & Marteijn, 1993), indicating the importance of aquatic insects for water management. In addition, also many biotic classification systems use the distribution of aquatic insects to define water and/or habitat quality (Resh, 1992). Examples are the widely used EPT-index, the benthic index of biological integrity (B-IBI; Fore *et al.,* 1996) and RIVPACS (River invertebrate Prediction and Classification System; Wright *et al.,* 1998). The construction of biotic indices that use riverine insects to assess pollution and other anthropogenic disturbances often requires insight in the sensitivity of taxa.

In contrast to the above, aquatic insects (and especially Ephemeroptera, Plecoptera and Trichoptera) have rarely been included in standardized ecotoxicological test schemes and most sensitivity data is based on field observations only and are often highly subjective (Metcalfe, 1989). Consequently, the responsible environmental or anthropogenic variables limiting the distribution of aquatic insect species are at this moment hardly known.

Objectives

This study aims to generate insight in how specialized river insects cope with large variations in water quality. Based on this understanding, the key environmental standards could be defined that allow rehabilitation of the original biodiversity in degraded rivers. To this purpose, basic eco(toxico)logical knowledge on representative riverine species will be provided. Riverine insects that play a key role in indicating ecological recovery, as argued above, are selected as model species and are to be developed as sensitive tools for the ecological management of rivers.

Test organisms

Test organisms frequently used in standardized test procedures were traditionally selected because of their ease in culturing, handling and testing (McCahon & Pascoe, 1988; Watts & Pascoe, 1996). However, since rehabilitation programs have become more location specific, the representativity of the test species for the ecosystem of concern has become increasingly important. Therefore, in river water quality assessment studies, several other more representative species (for example mayflies Frick & Herrmann, 1990) are chosen. In most of these studies, however, field-collected late instar individuals were used, because no culture methods were available. The major disadvantage of this approach is the relative insensitivity of late instars compared to young instars (Hutchinson *et al.,* 1998). The selection of the insect species that were used as test organisms in the present study was therefore based on two criteria: the past and present distribution of the species in the large European rivers and the ability to keep the organisms in the laboratory under controlled conditions. Attempts have been made to develop laboratory cultures for several caddisfly, mayfly and midge species. Based on the results of these attempts, two caddisflies (*Hydropsyche angustipennis*; Hydropsychidae, Trichoptera and *Cyrnus trimaculatus*; Polycentropodidae, Trichoptera) and one mayfly (*Ephoron virgo*; Polymitarcidae, Ephemeroptera) were selected (Fig. 2).

E. virgo was in the beginning of the previous century present in mass numbers in the Dutch rivers (Schoenemund, 1930; Albarda, 1889) but was observed for the last time in 1936 (Mol, 1985). It was extinct in The Netherlands for more than fifty years until Bij de Vaate *et al*. recorded some nymphs near the German/Dutch border in 1991 (Bij de Vaate *et al.,* 1992). *E. virgo* is nowadays present in the River Rhine (and some of its large tributaries, the Mosel, Main and Neckar) downstream from Mannheim where the River Neckar flows in the River Rhine (Schöll, 1996). Also the two caddisfly species used to be present in the rivers Rhine and Meuse. Originally, *H. angustipennis* was widely distributed in small streams as well as in large rivers (Eddington & Hildrew, 1981), but nowadays this species is not found in the lower reaches of the rivers Rhine and

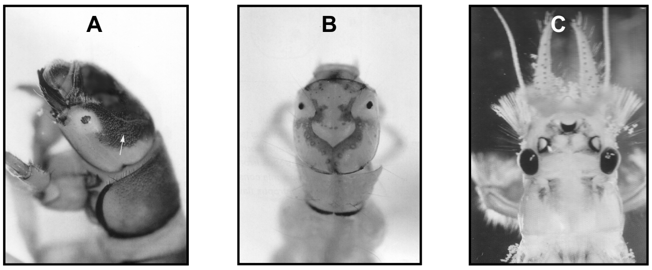


Figure 2. The three selected test organisms: A. *Hydropsyche angustipennis* (Hydropsychidae), B. *Cyrnus trimaculatus* (Polycentropodidae), C. *Ephoron virgo* (Polymitarcidae).

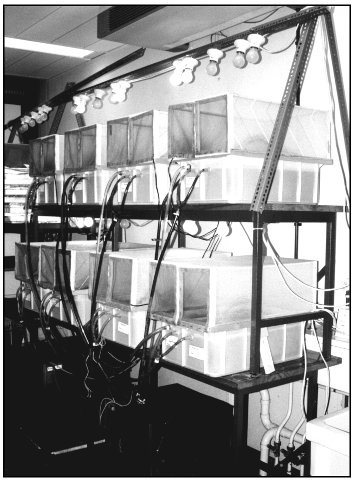
Meuse. *C. trimaculatus* usually appears in the lower reaches of large rivers, but also occurs in ponds en lakes (Eddington & Hildrew, 1981). During the previous century, however, also this species was not found in the rivers Rhine and Meuse for a long period of time. Only since the last two decades of the previous century, specimens of *C. trimaculatus* were recorded again. Based on their past and present distribution, the three selected species are potential useful species as indicators for ecological recovery of river ecosystems.

HANDLING OF RIVERINE INSECTS

Caddisflies and mayflies in the laboratory

Caddisfly cultures were started by collecting larvae in the river Erft, a tributary of the river Rhine, in Germany. In the laboratory, the larvae were placed in large rearing containers (Fig. 3A) filled with 30 L of Dutch Standard Water (DSW) and a stony substrate of gravel and stones. A flow-through system provided a continuous water flow inside the rearing containers. The emerged adults were collected in the net cages that were placed on top of the rearing containers and released in a cage where they could mate and deposit their egg masses. This mating cage was filled with a small layer of DSW and a few partly submerged stones on which the egg masses were deposited by the females. The eggs were removed afterwards and placed back in the rearing containers or used for experiments. This setup provided a continuous supply of larvae with a known history and age which is essential for the reliability of ecotoxicity tests. Simultaneously insight was gained in the autecology of the species. Maintaining a steady laboratory culture of these caddisflies, however, is laborious and time consuming.

Since it is impossible to make containers large enough to allow adults of the mayfly *E. virgo* to mate, a different setup was developed to ensure a continuous supply of mayfly nymphs: *E. virgo* eggs were collected from a population in the River Waal by attracting adults with a light-trap during twilight (Fig. 3B). Because eggs attach to the substrate after being deposited, they can be collected and stored on glass slides at which sand was glued with an inert epoxy resin. Approximately five hundred of these slides were placed in containers, which were filled with river water and placed beneath the light-trap. Each female attracted by the light deposited two egg masses immediately after touching the water surface in the trays. The egg masses sank to the bottom of the trays where they fell apart in thousands of eggs, which stuck to the glass slides (Fig. 3B). In the laboratory, the glass slides with eggs were placed in aquaria filled with DSW, and stored at 20 °C. At this temperature the embryos developed and after 4 weeks the development stagnated and diapause was entered. Two weeks later the eggs were transferred to a refrigerator where the temperature was maintained at 4°C. In this way the eggs can be stored for at least 3 years. After a minimum of three months at 4°C, the diapause can be deactivated by transferring the eggs from ± 4 °C to a temperature of 20°C. After 4 to 6 days at this temperature the nymphs hatch and can be used to perform (toxicity) experiments.

A. 

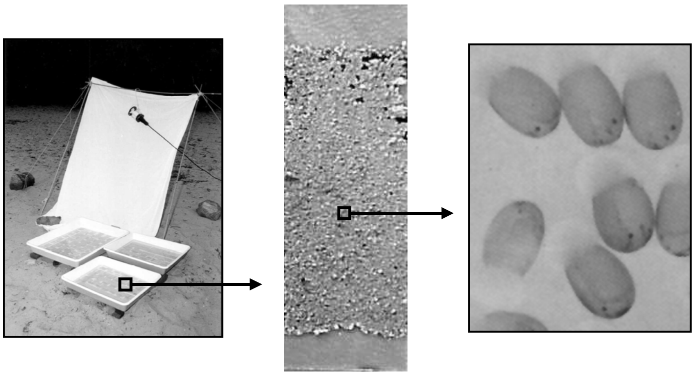
B. 

Figure 3. Newly developed methods for ensuring a continuous supply of young larvae with a known history and age: A. Caddisfly laboratory culture (Greve *et al.,* 1998) and B. Collecting and storing of eggs of the mayfly *Ephoron virgo* (Greve *et al.,* 1999).

Development of bioassays

By optimizing several test conditions (such as different types of substrate, water, food, aeration and different ages of first instars) and performing an extensive set of validation experiments, short term bioassays were developed for the three selected species. Details of test development and validation are provided by Greve *et al*. (1998, 1999). These newly developed bioassays combine the representativeness of the test species for river water and sediments with the availability of a continuous supply of young (and hence more sensitive) larvae with a known history and age. Also, the newly developed ecotoxicity test using newly hatched larvae of the selected riverine insects are reliable, reproducible and easy to perform.

RIVERINE INSECTS COPING WITH SELECTED CONTAMINANTS

To determine their sensitivity, the three selected riverine insects were exposed to two different model toxicants, copper and diazinon. These model toxicants were selected based on their occurrence in large European rivers and differences in mode of toxicity. Copper represents a micro-nutrient which is essential for a variety of physiological processes within organisms, but may become toxic at higher concentrations. Diazinon is an organophosphorous insecticide used to control a wide variety of insects in agriculture. Inhibition of the enzyme acetylcholine-esterase (AChE) is considered to be the most important mode of toxicity.

Survival tests

Using the newly developed bioassays, short-term LC50 values (*i.e.* the concentration of a compound at which 50% mortality occurs) of both model toxicants were determined for all three test species. As an example, in Fig. 4, a sensitivity distribution of aquatic macro-invertebrates, based on all known 96h LC50 values for diazinon in literature, is presented. The position of the three selected insect species is indicated by arrows. It is clearly shown that the three selected insects are among the most sensitive species to diazinon. The 96 hour LC50 for *C. trimaculatus* is even lower than for any other insect species known from literature. Also for copper a comparison with 96 h LC50 values from literature for other aquatic taxa was made. In contrast to diazinon, however, no general classification of sensitive and tolerant taxa can be made: in all taxa, both sensitive and tolerant representatives are found. The three species selected in this study are among the most sensitive insects, and in the middle range of all copper toxicity data.

The concentrations of diazinon at which effects on the selected insect species were detected in the laboratory, were relevant to the maximum concentrations of organophosphorous insecticides in, for example, the River Meuse, illustrating that such accidental peak concentrations are limiting

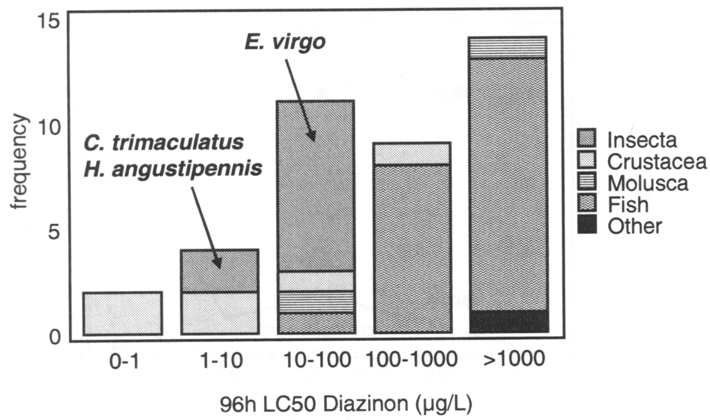


Figure 4. Sensitivity distribution of different groups of aquatic macro-invertebrates exposed to diazinon, based on all 96h LC50 available data in literature.

the distribution of riverine insects in the field. Maximum field concentrations of copper, however, do not reach levels at which acute effects are observed.

Behavioral tests

Behavioral experiments, using the impedance conversion technique (Heinis & Swain, 1986), were included in the present test scheme because a wide variety of pollutants can influence the function of sensory organs and alter the behavior of aquatic organisms (Blaxter & Ten Hallers-Tjabbes, 1992). Such behavioral changes are often the most sensitive reaction to chemical stress (Rand, 1985) and are likely to reduce the fitness of organisms under field conditions (Blaxter & Ten Hallers-Tjabbes, 1992).

In these behavioral experiments, activity patterns of larvae of the caddisfly *H. angustipennis* were recorded during 1 h after an exposure period of 48 h to different toxicant concentrations. From the activity signals provided by the impedance converter, three different types of behavior were defined according to the relative frequencies and amplitudes: undulatory movements or ventilation (mono-frequent with a relative high and constant amplitude), inactivity (signals below background noise) and other activity (multi-frequent with different amplitudes). For each larva, the time spent on these different types of behavior was determined and expressed as percentage of the total registration time. By comparing behavioral responses with those of controls under the same experimental conditions, the influence of variables potentially influencing results of these tests (for example method and moment of testing, temperature or current velocity) is reduced. In the experiments with copper, it was found that in all copper treatments, time spent on ventilation was significantly lower than in the control ($p<0.05$) (Fig. 5). Based on time spent on ventilation, the EC50 for 5th instars was 150 times lower than the 48 h LC50 for 1st instars. Furthermore, after an initial activity peak at low copper concentrations, larvae became more inactive at increasing copper concentrations with 100% inactivity at the highest test concentration. Consequently, time spent on other activities decreased at higher concentrations (Fig. 5). This reaction may be a strategy of 'waiting' until the pollution has passed by, as discussed by Gerhardt (1996).

The low effect concentrations for altered activity patterns may be indicative of chronic effects: In life-cycle tests with the caddisfly *Clistoronia magnifica*, Nebeker *et al*. (1984) demonstrated a significant reduction in adult emergence at concentrations similar to the behavioral effect concentrations. Therefore it may be expected that chronic exposure to the average load of copper in the River Meuse (5 µg/L) will provoke life-cycle effects on this caddisfly species.

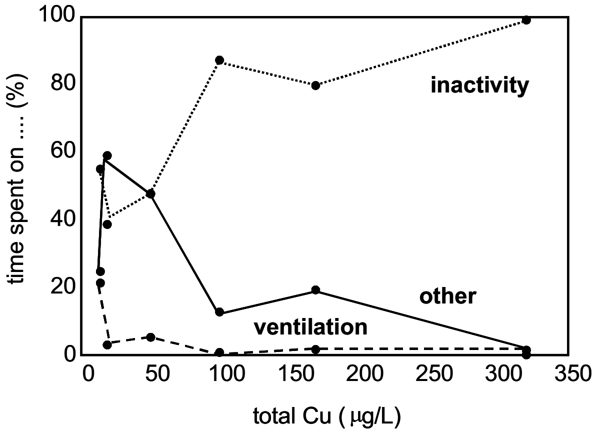


Figure 5. Time spent on different types of behavior (ventilation, other activities and inactivity) by fifth instar *H. angustipennis* larvae after 48 h exposure to different concentrations of copper, plotted as mean percentages against measured copper concentrations.

SURVIVAL OF RIVERINE INSECTS UNDER COMBINED STRESSORS

As mentioned before, most large rivers are nowadays suffering from a diffuse pollution by a wide range of compounds in low concentrations, often coinciding with sub-optimal environmental conditions (such as low oxygen concentrations). Therefore, this study also aimed to gain insight in the joint effects of chemicals (mixture toxicity) and multiple stressors on riverine insects. In this way, adverse effects of environmental relevant concentrations of contaminants can be quantified and compared to field observations on the occurrence of river insects.

Mixture toxicity

To gain insight in the joint effects of chemicals, the effects of a mixture of copper and diazinon on nymphs of the mayfly *E. virgo* were determined. It was demonstrated that although a less than concentration additive mixture effect was observed over the entire effect range, it was clear that the toxicity of the mixture was higher than that of the individual compounds. At the lowest calculated effect level in the mixture, the concentrations of copper and diazinon were relevant to the concentrations of both compounds as measured in for example the River Meuse. It is therefore argued that attention needs to be paid to mixture effects in defining standards for water quality and risk assessment procedures.

Multi-stress

Besides toxicants, there are several other key factors limiting the distribution of riverine insect species. One of the variables in the aquatic environment of considerable importance to benthic communities is the oxygen concentration of the water and the upper sediment layers (Ward, 1993). Fluctuating oxygen levels are often observed in inland waters, as a result of complex diurnal and annual variations, depending on both (a)biotic variables such as light intensity, current velocity or disintegration processes, as well as human activities like hydrological and geo-morphological modifications or additional input of organic matter. In most large European rivers, mean annual oxygen concentrations meet the environmental standards, but nevertheless temporary low oxygen conditions still occur frequently on a regional scale (for example the rivers Meuse, Scheldt, Mersey, Elbe, Tiber). In the River Meuse, for example, variations in oxygen concentration between almost 0 % and super-saturation are observed (RIWA, 1993-1997). Moreover, the periods of the lowest oxygen concentrations often coincide with the presence of the highest contaminant concentrations. Therefore, the combined effects of toxicants and oxygen depletion was determined. To this purpose, nymphs of the mayfly *E. virgo* were exposed to the two model toxicants, (copper and diazinon) under normoxia and hypoxia (50% air saturation, which causes no lethal effects after 96h of exposure).

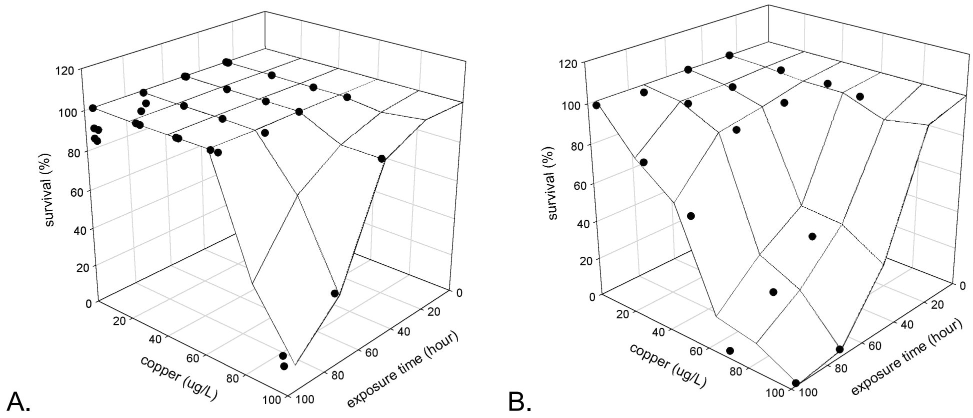


Figure 6. Survival of newly hatched *Ephoron virgo* nymphs after 0-96 h of exposure to different concentrations of copper, at 100% (A) and 50% (B) air saturation. The black dots represent the average survival per treatment.

Fig. 6 shows the survival of newly hatched *E. virgo* nymphs after 0-96 h of exposure to different copper concentrations at 100% and 50% air saturation. In both treatments, mortality increases with increasing copper concentrations and with increasing exposure times. In the 50% treatment (Fig. 6B), however, the observed mortality at each given exposure time and copper concentration was higher than the corresponding point in the 100% treatment (Fig. 6A). For example: after 96 h of exposure to ca. 50 µg copper/L, all nymphs survived in the 100% treatment, but in the 50% treatment survival was already reduced to 50%. Since 50% reduction in the oxygen content and 50 µg copper/L separately did not resulted in any mortality, it is concluded that the combined effect of copper and low oxygen is much higher than can be expected based on the effects of both factors separately.

In contrast to the copper experiments, no significant difference in response was observed between the 100% and 50% treatments in the presence of the insecticide diazinon. This difference between the influence of lowered oxygen on the toxicity of copper and diazinon is most likely caused by the difference in the modes of toxic action of both compounds: Inhibition of the enzyme acetylcholine-esterase (AChE) by an oxidative product of diazinon (diazoxon) is considered to be the most important mode of toxicity of diazinon. A possible inverse relationship between the oxygen concentration and the formation of this toxic oxygenated product (or of any other product causing oxidative stress (Choi *et al*., 2000)) at lower oxygen concentrations, may even reduce the toxicity of diazinon. Since this trade-off between respiratory stress and oxygen dependent transformation does not play a role in the toxic mode of action of copper, whereas it possibly does for diazinon, this could explain the observed differences in the influence of lowered oxygen on the toxicity of both compounds.

ENVIRONMENTAL AND ECOLOGICAL RELEVANCE

Understanding the distribution and abundance of species is one of the cornerstones of ecological science. Therefore, insight is required in the resource requirements of species, their life history, intra- and inter-specific interactions and their response to environmental conditions (Townsend *et al*., 2000). Similarly, understanding the distribution of species as affected by pollution requires a specific analysis of the parameters determining the persistence of species in disturbed environments. Therefore, environmental contaminants are thought of as being dimensions in a multidimensional niche, within which a certain species can maintain a viable population: we argue that a disturbance by environmental contaminants, due to anthropogenic activities, can cause niche dimensions to vary outside of the normal range or introduce previously non existing dimensions. Subsequent shifts in the abundance of species could be expected, therewith impairing the structure or functions of the riverine ecosystem.

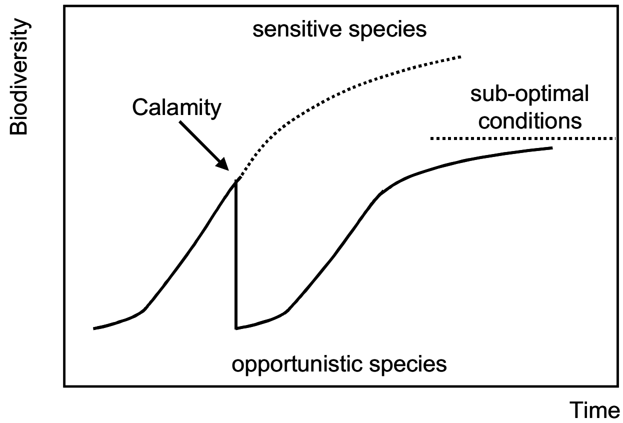


Figure 7. A theoretical model of the ecological recovery of riverine ecosystems over time, as influenced by incidental spills of acute toxic chemicals (calamities) and by ongoing sub-optimal conditions.

In the present study it was demonstrated that the maximum peak concentrations of organophosphorous insecticides in the field are in the same order as the observed effect levels for sensitive juvenile river insects determined in the laboratory. Riverine insects, that require stable environmental conditions because of their relative long life-cycle, are likely not able to maintain populations in rivers which are frequently disturbed by such accidental peak concentrations of contaminants. Simultaneously, this example shows that spatial and temporal aspects of pollution in relation to the life-cycle characteristics of the organisms, determine the extend to which contaminants impair the persistence of species. For example, not only the concentration and kind of chemical spilled are determining the effects on biota, but also the frequency and seasonal timing at which the organisms are exposed to it (Fig. 7). Considering this relationship between chemical stressors and life-cycle characteristics of the exposed riverine species, also chemicals present during longer periods at concentrations below acute lethal effect levels could prevent species to persist in certain habitats. For copper, maximum field concentrations do not reach acute effect levels, but the average load in for example the river Meuse is in the same order as the observed behavioral effect levels for *H. angustipennis*. These behavioral responses are likely to reduce the fitness of organisms (Blaxter & Ten Hallers-Tjabbes, 1992) and therewith indicative for potential life-cycle effects. Conditions that are steadily sub-optimal such as a continuous exposure to low concentrations of copper, as well as peak exposures such as pesticide spills, may therefore limit the distribution of species. Especially the species with a sensitivity above average and/or species with relative long life-cycles are prone to local extinction (Fig. 7).

It can be expected that chemicals interact with each other as well as with other environmental conditions. Mixture toxicity is an example of such an interaction: in the present study, experiments revealed that the tested model toxicants contribute to mixture toxicity below their individual effect levels. Adverse effects in the field are, therefore, not determined by the effects of (incidental discharges of) individual compounds only. In the field, mixture toxicity has indeed been demonstrated to be responsible for adverse effects on riverine invertebrates (Stuijfzand, 1999). Similarly, interactions between environmental contaminants and other limiting conditions can be expected. Considering the large differences in response types to these different dimensions, such interactions can be expected to appear in a variety of ways: For example, here it was demonstrated that the combined effect of copper and low oxygen is much higher than can be expected based on the effects of both factors separately. Since in the field the lowest oxygen concentrations often coincide with the highest toxicant concentrations, it was concluded that 'multiple stress' actually occur in large rivers carrying complex pollution and that under such conditions adverse effects of chemicals on riverine biota can be unexpectedly high. Therefore we argue that the joint effects of multiple stressors are a potential key factor hampering the progress of ecological recovery and that to adequately fulfill the needs of ecological recovery programs, attention needs to be paid to more realistic conditions in toxicity testing, especially to mixture toxicity and multiple-stress.

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