The effect of micropollutants on components of the Rhine ecosystem

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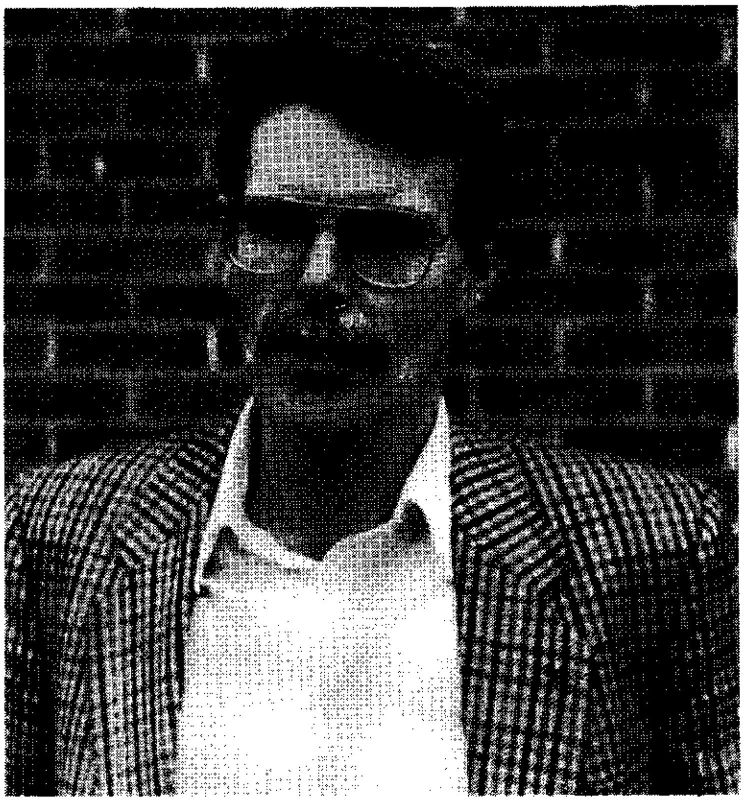

Drs. Geert van Urk (1944 - 1990).
Senior-biologist at the Institute for Inland Water Management and Waste Water Treatment.

"A pioneer and valued colleague in the research topics presented in this volume".

The effect of micropollutants on components of the Rhine ecosystem

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Introduction

Toxicological effects of micropollutants upon aquatic organisms are best known from standardised laboratory tests with individual chemicals or well known combinations of chemicals. This information is generally used for the derivation of ecotoxicologically based water-quality standards in the Netherlands, but also by the International Rhine Commission.

These standards are based on calculated risk using extrapolation techniques and they are therefore abstractions of reality. For the purpose of setting waterquality standards however this information satisfies.

The question arises wether the implementation of such standards gives an adequate protection of an actual ecosystem like the river Rhine. Also the environmental manager needs elective instruments in regulatory and decision making frame works: he likes to see watersystems are really improving once the pollution load is reduced. This is even more true when costly measures are necessary to remove historically pollluted sediments, as is the case in the downstream sedimentation areas of the Rhine in the Netherlands.

For these reasons it is necessary to have indications from the ecosystem itself about the present effects of pollutants. Indications which can be followed in time and may be used in evaluations of management policies.

In general there are a few methods which can be used in this way: measuring the level of toxicants in organisms and comparing them with possible effect-limits, measuring the structure of the ecosystem in relation to pollution or measuring lethal or sub-lethal effects on individual organisms or populations. This type of ecotoxicological work is rather limited at present. In the past it has not been very popular because very often it is difficult to link effects to their origin e.g. the presence of toxicants. Another reason may be, that it is also difficult to relate effects found in the biological community directly to pollution, because of many other constraints and pertubations.

In the last few years this type of research has received more attention. In the Dutch part of the Rhine ecosystem various investigations were carried out or inspired by **Geert van Urk** at the Institute of Inland Watermanagement and Waste Water Treatment (RIZA). Unfortunately he died in 1990 at the age of 46. We owe him a great deal as an esteemed colleague, someone with a profound knowledge of the Rhine ecosystem and most of all as one of the pioneers in this type of research.

The contributions brought together in this volume are elaborated bij the co-authors mentioned, based on his results or are results of investigations that were started by him or inspired by him. They are manuscripts in different stages of completing, meant to be published in scientific journals.

They may be considered as a tribute to an honoured colleague.

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Insects and insecticides in the Lower Rhine

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Summary

Two chemical accidents with insecticides in the River Rhine, i.e. the endosulfan accident in 1969 and the Sandoz accident in 1986 played an important role in public and political awareness regarding water pollution. The endosulfan accident gave cause for the development of a chemical monitoring and sanitation programme, whereas the Sandoz accident accelerated this sanitation programme and triggered a number of eco-(toxico)logical studies and their implementation in water quality control.

The gradual decrease in concentrations of organophosphorus insecticides since 1977 resulted in the recolonization of a number of insect species in the lower Rhine. First some species of chironomids appeared and later the caddisflies *Hydropsyche contubernalis* followed by *Ecnomus tenellus*. Ecological research before, during and after the Sandoz accident showed that chironomids and caddisflies occurring in large numbers in the IJssel, a branch of the River Rhine, were affected by the Sandoz accident. After the Sandoz accident, the pattern of recolonization showed the same sequence. Again, various chironomid species were among the first to recolonize, whereas recovery of caddisfly populations lasted longer. It appeared that for all species not more than one generation was needed for recovery, whereas the second generation showed complete recovery.

In general terms it means that species requiring stable conditions, e.g. because of their long life cycle, will not be able to maintain self-sustaining populations in the Rhine where different stress factors are operating.

Key words: Rhine, Sandoz, endosulfan, insecticides, chironomids, caddisflies.

Introduction

From the chemical monitoring programme of the Rhine, it can be demonstrated that insecticides have been present in the water of the Rhine for a long time, and that maximum concentrations occurred around 1975. The presence of these high concentrations of pesticides had not drawn wide attention, and there was no wide concern about possible effects. Public concern about toxic substances in the Rhine was evoked by two accidents. The first one was in the year 1969, when a large fish kill occurred in the Lower Rhine which could be ascribed to the insecticide endosulfan drained off by a chemical plant in the former Federal Republic of Germany. The second one was in November 1986, when water contaminated with pesticides flowed into the Rhine after a fire in a storehouse of Sandoz AG in Basel, Switzerland. These pesticides caused massive kills of aquatic organisms (Greve, 1971 and Capel et al., 1988).

At the time of the endosulfan accident, there were neither monitoring programmes of toxic contaminants in the Rhine nor warning systems to protect water supplies. The identification of endosulfan as the cause of the fish kill was due to the inventiveness of Greve and co-workers at the National Institute of Public Health in the Netherlands (Greve and Wit, 1971). The accident gave cause for the development of a monitoring and warning system as well as the development of a sanitation programme. Because no biological surveillance was carried out at the time of the endosulfan accident, no data are available on the possible effects of endosulfan on macroinvertebrate populations.

At the time of the Sandoz accident, biological monitoring studies were carried out, but these were not aimed specifically at the impact of toxic substances on freshwater biota. From both accidents it may be concluded that at least two groups of insecticides play an important part in the pollution of the Rhine: the organochlorine insecticides and the cholinesterase inhibiting insecticides, i.e. organophosphorus esters. From the former category, only lindane (gamma-hexachlorocyclohexane) is found regularly in detectable concentrations in the river water. Other organochlorine insecticides such as aldrin, dieldrin, DDT and derivatives, heptachlor and endosulfan were only detected in a small proportion of the river water samples, mainly in 1973 (Dijkzeul, 1982). This may be partly due to the rather high detection limit in water samples and their physicochemical behavior. In fish and mussel samples, most organochlorine insecticides are present above detection limits. Trends in the concentrations of these organochlorine insecticides from fish or mussel samples are difficult to establish, because no regular sampling has been carried out.

Concentrations of cholinesterase inhibitors in Rhine water show maximum values from 1975 to 1977. The highest value ever recorded during the monitoring programme was 134 µg/L in 1976. The median concentrations measured in 1975, 1976 and 1977 were 7.8, 3.4 and 3.2 µg/L, respectively. Since 1977, concentrations have gradually decreased to median values of 0.5 - 1.0 µg/L in recent years. An exception already mentioned was the Sandoz Accident in 1986. During that accident, the maximum measured concentration of cholinesterase inhibitors in the Rhine at Lobith was 5.5 µg/L (expressed as paraoxon equivalents). This is of course far lower than previously recorded values. The identity of the cholinesterase inhibitors is unknown as is their origin. It is suspected that the high levels of cholinesterase inhibitors were due to industrial discharges of waste waters from manufacturing processes, but there is no published information to confirm this suspicion.

Effects of cholinesterase-inhibitors on insect larvae

In Table 1, some data are summarized on the acute toxicity of organophosphorus and carbamate insecticides obtained in laboratory studies. Only data on species or groups commonly occurring in the Rhine are reported; data on Ephemeroptera, Plecoptera and Odonata were omitted. Moreover, a selection was made towards the lowest LC50 values reported in the literature. Although this procedure gives no insight in the full range of the toxicity data reported, the selection towards the lowest effect levels reported is a generally accepted procedure in ecological effects assessment (Van Leeuwen, 1990).
For most invertebrate species, information on chronic lethal effects and long-term effects on growth or reproduction is not available. An exception is *Daphnia magna* (Cladocera) which is very well studied. No-effect-levels (NELs) generally are a factor 10 - 20 lower than the 24h-LC50 or 48h-LC50 (e.g. Dortland, 1980). For *Hydropsyche* species, sublethal effects (i.c. effects on net-spinning activity) have been established for fenethcarb at concentrations of about 1 µg/L (Besch et al, 1977).
On the basis of acute toxicity data from laboratory studies, it may be concluded that concentrations of cholinesterase inhibiting insecticides in the order of magnitude of 1µg/L or more, would have deleterious effects. However, it should be kept in mind that the substances occurring in the Rhine were not identified and that the differences in toxicity between various cholinesterase-inhibitors may be considerable. This has not only been demonstrated for insect larvae (Table 1), but also for fish (De Bruijn, 1991). Furthermore, most of the toxicity data shown in Table 1 were obtained in laboratory studies in standard water. Reduced bioavailability in Rhine water due to e.g. adsorption of the pesticides to suspended matter is therefore not accounted for, which may hamper the prediction of effects in the field.
A lot of information on effects of insecticides on stream invertebrates can be derived from field studies carried out in connection with programmes to control nuisance species. From the different case studies reviewed by Muirhead-Thomson (1987), the Onchocerciasis Control Programme in the Volta River Basin is the most interesting in this context because organophosphorus insecticides, temephos (Abate) or chlorphoxim were used over a rather long period. In this case, the target organism was *Simulium damnosum*. Despite the great effort made to monitor side effects, it proved difficult to attribute faunal changes to the larvicide treatments because many other factors were influenced the distribution of non-target species. On the other hand it was possible to define three arbitrary groups of non-target invertebrates according to their reactions to Abate treatments. *Tricorythus* sp. (Ephemoptera) appeared to be a highly sensitive species. Moderately affected were two hydropsychid caddisflies and *Pseudocloeon* sp. (Ephemoptera). Among the least affected were several species of chironomid midges and another species of *Simulium*, i.c. *S. schoutedeni* (Muirhead-Thomson, 1987). Especially, this latter conclusion is striking, because it indicates, that taxonomical relationship does not imply a similar sensitivity to contaminants. No analysis was presented as to *S. damnosum* was sensitive and *S. schoutedeni* was resistant to pesticide treatment. Also intriguing is the conclusion that some Chironomidae, especially species belonging to the Orthocladiinae, may show great increases of numbers after treatment, probably because of absence of their predators.

Table 1. Acute toxicity of organophosphorus and carbamate insecticides to larvae of Chironomidae and Trichoptera.

Substance	Species	Exposure (h)	LC 50 (μ/L)	Ref.
Parathion	Chironomus sp	24	5.5	a)
	Chironomus riparius	24	0.60	b)
	Tanypus grodhausi	24	0.5	a)
	Hydropsyche contubernalis	24	0.64	b)
	Hydropsyche bettoni	48	0.48	c)
Malathion	Chironomus sp	24	2.1	a)
	Chironomus tentans	24	2.0	d)
	Hydropsyche bettoni	24	0.34	c)
Diazinon	Chironomus tentans	24	0.40	e)
	Chironomus tentans	48	0.10	e)
	Chironomus tentans	72	0.07	e)
	Chironomus tentans	96	0.03	e)
	Hydropsyche bettoni	48	3.54	c)
Chlorpyriphos	Chironomus tentans	24	6.4	d)
Dursban	Chironomus sp	24	0.42	a)
	Tanypus grodhausi	24	0.5	a)
Abate	Chironomus sp	24	0.7	a)
	Tanypus grodhausi	24	1.5	a)
Carbaryl	Chironomus tentans	24	1.6	d)
Carbofuran	Chironomus tentans	24	1.6	d)
Propoxur	Chironomus tentans	24	1.7	d)

References: a) Mulla & Khasawinah, 1969; b) Heinis & Crommentuyn, 1988; c) in Dortland, 1980; d) Karnak & Collins, 1974; e) Morgan, 1976, cited in McEwen & Stephenson, 1976.

Insects and insecticides in the Rhine and IJssel

Sample locations of macroinvertebrate studies in the Netherlands are shown in Fig. 1. In Fig. 2, the numbers of chironomid larvae recorded in the samples from the IJssel are shown, with the median concentrations of cholinesterase inhibitors. There has been an increase of the numbers of midge larvae, but the question whether this increase may be attributed to reduced concentrations of cholinesterase inhibitors or not, remains open. In the period from 1975 through 1983, other improvements of water quality have been observed, i.e. the oxygen content increased, whereas the concentrations of heavy metals decreased since 1975 (Dijkzeul, 1982).

A closer look at the 1977 and 1978 data on the Rhine and IJssel shows that the relation between cholinesterase inhibitors and numbers of insect larvae is not as simple as might

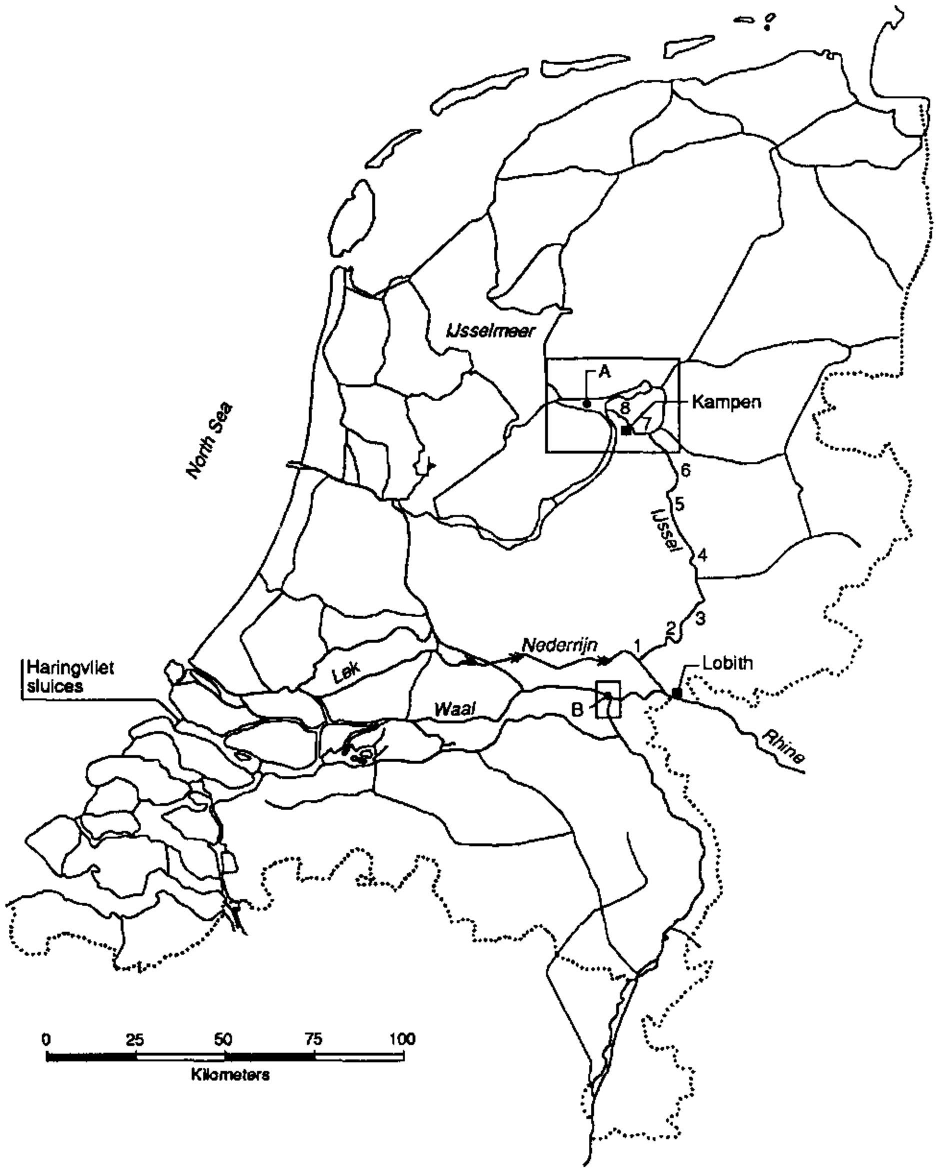

Fig. 1: Map of the Netherlands, showing the branches of the Lower Rhine. 1-8 = Sampling sites in the IJssel, A = Sampling area of mud and sand bottoms in the Lower IJssel, B = Sampling area of sand bottoms in the Waal.

be derived from Fig. 2. In 1977, peak concentrations of cholinesterase inhibitors well above 10 µg/L were recorded; in 1978, peak levels were recorded in spring, but maxima remained below 10 µg/L. In 1977, the numbers of midge larvae in samples from location Kampen (km 1000; Fig. 1) varied between 5 and ca. 5000. In 1978, the minimum number of midge larvae was 95 and the maximum was 1365. Although these numbers should be interpreted with extreme care due to the sampling frequency and the sampling method employed, these data show that high concentrations of cholinesterase inhibitors can coincide with high numbers of midge larvae.

During the summer of 1977 and 1978, species of the genus *Cricotopus* were dominant; these are small multivoltine species from the subfamily Orthocladiinae. Other abundant species, *Dicrotendipes nervosus*, *Parachironomus* sp., *Glyptotendipes pallens* and *Rheotanytarsus photophilus*, all belong to the subfamily Chironominae. Relative proportions of species differ from year to year and from season to season; the proportions established with different sampling methods are not quite the same (Van Urk & Bij de Vaate, 1990). In 1977, only a few ceratopogonid larvae and one damselfly larva (*Ischnura elegans*) were found in the IJssel, in addition to the large numbers of midge larvae. In subsequent years, other insect species were found. The caddisfly larva *Hydropsyche contubernalis* was first recorded in 1978 (Van Urk, 1981). Since then, the species was recorded every year, although the numbers showed strong variations. Only one other caddisfly species has colonized the IJssel successfully and established a permanent population: *Ecnomus tenellus* (Fig. 3). Other caddisfly species i.e. *Cyrnus trimaculatus*, *Cyrnus flavidus*, and *Ceraclea* sp were recorded only occasionally (Van Urk et al, 1990).

The numbers of *H. contubernalis* larvae and *E.tenellus* larvae show negative correlations both in time and space (Van Urk et al, 1990). When conditions are favorable for one species, they apparently are less favorable to the others. In the Middle Rhine, *H.contubernalis* was already abundant in the years 1975 till 1977, as were some species of chironomids (Caspers, 1980; Schiller, 1990). There is obviously a clear lag between the recolonization of the Middle Rhine and the Lower Rhine by this species.

In conclusion we may state that on the basis of laboratory toxicity data and the chemical data one would predict toxic effects in aquatic insect larvae in the Rhine, although the

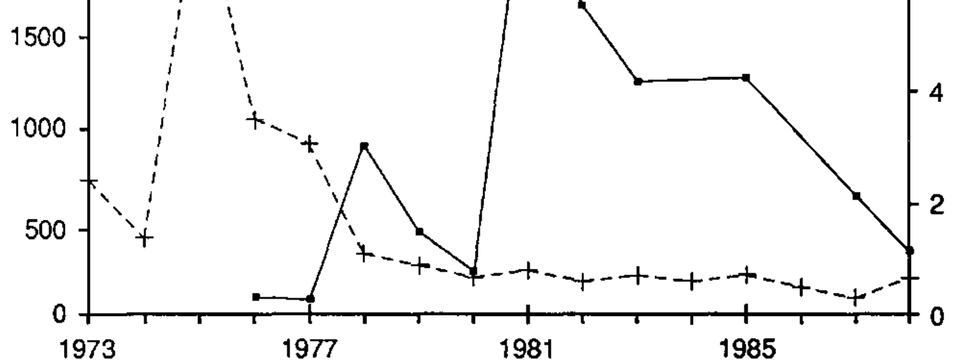

Fig. 2. Chironomids in samples from the IJssel and median concentration of cholinesterase inhibitors expressed as paraoxon equivalents.

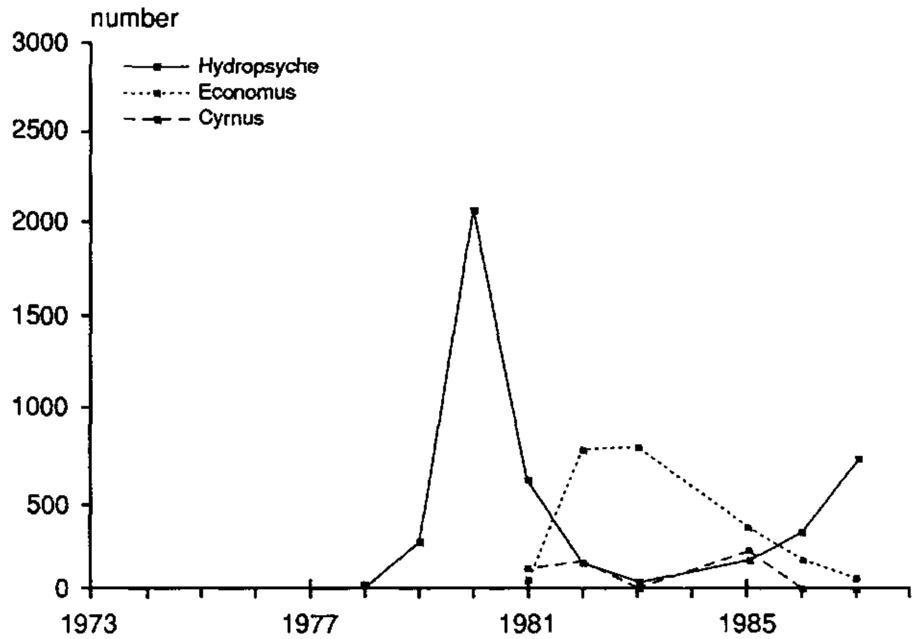

Fig. 3. (Re)colonization of some caddisfly species in the River IJssel.

field situation is too complex to draw final conclusions about this relation. An opportunity for additional studies on this relation came with the Sandoz accident that could be considered in this context as a large scale, but unfortunately ill prepared field experiment.

The Sandoz Accident

Among the chemicals accidently discharged during the Sandoz accident in 1986, were the fluorescent dye Rhodamine B, two organophosphorus pesticides, disulfoton and thiometon, and a fungicide, ethoxyethylmercuryhydroxide (Capel et al., 1988). Initial concentrations of disulfoton and thiometon exceeded 100 µg/L and 20 µg/L respectively, in the Upper Rhine near the point of discharge. This resulted in mass fish kills in this stretch of the Rhine (Lelek and Köhler, 1990). The downstream movement of the poisoned water was easy to follow by measuring the fluorescence of the water, caused by Rhodamine B. In Kampen, high concentrations of Rhodamine B were first measured on 10 November 1986. The highest concentration of this dye was measured on 11 November 1986.

A general picture of the concentrations of disulfoton occurring in the Rhine is presented in Fig. 4. At the German-Dutch border about 700 km downstream of the point of discharge, concentrations of disulfoton and thiometon had decreased to about 5.3 µg/L and 2.0 µg/L respectively, as a consequence of dispersion, transfer and elimination processes. These concentrations are not acutely toxic to fish and fish mortality was not observed in the Netherlands. However, from laboratory toxicity tests it is known that some

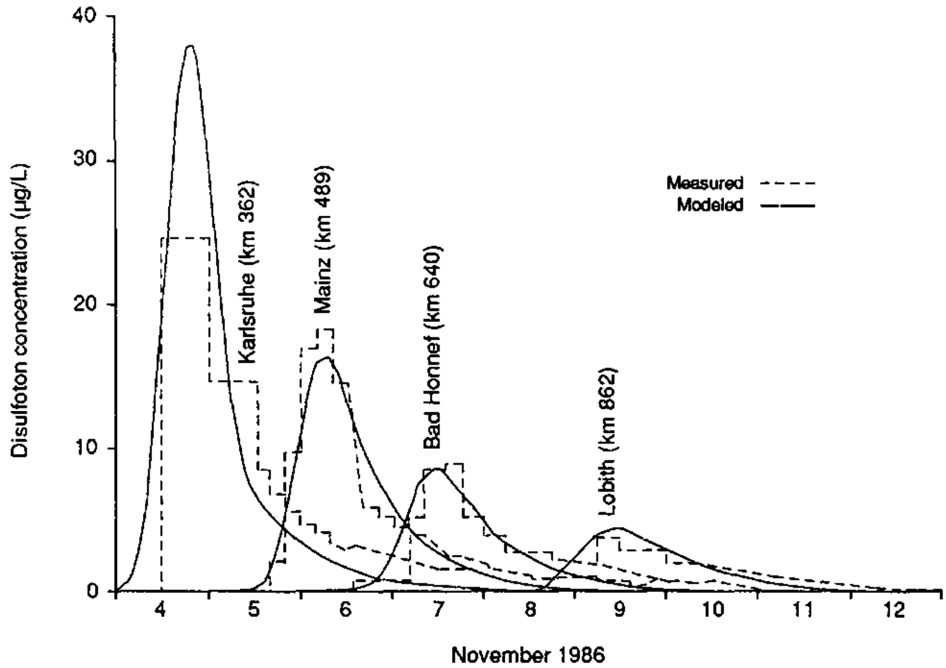

Fig. 4. Measured and predicted Disulfoton concentrations at several monitoring stations along the Rhine (µg/l) during the Sandoz accident in 1986.

aquatic insects and some crustaceans, are far more sensitive to organophosphorus insecticides than fish are (Capel et al., 1988). Toxicity tests with Rhine water sampled at Lobith showed that the above mentioned concentrations were still lethal for *Daphnia magna* within 48 h (Schäfer et al., 1986). As the data in Table 1 indicate that at least some insect larvae are equally sensitive to this type of pesticides, some damage to insect populations in the Lower Rhine seemed likely to occur; it should be realized however that there were no toxicity data on the effects of these particular substances, disulfoton and thiometon, on aquatic insect larvae.

Caddisflies and the Sandoz Accident

In September 1986, routine sampling had been carried out at 8 locations in the IJssel (Fig. 1). At each site, five stones by the waterside were carefully brushed down, either on the spot or in the laboratory. In this way a total surface of approximately 0.2 m^2 was studied per location. The material was sieved on a sieve with meshes of 0.5 mm. The animals were preserved, mostly in 70% alcohol. Due to peak discharges shortly before the contaminated water reached the IJssel (Van Urk & Kerkum, 1987; Fig. 5), these samples have to be considered the control samples for the Sandoz study. In Table 2 the numbers of caddisfly larvae recorded in September and shortly after the accident are presented. From Table 2 it can be concluded that the numbers of *E. tenellus* larvae in November 1986 were far less than in September, whereas the numbers of *H. contuber-*

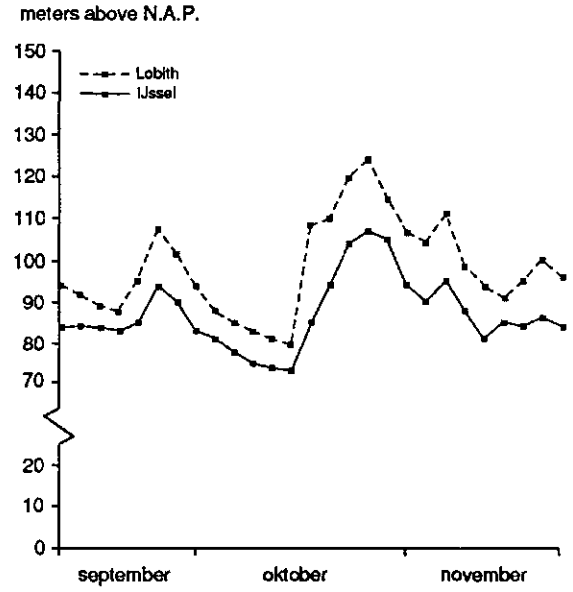

Fig. 5. Peak discharges of the IJssel in autumn 1986 expressed as meters above New Amsterdam Level (N.A.P.).

nalis larvae were not significantly different. When sampling was repeated in December 1986 at some of the locations, hardly any *Hydropsyche* larvae were recorded. Although the differences of numbers of *E. tenellus* were statistically significant (Mann-Whitney tests; $P < 0.05$), this does not allow us to establish the cause of these differences. In fact, information on the life cycle of *E. tenellus* is scarce, and therefore our results are difficult to interpret.

Table 2. Numbers of larvae of E. tenellus and H. contubernalis on 8 locations in the IJssel in September 1986 and November 1986.

Location	*E. tenellus*		*H. contubernalis*	
	Sept.	Nov.	Sept.	Nov.
1	-	-	32	128
2	-	-	48	15
3	-	-	23	27
4	15	-	12	27
5	33	-	13	10
6	16	4	170	13
7	83	6	12	7
8	12	-	2	-
Total	159	10	312	227

The shortcoming of sampling macroinvertebrates occurring on stones in the littoral zone due to the unpredictability of peak discharges is evident. The alternatives studied - sampling of insect adults and exuviae - had in this particular case the disadvantage that these methods do not yield any direct information on the densities of the larvae. The variables recorded are the numbers of emerging adults or flight activity of the adults. There can only be a relation between larval mortality after the Sandoz accident and adult numbers in the next summer if the mortality of the larvae in the winter is not density dependent.

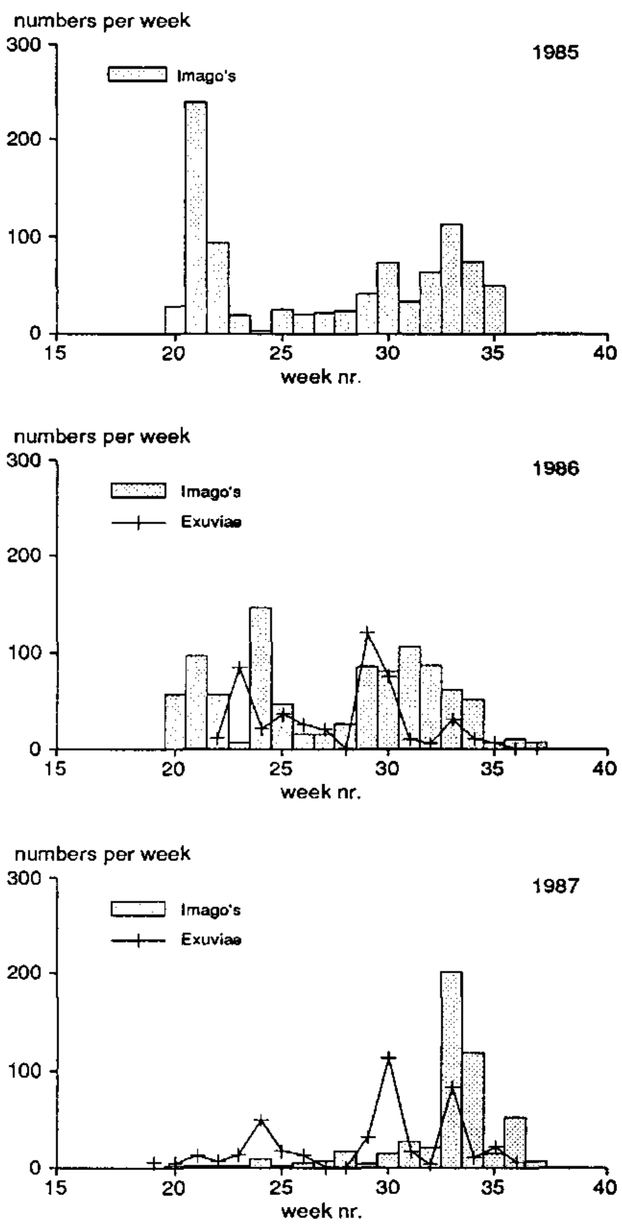

Fig. 6. Adult H. contubernalis caught in light traps and number of exuviae collected in weekly samples near location 8 at Kampen during the years 1985-1987.

The numbers of adult *H. contubernalis* collected in a Rothamsted type light trap on the location near Kampen during the years 1985-1987 are presented in Fig. 6, together with the numbers of exuviae collected by means of a conical drift net (maximum diameter 0.5 m; mesh size 1.0 mm) in weekly samples during the summer of 1986 and 1987.
H. contubernalis has two generations a year (Becker, 1987), but in our studies some overlap between generations was observed as at least some adults were collected in the light trap between the maxima in spring and in summer. From Fig. 6 it is clear that in 1987 the spring generation of *H. contubernalis* was virtually absent in the light trap collections. The number of exuviae collected was also less than in the previous year, although the difference is not as large as in the case of the adults. The weather conditions in the spring of 1987 were unfavorable which may have influenced the flight activity of *Hydropsyche* adults, but cannot explain the total absence of this species.
In Fig. 7 corresponding data on *E. tenellus* are shown. There are few literature data on the life cycle of *E. tenellus*. In the light trap at Kampen, peaks of flight activity were only recorded in August. In the exuviae collections in 1986, there was also only one peak just preceding the peak of flight activity. Both peaks were absent in the 1987 data. As weather conditions were favorable in August, there is no other explanation for the low numbers of adults than a severe reduction of larval populations. In 1988, the number of adult *E. tenellus* in the light trap collections were much higher than in previous years.
All data collected support the hypothesis that both caddisfly species occurring in large numbers in the IJssel were indeed affected by the Sandoz accident; there are no indications leading to rejection of this hypothesis. For both caddisfly species, the first adult generation after the Sandoz accident seemed to be reduced in numbers, whereas the second generation showed complete recovery.

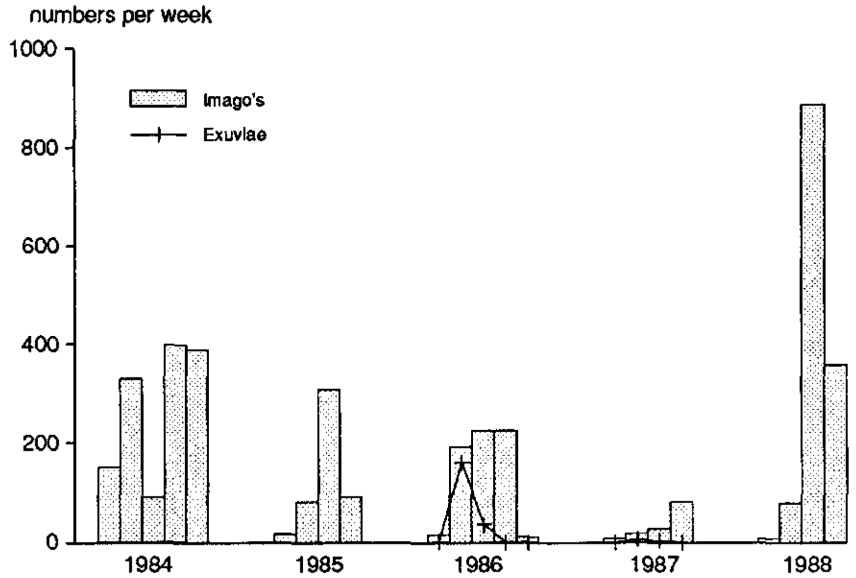

Fig. 7. Adult E. tenellus caught in light traps in August and number of exuviae collected in weekly samples near station 8 at Kampen during the years 1984-1988.

Chironomidae and the Sandoz Accident

Sampling of chironomid larvae from stones, was largely restricted to the most downstream location in the IJssel because at this place minimum interference was expected of water level fluctuations. The total surface studied per location was approximately 0.2 m^2. Table 3 shows that on the 12th of November, the species and numbers of chironomid larvae recorded in a sample from the groynes at this location were somewhat different from those in a corresponding sample collected in September.

Table 3. Chironomids on groynes in the IJssel near Kampen in 1986.

	16 September	12 November	27 November		
			dead	alive	total
total number present	779	474	300	49	349
Size of subsample[a]	134	166	110	49	159
Dicrotendipes	82	75	64	11	75
Glyptotendipes	7	34	28	7	35
Xenochironomus	-	6	1	-	1
Cricotopus	25	51	12	30	42
Nanocladius	1	-	-	-	-
Orthocladius	18	-	-	-	-
Others	8	-	-	-	-
Unidentified	-	-	190	-	-

[a]Only species in the subsample were identified.

It should be noted that larvae from the genera *Parachironomus* and *Rheotanytarsus*, although abundant in the light trap collections and the exuviae samples from the same site (Van Urk & Bij de Vaate, 1990) were not recorded in these samples taken from the groynes in the littoral zone. The presence of chironomid larvae in the sample taken on 12 November confirmed the findings by Klink (1986) that these larvae did not react to the Sandoz pesticides by entering the drift. All larvae in this sample had their normal appearance.

On 27 November, the location near Kampen was sampled again. Living larvae were separated from dead larvae and larval remains. It appeared that dead larvae comprised a large proportion of the total sample which was never observed in previous years. A total number of 349 live and dead larvae and larval remains were counted. Out of these, only 49 live larvae and 110 dead but still identifiable larvae, were counted, whereas 190 individuals could not be identified (Table 3). The proportions of identifiable larvae were about the same on the 12th and 27th of November 1986, but the percentages of dead and live larvae differed according to species.

A large proportion of dead and moribund larvae were found in bottom samples collected with an Ekman grab near the location Kampen. In Table 4 the combined results of all samples taken after the Sandoz accident are shown because the total numbers in bottom samples were low.

Table 4. Dead and live larvae in bottom samples taken in the IJssel near Kampen (km 1000) after the Sandoz Accident in November 1986.

	Dead	Live	Total
Chironomus	1	4	5
Cryptochironomus	1	9	10
Dicrotendipes	6	-	6
Glytotendipes	6	4	10
Endochironomus	20	3	23
Polypedilum	4	27	31

Among true bottom-dwelling species (*Chironomus* sp, *Cryptochironomus* sp, *Polypedilum* sp), few dead individuals were recorded. Many *Endochironomus albipennis* larvae were dead. *E. albipennis* is a species living on macrophytes and macrophyte remains. It is normally found in bottom samples during summer, but the species hibernates in a cocoon on the bottom (Moller Pillot, 1979). A population study on *Polypedilum breviantennatum* living in sandy substrates in the Waal indicated that there was a sharp decrease of the total number of *P. breviantennatum* between October 1986 and December 1986. In 24 samples taken with an Eykelkamp mudsampler (sample size 25 cm^2) the total number of *P. breviantennatum* decreased from 62 to 21.

It seems plausible that endobenthic species living below the mud surface were exposed in a different way than epibenthic species living on the mud surface or on solid substrates like stones. In the Ketelmeer downstream of the location near Kampen, no abnormal mortality was recorded among *Chironomus* larvae after the Sandoz accident. During this period, a population study of *Chironomus* was carried out in this area to assess the impact of sediment contamination on the bottom fauna. The results did not suggest that at this site *Chironomus* sp was affected during the period of the Sandoz accident.

Unlike the caddisfly species, most chironomid species are multivoltine, having several generations a year. Under favorable conditions, some species (notably of the Orthocladiinae) can complete a generation within 14 days. Under normal field conditions in the IJssel, the development of a new generation takes about 4-6 weeks for most species, as can be derived from the exuviae collections and the light trap samples from Kampen (Van Urk & Bij de Vaate, 1990). Some large species, e.g. *Chironomus plumosus*, are an exception to this.

From the light trap samples and the exuviae collections taken in 1987, the year after the Sandoz accident, the following observations can be made: (1) *Cricotopus* species

(mainly *C. bicinctus*) were more abundant in 1987 than in 1986, (2) A large spring generation of *Rheotanytarsus* was present, however, mainly male individuals were collected. This peak of males was not followed by a peak of flight activity of females. The summer generations of *Rheotanytarsus* only occurred in small numbers, (3) The spring generation of *Dicrotendipes* was smaller in 1987 than in previous years. In the summer, few individuals were recorded, (4) Very few individuals of *Glyptotendipes*, *Endochironomus* and *Polypedilum nubeculosum* were recorded during the whole year 1987 in the light trap.
A serious problem in the interpretation of results from the light trap collections is that only males were identified. In populations dynamics, numbers of females are often more important. Only in exceptional cases (e.g. *Rheotanytarsus*) some conclusions could be drawn about occurrence of the females, because the peak of *Rheotanytarsus* flight activity is well separated from those of all other species. In other cases, peaks of flight activity more or less coincide, so females cannot be separated. The proportion of females caught in the light trap is 80 - 90 %. For all species the total catch of males in 1987 was lower than in 1986, whereas the total catch of females was about equal in 1987 and in 1986.
All data lead to the conclusion that there is no relation between survival or mortality of larvae of different chironomid species as was observed directly after the Sandoz accident in 1986 and their abundance in exuviae and light trap collections in 1987.

Discussion

For the few macroinvertebrate species for which NELs for cholinesterase inhibitors exist (mainly Cladocera; Dortland, 1980), there is only a factor 10-20 between the acute LC50 and the NOEC determined in chronic toxicity studies. For ecological reasons, it seems therefore justified to focus on peak concentrations.
If, tentatively, concentrations of cholinesterase inhibitors of 1 - 10 µg/L are considered critical, organisms in the Rhine have been exposed in three ways. The first possibility is that concentrations are permanently above this critical level. This was the case during the year 1975 and the first half of 1976. The second possibility is that occasionally peak concentrations of cholinesterase inhibitors are recorded, whereas the majority of analyses indicates concentrations well below critical levels.
This was the case in the years 1977 and 1978. The Sandoz accident itself is an example of such a situation. The third situation is that concentrations are permanently below the critical level. This was the case from 1979 till November 1986, when the Sandoz accident took place.
In situations where peak concentrations of cholinesterase inhibitors are recorded occasionally, habitat suitability for a particular species is variable and unpredictable. Ecological theory predicts that in such a situation opportunistic species (or r- selection) will dominate. K-selection favours species with high competitive ability, delayed reproduction, larger body size, repeated reproduction and long life cycles, whereas r-selection favours species with rapid development, high intrinsic rate of increase, small body size and short life cycles (Krebs, 1978). All species reach some sort of compromise between

the extremes of r- and K-selection. From the insect species in the Rhine, the chironomids tend more to the extreme of r-selection than the caddisfly species; at least the larger species of Ephemeroptera and Odonata would tend most to K-selection out of all insect species. Because there are no recent observations of these groups in the Rhine, it can be concluded that in the Rhine r-selection operates.

The (re)colonization of the Rhine after 1977 by aquatic insect species showed the following pattern. First some species of chironomids were observed, secondly, *Hydropsyche contubernalis* and then, *Ecnomus tenellus*. This roughly corresponds to the numbers of generations each year, most of the chironomids being multivoltine, whereas *H.contubernalis* is bivoltine and *E. tenellus* seems to have only one generation each year.

In this context it should be noted that most species dominant in the chironomid fauna of the IJssel, may also be abundant in stagnant waters, which may increase their recolonization potential. This applies to *Cricotopus*, *Chironomus*, *Endochironomus*, *Glyptotendipes*, *Dicrotendipes* and most *Polypedilum* species. Not abundant in stagnant waters or even restricted to the river itself are *Parachironomus* species, *Rheotanytarsus* species and a species not mentioned so far, *Rheopelopia ornata*.

After the Sandoz accident, the pattern of recolonization showed the same sequence. Again, various chironomid species were among the first to recolonize, whereas recovery of *E. tenellus* populations lasted longer. For all species however, not more than one generation was needed for recovery. In this respect, there is no basic difference between the various species occurring in the Rhine. This recolonization pattern can be explained from the unpredictable nature of the stress factors involved. It should be noted that any environmental factor showing the same unpredictable pattern, will cause the same pattern in community composition, unless there is a very large difference of sensitivity between r- and K-strategists. But it may be assumed that stress factors other than insecticides operate in the Rhine as well. So species requiring stable conditions, e.g. because of their long life cycle, will not be able to maintain self-sustaining populations in these conditions. Because different environmental stress factors would cause the same community changes, the nature of the stress factor cannot be derived from community composition. Community composition can only be indicative of the nature of the stress if this stress factor exerts a more or less constant selection pressure.

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Life cycle patterns, density and deformity incidence in Chironomus larvae (Diptera: Chironomidae) in relation to a toxic pollution gradient

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Abstract

Chironomus cf muratensis larvae were studied in a gradient in the degree of sediment contamination in a sedimentation area of the Lower Rhine in the Netherlands. Deformity incidence was positively and density was negatively related to the grade of pollution. At the least polluted sites only the deformity incidence was raised compared to reference values. At an intermediately polluted site the development in the pre-pupation and pupation phase was delayed. At the most polluted sites larval density was strongly reduced.

At less polluted sites *C. cf muratensis* had a bivoltine life cycle and hibernated in the larval instar IV. At strongly polluted sites one generation at most was recognized from density data. In the spring of 1987 the percentage of larvae with swollen thorax was significantly lower at a moderately contaminated site compared to the least contaminated sites. The first appearance of pupae at this site was also delayed by two weeks. At strongly polluted sites no pupae were observed at all. The percentage of deformed larvae was higher from November to April than from July to October. Generally, the incidence increased between November and April during the larval stage IV. A maximum was observed during emergence of the overwintering generation. The period between February and April is probably most suitable for the assessment of mentum deformity incidence in *Chironomus* larvae. When combined with deformities incidence density might be a suitable additional indicator of toxic stress.

Introduction

Several studies have demonstrated the occurrence of deformities in the mouth parts and antennae of Chironomid larvae (Hamilton & Saether, 1971; Hare & Carter, 1976;

Wiederholm, 1984; Van Urk & Kerkum, 1987; Pettigrove, 1989; Warwick, 1988). The incidence of deformities is generally higher at localities where high concentrations of pollutants were demonstrated to be present in the environment or might be assumed to be present because of the vicinity of waste water discharges (Wiederholm, 1984; Van Urk & Kerkum, 1988; Warwick, 1990).

Warwick (1988) reviewed current literature and concluded that there seemed to be good possibilities for using chironomid deformity incidence as an indicator of toxic stress in aquatic systems. A major problem is the scarcity of experimental data on the induction of deformities by particular contaminants; there is some experimental evidence that heavy metals cause deformities of the epipharyngeal pecten in Chironomus species (Kosalwat & Knight, 1987; Van de Guchte & Van Urk, 1989), but so far no single pollutant has been found to which induction of mentum deformities can be attributed. Consequently the nature of information contained by deformity incidence contains, is still unknown. Do elevated levels merely indicate the presence of toxic stress, or does the variable give a more or less reliable indication about the pollution level?

An other problem related to the usage of deformity incidence as a biological indicator, is the lack of data on variations in deformity incidence in a population. Large temporal variations at a locality may render the interpretation of data more difficult (Warwick, 1988).

We wished to quantify the relation between pollution degree and deformity incidence under field conditions as much as possible. If elevated deformity incidence is induced by toxic components, it is to be expected that population dynamical parameters -like growth rate, rate of emergence and density- also respond to differences in contamination levels. We therefore explored the relation between pollution degree and some population dynamical parameters as well.

In most studies, either the deformity incidence was low (<10 %) or the densities of Chironomid larvae were low at the sites most affected (e.g. Hare & Carter, 1976), so that it would require a considerable additional effort to establish seasonal variations of deformity incidence. However, in our study area in the Netherlands, the joint conditions of relatively high densities and high deformity incidence are not unusual, and this provided an opportunity to study seasonal variations of deformity incidence and population dynamics in *Chironomus cf muratensis* larvae.

Materials and methods

Study area

The study area is situated near the mouth of the River IJssel, a branch of the River Rhine in the Netherlands. The IJssel flows into the IJsselmeer, a freshwater reservoir created in 1932 by the closure of a dike separating the Zuiderzee, an inland sea, from the North Sea. The study area, now called the Vossemeer, is a long narrow lake between the former coast line and the dike of the Flevoland polder (Fig. 1). At the west end, the lake has a depth of about 2-3 m. In this area, large amounts of the suspended matter car-

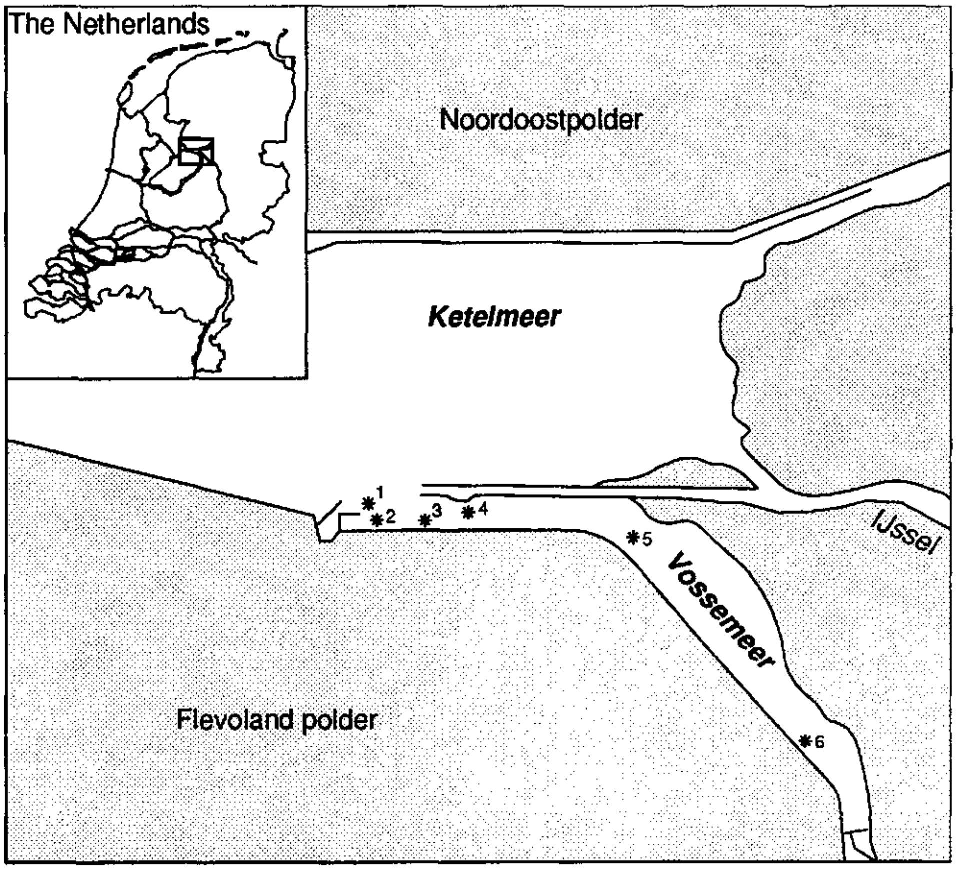

Fig. 1. Position of the study area in the Netherlands. Sampling sites are indicated by the numbers 1 to 6.

ried by the IJssel are deposited, resulting in a mud layer: the thickness of this mud layer decreases with increasing distance from the mouth of the IJssel. In this area, four sampling stations were chosen (Fig. 1). The remainder of the lake is shallow, except for the shipping channel. The bottom mainly consists of sand; only at the south end of the lake some mud is present in the shipping channel, resulting in a bottom composition roughly similar to that at site 4. Two additional sampling sites (5 and 6) were chosen in this area. A reference site (R) was chosen in the unpolluted neighbouring Veluwemeer (coordinates: 52°22' NB; 5°38' OL).

Grain size distributions at the study sites showed some differences (Table 1). At the sites 4-6 the mean grain size was considerably greater than at the sites 1-3. The content of Kjeldahl-nitrogen at site 4 was intermediate between the low values at the sites 5 and 6 and the high values at the sites 1-3.

The sediments at sites 1-4 clearly show the pollutant input from the Rhine (Table 2). After correction for differences of organic carbon contents, the most persistent pollutants such as PCB-180 and some heavy metals show almost the same concentrations at these sites. Less persistent or relative volatile substances such as PCB-52 show a concentration gradient, with highest concentrations near the IJssel. The relative low concentrations of mercury and hexachlorobenzene (HCB) at site 1 are the result of a de-

crease of the load carried by the IJssel in recent years. For other substances such as cadmium, the decrease of the load observed in recent years has not yet resulted in a decrease of sediment concentrations.
At sites 5 and 6, heavy metals contents of the sediment are still higher than the background concentrations at site R. Concentrations of organic contaminants at these sites were below the detection limits.

Table 1. Sediment characteristics of the upper 10 cm at the sampling sites in the Vossemeer.

Parameter	1	2	3	4	5	6
Dry matter %	42	35	33	45	48	49
Organic carbon %	3.4	6.1	6.0	2.8	2.2	2.4
Kjeldahl-N $mg.g^{-1}$	14.3	24.8	21.4	9.7	2.1	1.8
Total-P $mg.g^{-1}$	0.6	0.8	0.9	0.5	0.6	0.5
Sand > 60 μm	35	13	12	53	48	62
Silt 2-60 μm	59	81	81	45	50	36
Lutum < 2 μm	6	6	7	2	2	2

Table 2. Contents of selected contaminants at 6 sites in the Vossemeer and a reference site (R) in the unpolluted Veluwemeer.

Parameter	1	2	3	4	5	6	R
Cd $mg.kg^{-1}$	3.4	6.2	6.7	3.1	2.7	1.6	<0.3
Hg „	1.1	2.7	3.9	1.3	0.7	0.5	0.2
Pb „	85	134	135	71	44	38	8
Cu „	64	109	111	55	29	20	7
Fluoranthene „	1.8	2.8	2.1	0.4	<0.1	<0.1	<0.1
Benz(a)pyrene „	1.2	1.6	1.1	0.4	<0.1	<0.1	<0.1
Indenopyrene „	3.1	1.5	0.9	0.5	<0.1	<0.1	<0.1
HCB $\mu g.kg^{-1}$	47	195	35	11	<1	<1	<1
PCB-52	38	18	16	9	<1	<1	<1
PCB-153	27	41	31	14	<1	<1	<1
PCB-180	14	22	17	9	<1	<1	<1

Sampling and analysis

Ten replicate samples were taken fortnightly from February through November 1987 and monthly from March through May 1988 with an Eckman-grab with a surface area of 0.15 x 0.15 m^2. At least the upper 0.15 m of the sediment was sampled. Preliminary observations had demonstrated that most *Chironomus* larvae occurred at a depth of 0.05 - 0.10 m; below 0.15 m very few larvae were found. The material was sieved on a sieve with meshes of 0.5 mm. It has been assumed that at sieving, the efficiency for instar III and IV *Chironomus* larvae as well as for pupae is nearly complete. The larvae and pupae were removed up from the sieve by hand in the field, and preserved in 80 % ethanol.
In the laboratory, the larvae were identified and checked for mentum deformities. For this purpose, the larvae were cleared by heating in 40 % potassium hydroxide and mounted in temporary slides in glycerol for microscopical examination. All Chironomus larvae were identified to the species level according to Webb and Scholl (1988). In April 1987 about 100 larvae were brought to the laboratory and fed with tetramine. The emerged male adults were identified according to Pinder (1978) and Strenzke (1959) to check for the larval identification. Although the larvae had been identified as *C. muratensis*, the adult males were identified as *C. plumosus*. Therefore the species dealt with in this study has been named *C. cf muratensis*. The mentum deformities were scored, using the criteria from Warwick (1988), which means that all deviations from an ideal pattern of the mentum teeth were considered a deformity. Deformities of other parts of the head capsule such as the antennae and the mandibles were not considered. Head capsule width of the larvae was measured with an eyepiece micrometer, and larvae were assigned to the instar III or IV on basis of the measurements.
For the calculation of 95 % confidence intervals densities were log-transformed and deformity incidence were arcsin-square root transformed.

Results

Population dynamics

Temporal patterns of larval density.

In the winter and spring of 1987, only instar IV larvae were found at all sites (Fig. 2). From April on, the larval densities gradually decreased. The rate of density decrease was not significantly different at different sites or different periods, due to the great variance of the numbers in replicate samples at sites 5 and 6. Minimum densities were observed in June, with values of about 200 individuals m^{-2} at sites 4 - 6 (Fig. 2). During the summer, instar III larvae were found on all sampling dates, but the percentage of instar III larvae in the total of instar III and IV remained low until September. In that month densities were significantly lower than in August and October at sites 4, 5 and 6. No density decrease was observed at site 3. In October 1987, a decrease of the frequency of instar III larvae was again observed, and from November 1987 till the next spring, only instar IV larvae were found.

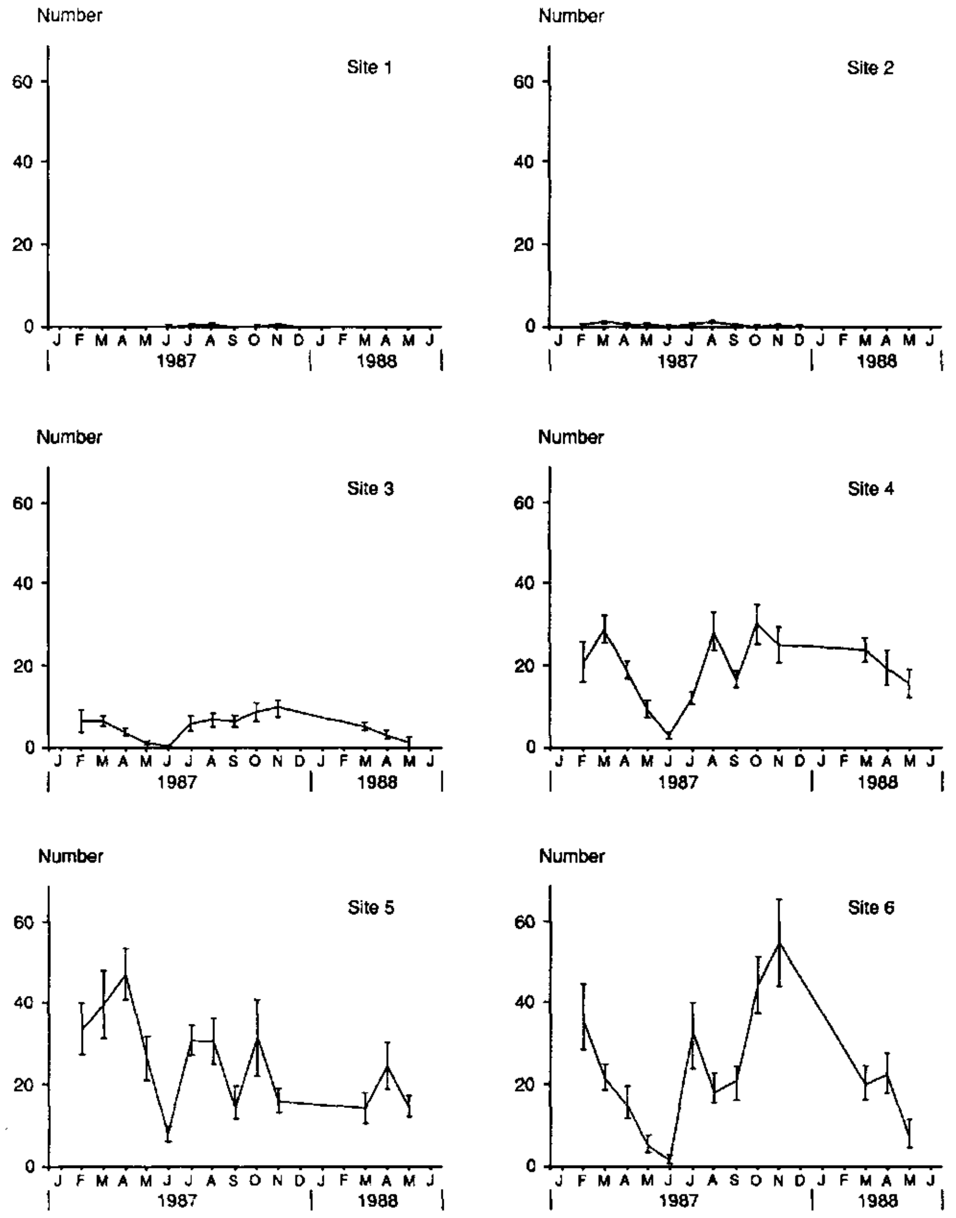

Fig. 2. Average (+ 95% CL) number per sample of instar IV C. cf muratensis larvae. Data are averages over each month.

Spatial patterns of larval density.

Densities were the lowest at site 2 and showed a significant increase (Wilcoxon $p<0.01$) at each of the sites 2, 3 and 4. Densities at the sites 4, 5 and 6 were not significantly different ($p>0.15$). At site 1, hardly any larvae were found, whereas at site 2, the frequency of occurrence of at least one larva in a single grab sample was less than 50 %. At site 3, the frequency of occurrence of at least one larva in a sample reached nearly 100 %; the frequency distribution in the samples was in agreement with a random (Poisson) distribution at this site.

Incidence of swollen thorax.

Swelling of the thorax segments is an easily observed external character of larval development. It coincides with the phase 9 the instar IV, as illustrated by Wülker & Götz (1968). The incidence of larvae with swollen thorax segments at site 4 was always lower than those at sites 5 and 6 on the corresponding dates in the spring of 1987 (Fig. 3). This indicates a delayed development at site 4 compared to the sites 5 and 6. At the sites 1-3 very few larvae with swollen thorax were observed. At these sites also the total number of larvae in May was too low to calculate the incidence of larvae with swollen thorax segment with any degree of precision.

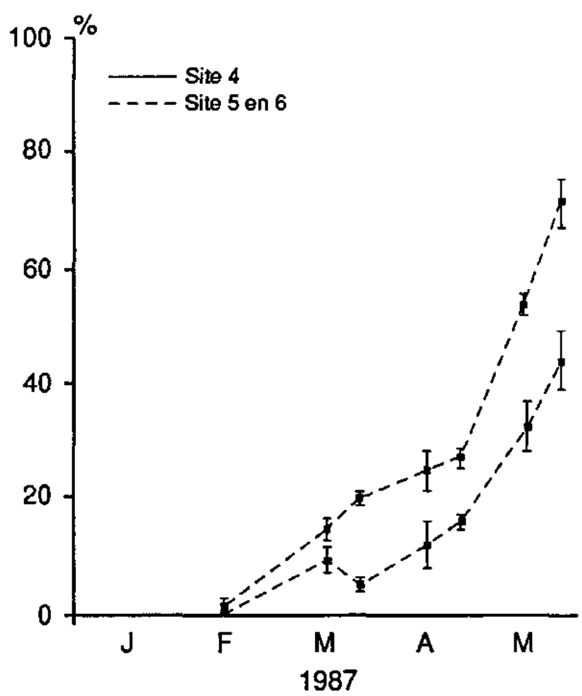

Fig. 3. Frequencies of instar IV C. cf muratensis larvae with swollen thorax segments in the spring of 1987.

Densities of Pupae

At site 4, the occurrence of pupae was somewhat delayed compared to the sites 5 and 6: the maximum number was found by the end of May instead of the beginning of May, and during June 1987, pupae were still found (Fig. 4). At the sites 1, 2 and 3 no pupae were found at all. In general the densities of pupae were low; observed values did not exceed 50 pupae.m^{-2}. Pupae occurred on all sampling dates from May until September 1987. At site 3, the densities of larvae were so low that no pupae were to be expected in the samples when the same ratio between the densities of larvae and pupae as that observed at sites 5 and 6 is taken as a starting point. In the spring of 1988 similar differences were observed as in 1987: pupae were absent at site 4, few were found at site 5 and most at site 6.

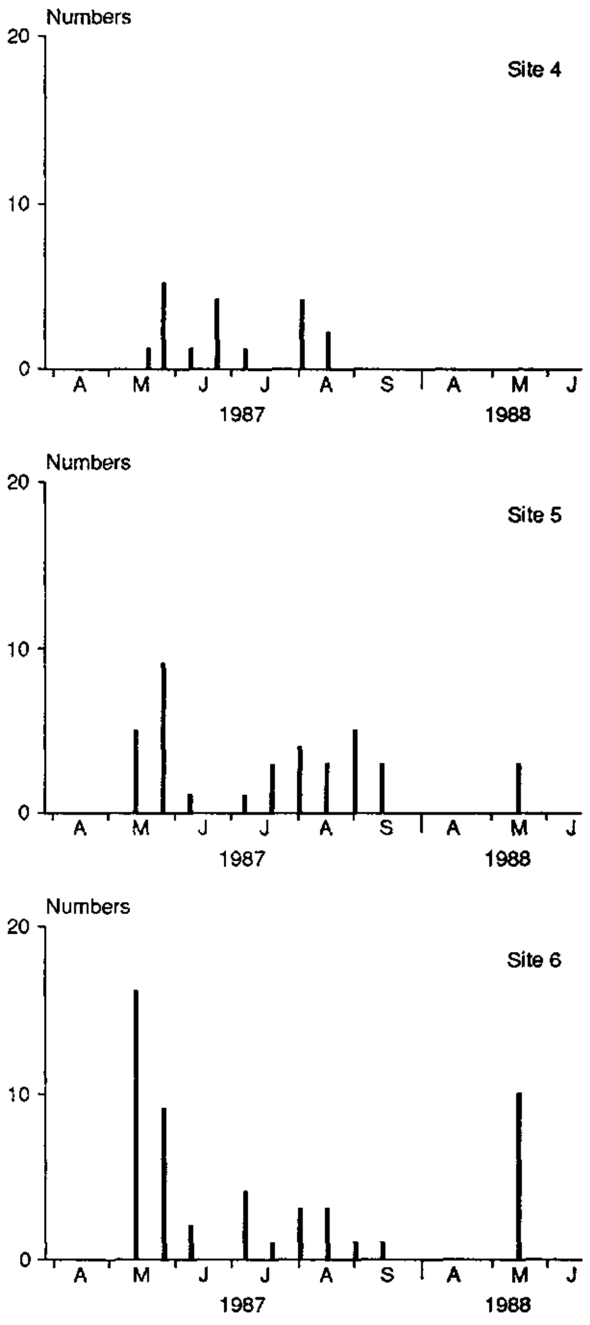

Fig. 4. Total number of Chironomus cf muratesis pupae in 10 grab samples at three sites differing in pollution degree.

Deformity incidence

Temporal patterns

At the sites 4, 5 and 6 deformity incidence was lower from July to November than from February to May 1987 and from March to May 1988 (Fig. 5). At these sites values increased between February and April 1987 and between November 1987 and March 1988. A somewhat different seasonal pattern was observed at site 3: in the spring of 1987 no increase was observed in the period of emergence. In the summer of 1987, the frequency of deformed larvae was lower than that in the preceding winter, but increased from July to November. In April and May 1988, the frequency of deformed larvae was

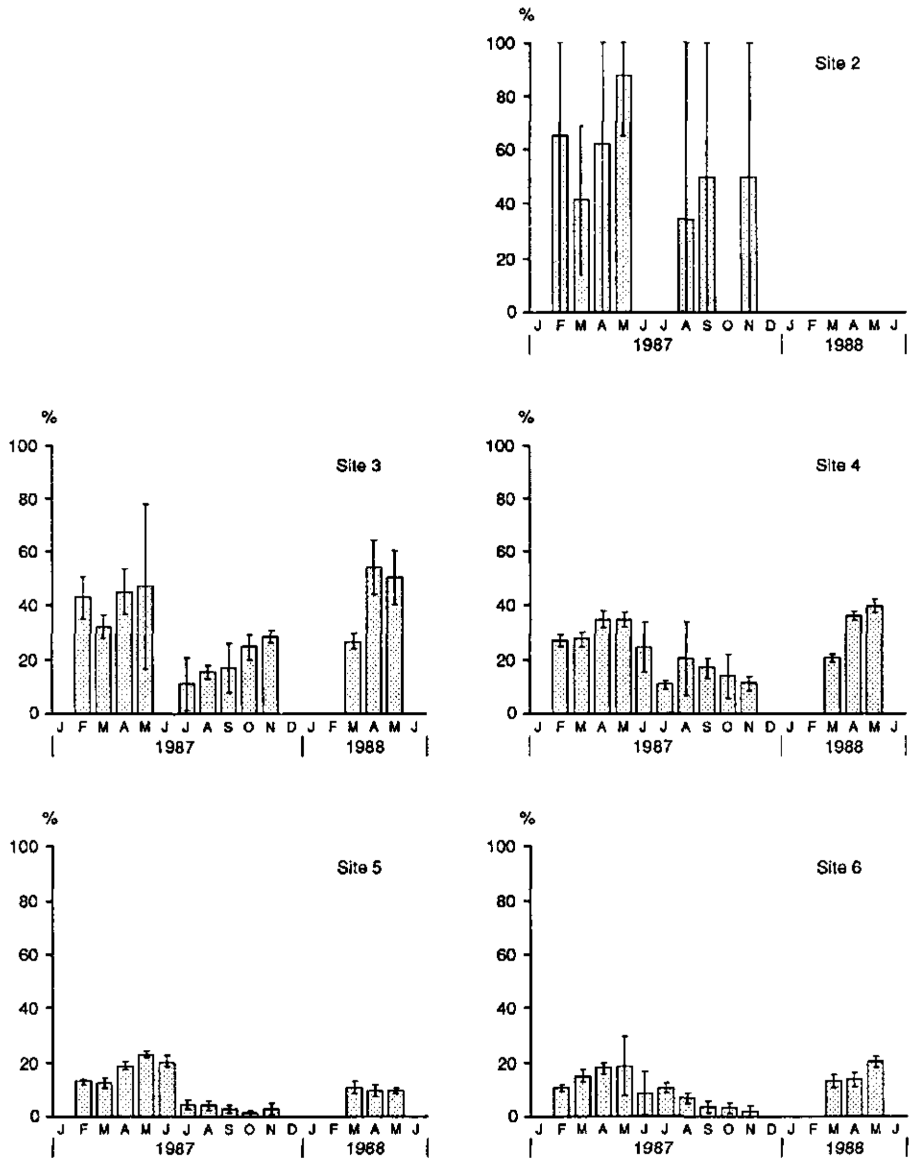

Fig. 5. Temporal patterns of deformity incidence (% + 95% CL) at sites 2-6.

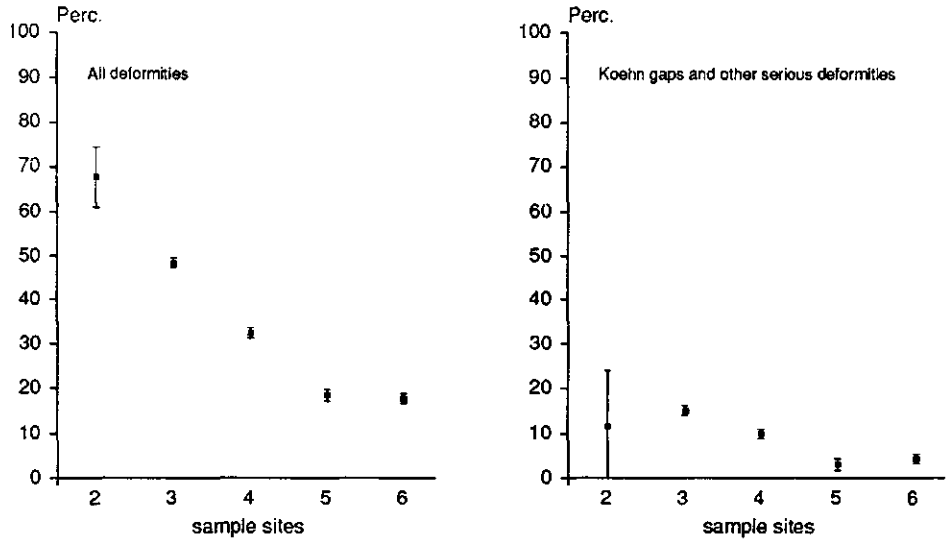

Fig. 6. Incidence of all deformities and more serious types of deformities like Köhn-gaps (Köhn and Frank, 1980) at the sites 3-6. Average requency (% + 95% CL) over the period from February through April 1987.

again higher. At site 2 too few individuals were found to notice any significant temporal difference. Deformity incidence in March, April and May 1988 was not significantly different from the incidence in the corresponding months in 1987. In a few cases however, values were lower in 1988 than in 1987.

Spatial patterns

Deformity incidence was lowest at the sites 5 and 6 (no significant difference) and showed a significant increase at each of the sites 4, 3 and 2 (Wilcoxon $p<0.01$ between each pair of the sites 5, 4 and 3; $p<0.05$ between the sites 3 and 2). The deformity incidence at sites 5 and 6 is still higher than the reference value of 1% at site R and the values considered to represent background values by most authors (e.g. Warwick, 1988; Wiederholm, 1984).

At the sites with the highest deformity incidence, also the more serious types of deformities - by which "Köhn-gaps" (Warwick, 1988; Köhn & Frank, 1980) and the total absence of a part of the mentum are understood - were observed more frequently than at the sites with low deformity incidence (Fig. 6).

Discussion

Most authors (e.g. Matena, 1989; Beattie, 1978; Hilsenhoff, 1966) describe *Chironomus plumosus* as being bivoltine. Sokolova (1983), who compared data from a wider geographical area concluded that *C. plumosus* might be univoltine in northern latitudes, whereas three generations were observed in the southern part of the USSR. In Lake

Suwa, Japan, *C. plumosus* produced three generations a year in the littoral whereas it lacked a summer generation in the profundal (Yamagishi & Fukuhara, 1971). The *C. cf muratensis* population at sites 4-6 in the Vossemeer showed essentially the same pattern of density changes as the populations studied by Matena (1989) and Beattie (1978): at least a part of the population is bivoltine. However, pupae were found during a long period in summer, as well as instar III larvae. This indicates a poor synchronization, so it cannot be excluded that a part of the population has a number of generations differing from two. At site 3 at most one generation per year may be supposed, since no density decrease of instar IV larvae was observed in September and no pupae were observed.

Larval development at site 4 is delayed compared to that at sites 5 and 6: a lower percentage of larvae with swollen thorax was found in spring followed by a later appearance and disappearance of pupae. This delay may be caused by the presence of pollutants in the sediments or the water at this site; It may however not be excluded that these difference are caused by other differences in environmental conditions. At site 4 the higher deformity incidence and the retarded growth did apparently not yet result in a lowered density.

The incidence of deformities differs within a generation. The increase as observed between November 1987 and March 1988 at the sites 4, 5 and 6 also becomes apparent when data from October 1986 (Van Urk & Kerkum, 1987) are compared with those

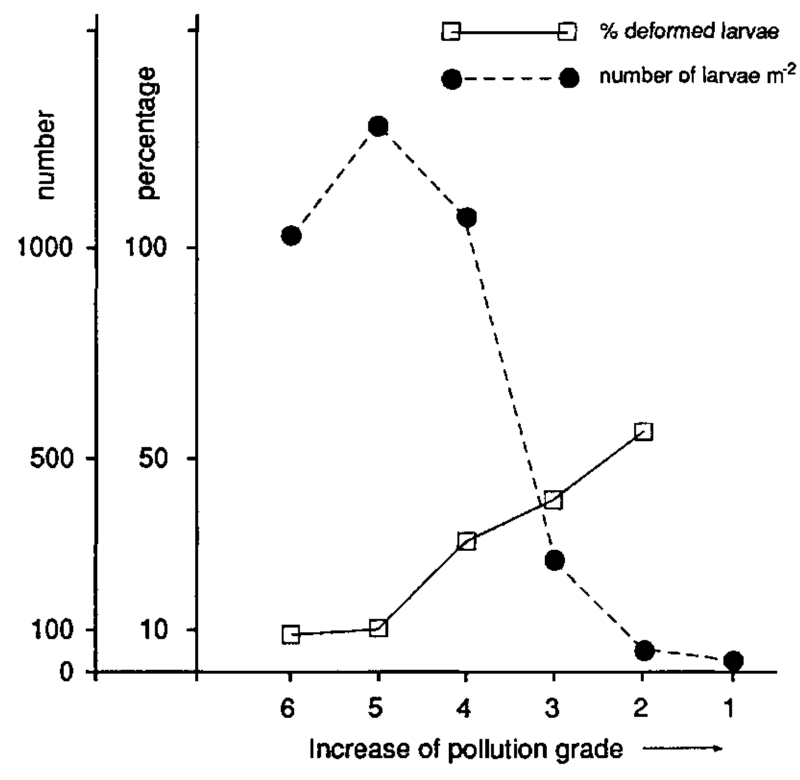

Fig. 7. Relation between the pollution degree and densities and deformity incidence in the Vossemeer, near the mouth of a R. Rhine branch. Data are averages over the February through April 1987.

from the spring of 1987. Since no recruitment or emergence occurs in the winter period it must be assumed that deformities do not only develop at the transition of the instar III to IV, but can also develop during instar IV. We assume that this is the result of an improper regeneration at the transition from instar III to IV, after which the mentum would become more susceptible to wearing and fracturing. The marked rise observed in the period of emergence of the winter generation might be the result of the same process, but might also be due to delayed development of the deformed larvae. However, Janssens de Bisthoven (1989) could not find a difference of emergence rate between deformed and non-deformed larvae in rearing experiments. But conditions in rearing experiments may be different from those encountered in the field, so that less selective pressure is exerted.

Both the density and the incidence of mentum deformities showed a clear gradient, corresponding to the pollution gradient, illustrated in Fig. 7. At the lowest pollution grades (sites 5 and 6) only the deformity incidence is elevated compared to reference levels. With increasing pollution grades, the pre-pupation and pupation is also retarded (site 4), and with a further increase of sediment pollution (sites 1-3) densities are reduced as well. This sequence of effects along the pollution gradient is in good accordance with the toxicological experience principle that sublethal effects very often precede lethal ones and increases the probability that the observed relation between deformity incidence and pollution grade has a causal basis. The positive relation between mentum deformity incidence and pollution degree, suggested by Warwick (1988), has now been confirmed in a field situation.

With systematic sampling, differences between locations can be recognized more easily. In the Vossemeer, the gradient of deformity incidence between locations was present at most sampling occasions, but was most pronounced from February to April. This is probably the most suitable period for the assessment of mentum deformity incidence in *Chironomus* larvae.

When combined with deformities incidence density might be a suitable additional indicator of toxic stress.

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Macroinvertebrates and quality assessment of Rhine sediments

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Abstract

In the flood plains of the river Rhine the contamination of deposited sediments is a major concern with respect to aquatic ecosystem health. To enable rehabilitation remediation programmes are being developed, in which the assessment of sediment quality is a first step. In the sediment quality triad approach combined chemical analyses, sediment toxicity tests and *in situ* bottom fauna studies combined give an integrated diagnose of sediment contamination.
Chemical analyses on 49 persistent sediment pollutants (heavy metals, PCB's, PAH's and organochlorine pesticides), bioassays using *Chironomus riparius, Spirosperma ferox, Tubifex tubifex* and *Daphnia magna,* and *in situ* macrofauna surveillances were used to assess sediments from 5 sedimentation areas of the River Rhine. According to the Dutch sediment quality criteria two areas, Lake Ketelmeer and Lake Haringvliet, are heavily contaminated. In the bioassays only chronic endpoints were sufficiently sensitive to discriminate between sites in the Dutch areas of concern. False positives were recorded from bioassays using *Tubifex tubifex,* which had not been adapted to the hypertrophic characteristics of the Dutch sediments. Field surveillances on benthic community structure revealed discriminatory responses among sites of the dominant macroinvertebrate taxa, oligochaetes being more sensitive to state of eutrophication than chironomid larvae. The incidence of deformities of the mentum in larvae of *Chironomus muratensis* seemed to be proportional to the amount of Rhine water in their habitat. The sediment quality triad, which has macroinvertebrates as major component in both bioassays and field studies, adequately indicated major areas of concern with respect to the direct impact of polluted sediments. In the sedimentation areas of the river Rhine priorities are directly related to the time period during which the sediments were deposited, sediments from the seventies being the most polluted, and to the degree of mixing with cleaner sediments due to wind and shipping. Before remedial programmes are initiated additional research is needed to clearly identify compound specific cause-effect relations.

Introduction

In aquatic systems, finely grained sediments form a sink for toxic contaminants because of the adsorption capacity of clay minerals and organic matter. The sediments may turn into an internal source of contaminants after reduction of inputs, and thus offer a potential long-term threat to aquatic biota.

For the assessment of sediment quality, Chapman (1986) proposed the "sediment quality triad approach" consisting of chemical analyses, sediment toxicity tests and in situ bottom fauna studies.

This approach is used for sediment quality assessment in the sedimentation areas of the River Rhine in the Netherlands. The River Rhine flows through one of the most densely populated and industrialized areas of the world, and several hundreds of potentially toxic pollutants have been identified in Rhine water samples so far. These include chlorinated hydrocarbon insecticides, polychlorinated biphenyls, organic solvents like chloroform, organophosphorous insecticides, herbicides, chlorinated phenols and anilines, nitrophenols and nitrobenzenes, mineral oil, polycyclic aromatic hydrocarbons etc. In addition, all heavy metals show more or less elevated concentrations in Rhine water.

A large amount of these contaminants is retained in the sediments of two freshwater reservoirs in the Netherlands, the Ketelmeer/IJsselmeer and the Hollandsch Diep/Haringvliet; these reservoirs are former estuaries of the Rhine closed off from the North Sea by the construction of dams and sluices in 1932 and 1970, respectively. The reservoirs are a major source of freshwater in the Netherlands, and great concern exists about the long term effects of sediment pollution.

A number of chemical and biological surveys has been carried out to assess the present situation. The mere fact that only a part of the contaminants present, has been chemically identified so far, gave cause for the use of biological methods. In the biological assessment of sediment quality, it was desirable to distinguish between the effects of toxic contaminants and those caused by eutrophication. In addition to toxic contaminants, the Rhine has a very high nutrient load greatly contributing to eutrophication of stagnant waters in the Netherlands.

In this paper, some problems associated with quality assessment of sediment from hypertrophic waters will be discussed, and results will be compared with the sediment quality approach. Emphasis is given to the use of macroinvertebrates in the assessment of contaminated sediments.

Site description

Fig. 1 shows the different branches of the Rhine in the Netherlands. The River Waal is the main branch, receiving about two third of the total discharge of the Rhine. The River IJssel, running north to the IJsselmeer area, is the smallest branch receiving about 15% of the total Rhine discharge at high flow conditions and about 25 % at low flow conditions, due to manipulation of the weirs in the Nederrijn/Lek. Water and sediment quality is similar in all branches; only in the Rotterdam harbour basins, sediment quality is markedly influenced by local discharges. The Hollandsch Diep/Haringvliet also re-

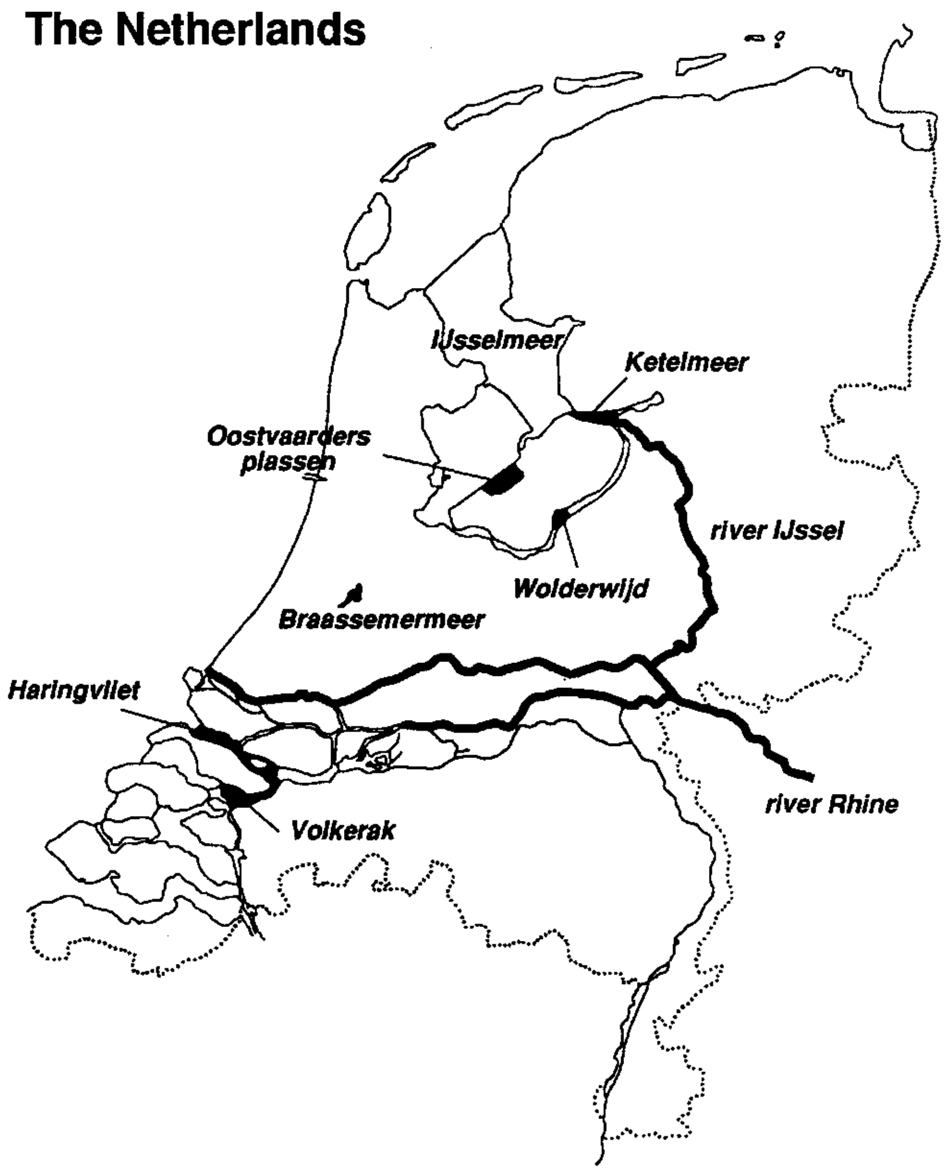

Figure 1: Study sites in the sedimentation areas of the Lower Rhine.

ceives the discharge of the River Meuse, but this has only a minor influence on sediment quality in the area.

Most of the sediment carried by the IJssel settles near its entry in the IJsselmeer area, called the Ketelmeer; due to this, the small discharge of the IJssel and the large surface area of the IJsselmeer itself, the sediments in the major part of the IJsselmeer are only slowly being loaded with contaminants. Those parts of the area isolated by the construction of dams and dykes for new polders, are only slightly contaminated and can be considered reference areas. This applies to the Oostvaardersplassen and to the Wolderwijd and the Veluwemeer bordering the polder Flevoland.

On the contrary, the Hollandsch Diep/Haringvliet area, which was closed off from the sea in 1970, was rapidly loaded with contaminants. The sedimentation process in this area was described in a study by Rijkswaterstaat (cited by Van Urk & Smit, 1989): sedimentation proceeds in a delta-like manner so that the sediments in the eastern part of the reservoir are more strongly contaminated than those in the western part.
The Volkerak, a recently constructed reservoir, is still only slightly contaminated and can be used as a reference area.
Water from the Rhine is also used for flushing canals and lakes in the western part of the Netherlands. This practice has resulted in a loading of these lakes (among which the Braassemermeer; Fig 1) with contaminants comparable to present levels in the IJsselmeer. Some indicative data on chemical sediment quality in the lakes mentioned are summarized in Table 1.

Table 1: Indicative values of the contaminant load of fine grained sediments in the sedimentation areas of the river Rhine. Concentrations given (mean, min., max.) are corrected for a defined Dutch standard sediment (particle size: 25 % < 2 um, organic carbon content: 5 % dm).

Sites	Cd mg/kg dm	HCB ug/kg dm	j-HCH ug/kg dm	PCB-153 ug/kg dm	B(a)P ug/kg dm	years of reference
Oostvaardersplassen	0.9 (0.8-1.0)	<1	<1	3.2 (2.5-3.9)	480 (<130-900)	'85-'90
Volkerak	1.6	3	<1	6.7	350	'87
IJsselmeer	6	6	1.7	6.3	300	'82-'85
Haringvliet	31	75	2.5	200	2500	'85-'88
Ketelmeer	18	50	3.5	30	1100	'87-'90

Source: LAWABO datafiles Rijkswaterstaat, RIZA, Lelystad, The Netherlands.

The lakes are all shallow, averaging in depth from 0.5 m in parts of the Oostvaardersplassen, and 2.7 m in the Ketelmeer up tp 4.5 m in the IJsselmeer. This implies that highly dynamic sediment conditions prevail in these waters. Due to wind conditions and navigation, the sediment is frequently resuspended which will have a strong impact on the composition of benthic communities.
All lakes, the Volkerak excepted, are eutrophic or hypertrophic, and show temporary or permanent blooms of Cyanobacteria. The Volkerak is a macrophyte dominated lake, with low phytoplankton densities.

Methods

Sediment sampling and chemical analyses

Composite sediment samples were made by mixing 4-5 aliquots from the upper 10-15 cm layer of the sediment. An Ekman or Van Veen grab was used. Within a maximal storage period of 6 weeks at 4 °C, subsamples were analysed for heavy metals, organochlorine pesticides, PCB's and PAH's, using dutch standard procedures (Anonymus, 1985-89). All the analyses were carried out at the RIZA in Lelystad. Bulk sediment concentrations were corrected for particle size distribution and organic carbon content, according to dutch standards (Van der Kooij *et al.*, 1991).

Bioassays - species selection and laboratory cultures

Sediment dwelling organisms stay in close contact with the sediment and the interstitial water and are therefore recommended as test organisms to assess toxicity associated with contaminated sediments (Giesy & Hoke, 1989; Milbrink, 1987; Nebeker *et al.* 1984; Van de Guchte & Reynoldson, 1991; Wiederholm *et al.*, 1987). Because Chironomid larvae and Oligochaete worms are the dominant taxa in the benthic communities of Rhine sediments, the test species used in this study (*Chironomus riparius, Spirosperma ferox* and *Tubifex tubifex*) were selected from these groups. *Daphnia magna* completed the macroinvertebrate test battery, as aquatic test procedures with this species are standardized and well documented (see: Giesy & Hoke, 1989). Although the same argument holds for the use of *Photobacterium phosphoreum*, and although also this species is widely recommended as a test organism in a standard set of sediment pore water bioassays (Giesy *et al*, 1988; Giesy & Hoke, 1989, Thomas *et al.*, 1986), we decided not to include this species in our test battery: in previous studies on fresh water sediments, heavily contaminated according to dutch sediment quality criteria, hardly any effects or no effects at all could be observed (Van de Guchte & Maas-Diepeveen, 1988).

Daphnia magna and *Chironomus riparius* are reared in synchronised cultures at the RIZA, Lelystad (The Netherlands) on a routine base. The daphnids are kept at 20 + 1°C in filtered (.45 μm) and sterilized (UV-light) water from the IJsselmeer. In times of large algal blooms Dutch Standard Water is used (hardness 250 mg/l as $CaCO_3$). Food (*Chlorella*) is being administered *ad libidum*.
Adult midges of *C. riparius* are held in flight cages, where they deposit their egg masses in a bath of demi-water diluted medium after Alabaster & Abram (1965) (hardness 100 mg/l as $CaCO_3$, pH = 7.7 + 0.2). Up to the second larval instar they are kept in water and fed daily with a finely grained Trouvit fish food suspension. The red coloured second instars are then transfered to 10-liter aquaria containing a 5 cm sediment layer from the Oostvaardersplassen, which is sterilized beforehand by γ-radiation (10 kGy). Trouvit is administered daily to the overlying water (see above), which is aerated continuously. At 20 °C it takes about 28 days from oviposition before the full-grown fourth instar larvae of *C. riparius* pupate. Adult midges are transferred into the flight cages again. The incidence of mentum deformities in cultured larvae is normally below 8 %.
For *S. ferox*, a new culture was established at the Agricultural University in Wageningen (The Netherlands). The organisms were kept at 20 ± 2 °C in a basin of 1 m^2 containing a mixture of oligochaete species in sediment from the Oostvaardersplassen. They were fed finely grained Trouvit every day. The overlying water (Dutch Standard Water, hardness 208 mg/l as $CaCO_3$) was aerated continuously. Two days before testing sufficient numbers of mature *S. ferox* were sorted out by hand.

Tubifex tubifex was cultured at the NEPB laboratories in Uppsala (Sweden). The bioassays with this species were performed. There as well the single species culture of *Tubifex tubifex* was maintained in glass aquaria on a substrate of sediment from a nearby uncontaminated natural lake. The organisms were fed Trouvit two times a week.
Overlying water originated from the same lake as the sediment and was aerated continuously.

Bioassays - test procedures

Before testing the sediments were seived through a 0.5 mm net to remove large objects and shells. Whole sediment bioassays were run as static renewal experiments in a 1:4 v/v sediment water ratio. This mixture was shaken vigorously for two days. After one day of settlement the bioassays were started. Pore water was sampled weekly by centrifugation (20 min., 7000 g) and subsequent filtration (glass fiber and cellulose acethate (pore size .45 m, Sartorius) under 20 psi vacuum. The whole sediment tests were renewed every week, the pore water assays twice a week. All tests were controlled for T (20 °C), O_2, pH, conductivity, salinity, hardness, ammonia and nitrate/nitrite using routine laboratory procedures.
In previous short term acute tests conducted in our laboratory to assess equally polluted dutch sediments, no effects or hardly any effects on survival of the test organims could be detected. Therefore only chronic endpoints were included in this test series (see Table 2).

Table 2: In the bioassays four different test species were used.

Test species	duration of test		endpoints
	whole sediment	pore water	
Daphnia magna	21 d	21 d	survival, growth, reproduction
Chironomus riparius	21 d	10 d	survival, larval development
Spirosperma ferox	23 d	-	survival, reproduction
Tubifex tubifex	64 d	-	survival, weight

D. magna neonates, less than 24 h. old, were used to start the bioassays. The tests were carried out in 10-fold: 10 young animals were placed individually in glass vessels containing either 50 ml of several pore water dilutions (0, 44, 67 or 90 % IJsselmeer medium added) or the sediment sample with IJsselmeer medium in a 1 to 4 ratio. Whenever oxygen levels dropped below 5 mg/l, the water was aerated for 10 min. Every day the daphnids were fed Chlorella (3 x 10^8 cells/l). Survival and offspring were counted daily.

Reproduction rate was calculated using the method of Van Leeuwen *et al.* (1985). After 21 days growth (length of the carapax of the adults) was also determined. Sediment from the Oostvaardersplassen served as a reference.
To start the chironomid assays second larval instars of *C. riparius* were collected from the laboratory culture. 25 individuals were brought into one glass vessel containing 50 ml of the settled sediment-water mixture. Tests were run in triplicate. Whenever oxygen levels dropped below 3 mg/l the overlying water was aerated for 10 min. The larvae were fed a fine by grained Trouvit suspension three times a week. Survival was determined weekly. At the end of the test period (21) the larval development in time was recorded by measuring the head capsule width to determine the larval stage of development. In preliminary studies in our laboratory this parameter appeared to be a more reliable indicator of larval growth then length or weight of the larvae, which both reveal a high variation between individuals. The pore water tests (for dilutions see above) were also carried out in triplicate. These tests started with the second larval instars and lasted for 10 days only, as recommended by Giesy and Hoke (1989). Longer test periods result in cannibalism of the animals. Other specifications are the same as mentioned for the whole sediment tests.
The oligochaete bioassays using *Spirosperma ferox* were carried out in 10 x 10 cm glass vessels, containing a 2.5 cm layer of sediment with 2.5 cm Dutch Standard Water above. 10 mature individuals were placed in one vessel, which equals a density of 1000 ex./m^2. This density resembles numbers found in field surveys and should affect neither survival nor reproduction of the test organisms (Reynoldson *et al.*, 1991). To get an indication of dose dependent effects, mixtures of the sediments in study and the reference sediment from Oostvaardersplassen were made in a ratio of 1:1 v/v. All tests were run in triplicate. During the test the overlying water was gently aerated. The water volume was made up to the original amount daily with distilled water. The animals were not fed. Every week the oligochaetes and cocoons were removed from the test vessels by seiving over 125 m. Living worms were replaced in newly prepared sediments. The tests were conducted for 23 days. The bioassays with Tubifex tubifex were started by placing five small individuals into one of every eight replicate test vessels of 6 cm wide. This equals a density of approximately 2500 ex./m^2.
Before testing the seived sediments were homogenized to eliminate all natural macrofauna including oligochaete cocoons. The tests were conducted in darkness. The worms were not fed during the experiment and water lost due to evaporation was replaced with acclimated test water. Dissolved oxygen and pH were measured intermittently during the experiment. After 64 days the tests were terminated and the sediments were sieved through a 0.3 mm net. The adults from each test jar were counted and weighed together. The number of young and cocoons were counted. As a reference previous experiences with swedish sediments were taken into account.

Results of the whole sediment bioassays were tested for significance of site specific differences from reference values using 2- and Student's t-tests (Sokal & Rohlf, 1981). LRCT-values (Lowest Rejected Concentration Tested) of the pore water bioassay results were calculated using the 2-test (Sokal & Rohlf, 1981) for the data on survival, and Williams' test (Williams, 1971; 1972) for data on the other parameters measured.

In situ bottom fauna studies

Samples were taken with an Ekman grab with a surface area of 0.15 x 0.15 m ; it was checked that at least the upper 0.15 m of the sediment was sampled. When necessary, a pole-operated device was used. The contents of the grab were sieved on a sieve of 0.5 mm mesh size. The material on the sieve was preserved in 80 % ethanol and sorted in the laboratory. This procedure is appropriate for 4th instar larvae of the larger species of Chironomids, the smaller bivalve Molluscs including *Pisidium* spp. and adult (mature) Tubificids. When Naididae and immature Tubificidae had to be sampled, a corer of 0.05 m internal diameter was used, and the material was sieved on a sieve with finer meshes (250 um). For determination of dry weights of *Chironomus* larvae, some larvae were sorted from the material remaining on the sieve in the field, transported to the laboratory in clean water, and after gut clearance weighed without any preservation. Oligochaetes were identified mostly according to Brinkhurst & Jamieson (1971); for *Peloscolex* spp., the revision by Holmquist (1978) was followed. *Chironomus* larvae were identified according to Webb & Scholl (1985). At identification, the incidence of mentum deformities was recorded, using the criteria formulated by Warwick (1990).

Assessment

The sediment quality triad comprises three complementary components: chemical analyses, bioassays and field surveys. Data on each of the components were classified in three classes: sediment bulk chemistry was classified according to the Dutch quality criteria system (Ministry of Transport and Public Works, 1989; see Table 3). The classification of the biological parameters from bioassays and field surveys is presented in table 4. The criteria are related to control measurements in the bioassays or reference values having site specific relevance for the field surveys. Total scores for each of the

Table 3: Dutch quality criteria of some selected contaminants for a defined Dutch standard sediment (particle size: 25 % < 2 um, organic carbon content: 5 % dm).

	Cd mg/kg dm	HCB µg/kg dm	γ-HCH µg/kg dm	PCB-153 µg/kg dm	B(α)P µg/kg dm
highly contaminated ↑					
'warning value':	30	500	500	100	3000
moderately contaminated ↕					
'target value 2000':	2	4	1	4	50
lightly contaminated ↓					

three Triad components were derived in a way similar to the one used to classify sediments based on chemical analyses: the most sensitive parameter determines the final classification. The integrated interpretation of the three Triad components is shown in table 5. Combining this interpretation with the classification mentioned above, a priority ranking is derived, the aim of which is to guide decision makers in developing remediation programmes (Table 6).

Table 4: Classification in the Sediment Quality Triad: examples.

score:	-	+	++
chemical analyses			
Cd	< 2 mg/kg	2 - 30 mg/kg	> 30 mg/kg
PCB-153	< 4 ug/kg	4 - 100 ug/kg	> 100 ug/kg
bioassays			
survival	> 90 %	90 - 50 %	< 50 %
reproduction	> 90 %	90 - 50 %	< 50 %
field surveillances			
mentum deformities	< 10 %	10 - 20 %	> 20 %
max. density *Chironomus*	> 1500 / m^2	1500 - 500 / m^2	< 500 / m^2

Table 5: The Sediment Quality Triad: some diagnostic interpretations.

chem. anal.	*bioass.*	*field surv.*	diagnostic interpretation
+	+	+	sediment is toxic and impacting resident biota, causative agents are suspected
-	+	+	impact is sediment related but due to unknown physico-chemical parameters
+	-	+	causative agents are suspected; effect is low-level and long-term; bioassays are not sufficiently sensitive
-	-	+	impact is due to unknown low-level toxicants, or due to a stress other than contaminated sediment
+	-	-	contaminants are not bioavailable or have no direct impact on resident biota

+ : significantly different from reference values

Table 6: Assessment and priority ranking using the Sediment Quality Triad.

chemical analyses	bioassays	field surveillances	need for remediation	urgence for remediation	need for additional research
++	++/+/-	++	yes	high	no
++	++	++ / + / -	yes	high	no
++	+/-	+	yes	intermediate	no
++	+	+/-	yes	intermediate	no
++	-	-	yes	low	yes
+	++/+/-	++	yes*	high*/intermediate	no*/yes
+	++	++/+/-	yes*	high*/intermediate	no*/yes
+	+/-	+/-	no		no*/yes**
-	++/+/-	++	no		yes
-	++	++/+/-	no		yes
-	+/-	+/-	no		no/yes**

-/+/++: see table 4 for scores; *: only when cause-effect relation is evident;
**: depending on objectives.

Results

Chemical data and sediment dynamics

Out of hundreds of chemicals so far identified in Rhine water, 49 substances are quantified regularly in the assessment of chemical sediment quality. These include the heavy metals, polycyclic aromatic hydrocarbons, chlorinated hydrocarbon insecticides and polychlorinated biphenyls. Typical concentrations of some selected sediment contaminants are already presented in Table 1.

Dynamics in sedimentation and erosion results, in some of the water systems studied, in inhomogenous patterns of sediment contamination in space and time. For example, relatively high concentrations in surficial sediments are found in the eastern part of the Hollandsch Diep/Haringvliet; in this area, silt deposited in the 1970s still forms the top layer of the sediment. The material recently transported by the Rhine contains less contaminants, but this material is deposited mainly in the western part of the Hollandsch Diep/Haringvliet area. The latter was included in this study.

In the Ketelmeer, the deposition of silt is more evenly distributed, although here too sedimentation and erosion areas can both be discerned. In the connection between the Ketelmeer and the lakes bordering the new polders, a gradual improvement of sediment quality is observed due to decreasing sedimentation intensity. In this study a site in the sedimentation area near the inflow from the River IJssel was selected, were the sediment top layer contains a high amount of material deposited in the mid seventies.

The composition of finely-grained sediments in the IJsselmeer is rather homogeneous, due to the long residence time of water and sediments in the lake and an intensive mixing. At the sites in the other areas studied, including the reference sites, the top layer of the sediment is also relatively homogeneous because the waterflow is generally low and because wind and shipping give rise to turbation and mixing.
In Table 1 only some indicative data on relevant sub-sites, *id est* fine by grained sediment areas (particle size: more then 30 % < 16 um, organic carbon: > 2.5 %) suitable for macrofauna colonisation, and of relevant years are summarized. The sampling for biological surveys was carried out on typically identical sediments. Results of the chemical analyses on the fine by grained samples used in the bioassays were within the ranges mentioned in Table 1. From this table it can be discerned that the the Oostvaardersplassen and the Volkerak are the least polluted, while Haringvliet and Ketelmeer are the most polluted sites in this study. The last two datasets represent actual contaminant loads of the River Rhine, which in general can be characterised as "moderately polluted" according to dutch sediment quality criteria (Table 3). For some contaminants, however, the lable "heavily polluted" is applicable, especially when sediments date from the mid seventies.

Bioassays

As an example the data on survival of the different test organisms in undiluted samples are summarized in Table 7. Observed effects on the other parameters are dealt with in the presentation of the TRIAD scores in Table 8.
In general the observed effects were most pronounced in the pore water bioassays. In the *Daphnia* tests with IJsselmeer sediment and pore water the observed effects could be attributed to ammonia released from the sediment.
Percentage survival showed significant differences from control (Oostvaardersplassen) for the Ketelmeer pore water in both the *Daphnia* and *Chironomus* bioassays. Sublethal effects (not tabulated; see table 11) were found in the *C. riparius* assay with pore water

Table 7: Percentage survival in the bioassays.

	D. magna		C. riparius		S. ferox	T. tubifex
site	s	pw	s	pw	s	s
Oostvaardersplassen	100	100	100	90	82	37*
Volkerak	100	-	100	-	90	70
IJsselmeer	r	r	100	-	40	35*
Haringvliet	100	100	85	90	28	47*
Ketelmeer	100	50*	100	50*	37	0*

-: not determined; r: rejected due to high ammonia concentrations;
*: significantly different from reference.

Table 8: Classification of Rhine sediments for the grouped Triad parameters. (49 chemicals, 9 chronic bioassay endpoints, 3 field effect parameters)

site:	Oostvaardersplassen				
chem. analyses		bioassays		field surveillances	
metals	-	*D.magna*	-	*Chironomus* sp.	
PCB's	-	*C.riparius*	-	abundance	-
PAH's	+	*S.ferox*	-	deformities	-
OCP	-	*T.tubifex*	++	Oligochaeta	
				species comp.	-

site:	Volkerak				
chem. analyses		bioassays		field surveillances	
metals	-	*D.magna*	-	*Chironomus* sp.	
PCB's	+	*C.riparius*	-	abundance	-
PAH's	+	*S.ferox*	nd	deformities	++
OCP	-	*T.tubifex*	+	Oligochaeta	
				species comp.	-

site:	IJsselmeer				
chem. analyses		bioassays		field surveillances	
metals	+	*D.magna*	r	*Chironomus* sp.	
PCB's	+	*C.riparius*	-	abundance	r (B: +)
PAH's	+	*S.ferox*	-	deformities	r (B: +)
OCP	+	*T.tubifex*	++	Oligochaeta	
				species comp.	-

site:	Haringvliet				
chem. analyses		bioassays		field surveillances	
metals	++	*D.magna*	-	*Chironomus* sp.	
PCB's	++	*C.riparius*	+	abundance	+
PAH's	++	*S.ferox*	nd	deformities	+
OCP	-	*T.tubifex*	++	Oligochaeta	
				species comp.	+

site:	Ketelmeer				
chem. analyses		bioassays		field surveillances	
metals	+	*D.magna*	+	*Chironomus* sp.	
PCB's	+	*C.riparius*	+	abundance	++
PAH's	++	*S.ferox*	++	deformities	++
OCP	+	*T.tubifex*	++	Oligochaeta	
				species comp.	+

from the Haringvliet and in all testspecies exposed to Ketelmeer sediment or pore water, when compared with the reference Oostvaardersplassen. Survival of the oligochaete *Spirosperma* was not affected. However, the production of cocoons was significantly reduced (60 % reduction) after exposure to Ketelmeer sediment. The exposure to Haringvliet sediment resulted in a reduction of the number of eggs per cocoon. Other significant effects on reproduction of *Spirosperma ferox* were not found. The bioassays with *Tubifex tubifex* revealed significant differences from the Swedish reference values with respect to survival for all samples except Volkerak (Table 7). Even in the sediment from the Oostvaardersplassen survival was rather low compared to the reference from swedish experiences. Differences between treatments were significant only when Ketelmeer was compared with Volkerak. In the data on weight of the oligochaetes after 64 days only one significant difference between treatments was found: as well the worms which survived the exposure to the IJsselmeer sediment (mean wet weight: 3.73 mg/individual) were heavier then those exposed to Haringvliet sediment (mean wet weight: 0.82 mg/individual). Differences between Haringvliet and Oostvaardersplassen were significant only when two outliers were excluded. Data on individual weight after exposure to Ketelmeer sediment are not available because no tubificid worms survived.

Field data on macroinvertebrates

In the Ketelmeer, sedimentation rates are so high that, despite frequent resuspension of a part of the sediment, a permanent habitat is present for mud-dwelling macroinvertebrates. Near the mouth of the IJssel, no *Chironomus* larvae were found in the samples; at an increasing distance to the river's mouth, the densities of *Chironomus* spp. (mainly *C. muratensis*) increased. At the study site numbers were below 500 individuals / m^2. In the finely-grained sedimentation areas of the lakes bordering the new polders (Wolderwijd, Veluwemeer) the highest maximum densities are observed (1800 ind./ m^2; see: Van Urk, Kerkum & Smit, this issue). The incidence of deformities of the mentum in *Chironomus* spp. was highest near the IJssel (50 %) and decreased with increasing distance from the river towards the bordering lakes (10 %).
The densities of *Chironomus* spp. (*C. muratensis*) larvae in the Volkerak were much higher than in the Ketelmeer and comparable to those in the Oostvaardersplassen (2000 ind./ m^2); however, the incidence of mentum deformities in *Chironomus* was as high as at the most polluted sites in the Ketelmeer (40 - 50 %). In the IJsselmeer hardly any chironomids are found due to the higher mean depth of this water reservoir and its relative by high turbidity. In addition observations in the Braassemermeer are presented. This lake resembles the IJsselmeer exceptionally well with respect to sediment contamination, sediment grain size and state of eutrophication. The Braassemermeer has intermediate densities of *Chironomus* spp. (1200 ind./ m^2). Mentum deformities also show intermediate values (18 %), compared to the Ketelmeer and the reference areas.
Maximum chironomid densities and deformity incidences at the sites of study are classified in Table 8.
In the Ketelmeer, the IJsselmeer, the Oostvaardersplassen and other reference sites except the Volkerak, *Limnodrilus claparedeianus* and *L. hoffmeisteri* were the dominant

Tubificid species. Inability to separate the immature individuals of these species prevented detailed population studies. Relative proportions of the mature individuals are given in Figure 2. Another species frequently found was *Potamothrix moldaviensis*; occasionally, *Quistadrilus multisetosus* was found in rather high numbers in the Ketelmeer.

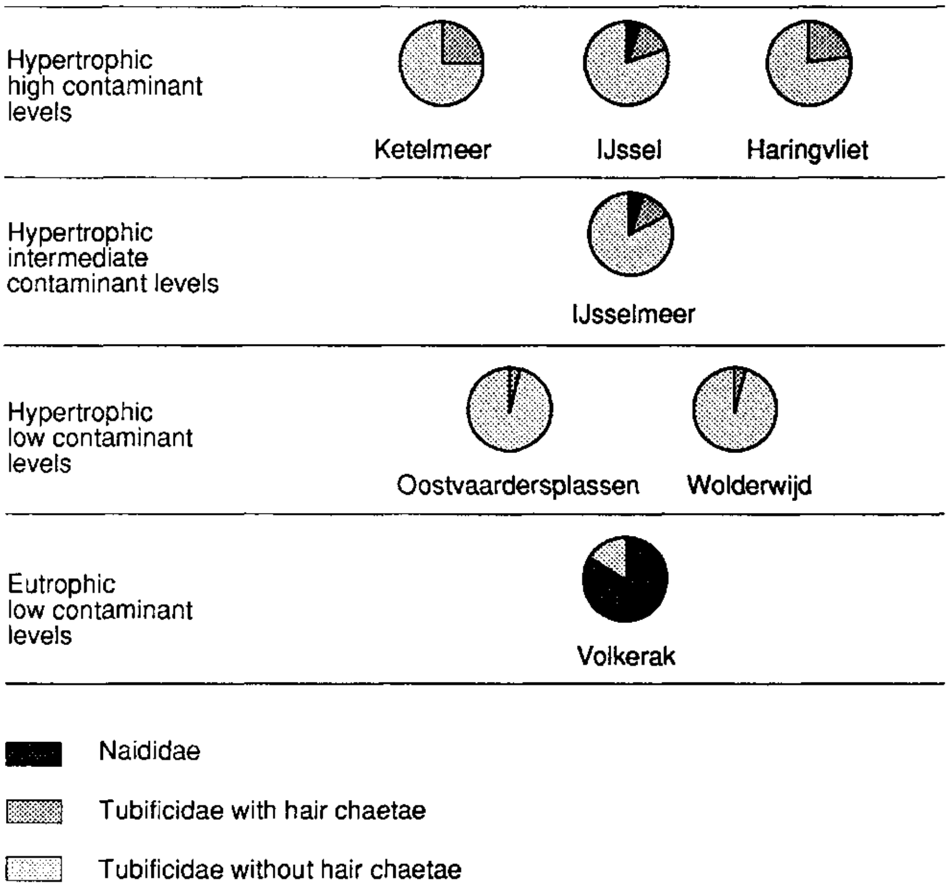

Figure 2: Oligochaete species composition in Rhine sediments.

Naididae were never found in significant numbers in mud bottoms in the IJsselmeer and the Ketelmeer; however, they were abundant in samples from the stones of groynes and bank revetments in the area. Dominance of Naididae in bottom samples was only observed in the Volkerak.

Quality assessment: the Triad approach

The classification of the different Triad components in this study is comprised in tables 8. In the final classification or priority ranking of the sediments (Table 9) data from the bioassays with *Tubifex tubifex* were excluded. The reason for this was the strong im-

pression that the swedish control sediment differed too much from the dutch sediment characteristics with respect to eutrophic state.
Some diagnostic interpretations of the final classification can be read from Tables 5 and 6. Because compound specific cause effect relations are still clouded in the cases of Volkerak and Haringvliet, additional research is recommended to identify causative agents.

Table 9: Priority ranking of Rhine sediments using the Sediment Quality Triad.

site	chemical analyses	bioassays*	field surveillances	need for remediation	urgence for remediation	need for additional research
Oostvaardersplassen	+	-	-	no	-	no
IJsselmeer	+	-	+ (b)	no	-	no
Volkerak	+	-	++	no (c)	-	yes (d)
Haringvliet	++	+	+	yes	intermediate	yes (d)
Ketelmeer	++	++	++	yes	high	no

*: the data from the *Tubifex* bioassays were excluded; b: data from Braassemermeer; c: cause of effect seems to be related to Rhine water rather then to sediment quality; d: to identify direct cause-effect relations.

Discussion

In the sediment quality triad approach, two basic problems should be distinguished: the approach as such, with equal emphasis on chemical analyses, sediment toxicity tests and in situ bottom studies, and, secondly, the methods used to fill up the various components of the triad approach.
With regard to the latter aspect, it can be concluded that the most appropriate methods can only be selected by experience in a particular situation.
When a wide range of toxic contaminants is present in addition to high concentrations of nutrients, like in the Ketelmeer, all types of organisms appear to be affected. This applies to macroinvertebrates, but also to other benthic organisms like Nematodes (Bongers, 1991). It can also be shown that piscivorous birds like cormorants are affected in regions with polluted sediments (see: Van der Gaag *et al.*, this issue). Thus, a wide range of organisms may potentially be used as indicators of sediment contamination.
When all species are affected, but not replaced by other more tolerant species, there is no reason to expect shifts of relative abundances of species. It was indeed established that species composition of the macroinvertebrate benthic community in the sedimentation areas of the Lower Rhine was not significantly different from that in hypertrophic waters free of chemical pollution such as the Wolderwijd and the Oostvaardersplassen. However, studies in a pollution gradient in the Ketelmeer/Vossemeer showed that densities of Oligochaetes as well as Chironomids may be reduced.
When a narrow spectrum of toxic contaminants (e.g. only heavy metals) is present, vari-

ous species may be affected to a different degree so that there are conspicuous shifts in relative abundance. In situ bottom fauna studies should be started with a general survey, before studying any group or species in detail. Detailed studies are needed to detect more subtle impacts of contaminants.
However, not only contaminants affect species abundance. E.g. densities of all groups of Chironomids are low in the River IJssel, and it is supposed that physical conditions determine species composition and biomass of Chironomids and other bottom macroinvertebrates rather then the presence of contaminants. At least at this site the bottom fauna does not reflect changes of the pollutant load in the Lower Rhine (Van Urk and Kerkum, 1988).
In the finely-grained sediments of this study the dominant Chironomid species were the same at all sites. These species, *Chironomus* spp. and *Procladius* spp. represent the extreme eutrophication stage in the scheme by Saether (1979).
Species composition among the Oligochaetes, however, is changed by eutrophication, as is also established by other authors (e.g. Lafont et al, 1988; Milbrink, 1987). Our results indicate a more sensitive response of the Oligochaete communities to the state of eutrophication than to the degree of chemical pollution as compared to the Chironomid communities. Therefore one should be cautious to classify polluted sediments based on these parameters when different levels of nutrient enrichment are of concern.
Deformity incidence in *Chironomus muratensis* larvae was proportional to the amount of Rhine water in a surface water, even when contaminant concentrations in the sediments are still low as is the case in the Volkerak. In the case of the Rhine, an increase of deformity incidence in *Chironomus* larvae seemes to provide the earliest response to the presence of toxic contaminants among the macroinvertebrates. This conclusion, however, does not necessarily hold for other situations. Furthermore a direct relationship between a chemical cause and effects upon the incidence of deformities has not been established yet (Van de Guchte and Van Urk, 1989; Van de Guchte, 1991).
Development of the larvae from the most polluted sites was found to be retarded compared to reference sites; this was derived from the developmental phases of the larvae found, the appearance of pupae in the samples at the different sites and from rearing experiments. This retardation in development correlates well with the incidence of deformities at the different sites (see: Van Urk, Kerkum and Smit, this issue).

In conducting toxicity experiments with sediments, the selection of test organisms is a critical phase. When only the effects of toxic contaminants are to be studied, a basic requirement to the test species is that it should be able to survive under the same conditions where the toxic contaminants are absent; this means that the test species should be able to withstand the hypertrophic conditions found in Dutch water systems. The best proof for this is that the test species is abundant in the field at a wide range of environmental conditions. *Chironomus* species fulfil this requirement, but with regard to different Oligochaete species there is some uncertainty which can only be solved by additional experiments. E.g. the species *Tubifex tubifex* is not abundant in hypertrophic Dutch lakes, where normally *Limnodrilus* species are dominant. But this may also be a result of competition rather than of a limited tolerance of *T. tubifex* to hypertrophic conditions. Nevertheless, in the bioassays it was noted that survival of *Tubifex tubifex* was highest in the Volkerak sample, which resembles the Swedish sediment conditions best. It was felt that the organisms, cultured on Swedish reference material, were not acclimised well enough to the hypertrophic conditions of the Dutch sediments. Also when Oostvaardersplassen sediment was regarded as a reference in the bioassays, no additional information was gained from the experiments. For this reason the results of the *Tubifex* assays were not included in the Triad assessment and classification. The experience with the *Tubifex* assay illustrates how essential a basic knowledge of the environmental requirements of the testorganisms is. Additional measurements of indicative physicochemical parameters and the use of adequate reference material are definitely essential to interprete bioassay results correctly.

The use of the results from the three different components of the triad approach can be considered separately from the technical details of the methods involved. For this purpose, the only important thing is whether or not impact has been established. Basically, the information obtained by the three components of the triad approach is independent. E.g. it is quite conceivable that sediment toxicity tests and *in situ* bottom fauna studies indicate impact by toxic contaminants whereas chemical analyses do not show elevated concentrations of the substances selected for analysis, as is the case in the Volkerak. In such a case, it may be supposed that substances not included in this selection are the primary cause of the effects observed. Even waterborne chemicals, which are not included in the Sediment Quality Triad, might be the causative agents. In some small surface waters in the Netherlands similar situations have been met.
In Table 9, the situations encountered in this study are summarized, Lake Ketelmeer showing the highest priority for further action. Although basically independent, there is in most cases an agreement in the information derived from the various components of the triad approach. The bioassays with isolated sediment samples give insight in the question whether effects observed in field studies are to be related to the quality of the sediment or not. However, this does not necessarily imply that the substances showing elevated concentrations in Rhine sediments are the primary causative factors in the biological effects observed. For some chemicals causal relations are derived from compound specific studies with spiked sediments. For chironomids, however, laboratory studies with persistent priority pollutants only reveal direct relations between causative agent and effects on larval development at concentration levels of the Dutch 'warning value'. Therefore bioassays with chironomids are not sufficiently sensitive to identify cause-effect relations at lower levels of sediment contamination (Van de Guchte, 1991). Furthermore, e.g. in lake Ketelmeer, the effects *in situ* cannot be explained by the presence of PAH's, which classifies this sediment as 'heavily polluted', simply because ecotoxicological data on the sensitivity of chironomids and oligochaetes to PAH's are lacking at this moment. If species specific information is not available or not sensitive enough, compound specific quality criteria based on ecotoxicological information of other taxonomic groups still enable decision makers to set priorities for remedial action programmes. However, any predictions on rehabilitation of the aquatic community remain uncertain as long as causative agents are not identified. For this reason the Triad approach, in which biological information is integrated in the decision making process (see Table 5), is very useful as a diagnostic instrument only.

The study presented here shows that the use of macroinvertebrates in field surveys and bioassays reveals sufficient information to assess the quality of sediments in the Rhine delta to adequately set priorities for further action. Obviously top layer sediments that mainly consist of deposited Rhine particulate matter from the mid seventies need the highest attention.
Before corrective measures are carried out additional research might be needed to identify cause-effect relationships.

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Trends of some IRC-priority pollutants in zebra-mussel (*Dreissena polymorpha*) and yellow eel (*Anguilla anguilla*)

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Abstract

Concentration trends for selected IRC-priority pollutants (mercury, cadmium, lead and PCB's) in water, sediment and biota (zebra-mussel and yellow eel) are presented for the Lower Rhine from Lobith to lake IJsselmeer. An assessment of concentrations measured is made by using ecotoxicologically derived quality criteria for water and sediment and recalculated criteria for biota using the equilibrium partitioning theory. Recent data on dioxines and coplanar PCB's are presented too.
For heavy metals decreasing concentrations in water reached ecotoxicologically 'acceptable' levels. In 1988 this decreasing load in the River Rhine was not yet reflected in analytical data on sediment quality at several of the sites studied. Historical loading of the sediment, sediment dynamics and spatial heterogenicity of the sediment top layer contribute to this. Organism tissue levels do not always reveal identical information because the generalised bioconcentrationfactors and sediment-water partitioning-coëfficients in the EP-model oversimplify site and species specific factors determining 'real world' bioaccumulation.
For the PCB's concentrations in the compartments sediment and biota all indicate persistent ecotoxicological pressure on the ecosystem, for ecotoxicologically derived quality criteria are exceeded by up to a factor ten.
Concentrations of dioxines plus toxic PCB's in yellow eel from the Lower Rhine are at the same level as indicated by the Canadian standard for fish.

Introduction

Molluscs and fish are widely used in biomonitoring programmes. The most general objective is to study trends in the bioavailability of persistent chemicals like heavy metals and organic micropollutants. Attention mostly focusses on tissue residues of contaminants, but sublethal effects are subject of biological investigations as well (Marquenie et al., 1987, not published; Bowmer *et al.*, 1991).
In the River Rhine, Van Urk and coworkers recorded an increase in population densities of *Dreissena polymorpha* after a period of low abundancies in the mid seventies.

Growth retardation in that period could partly be attributed to the relatively high levels of heavy metals, i.c. cadmium. Nowadays average treshold levels of cadmium in Rhine water (0.1 ug/l) are well below critical levels which exert acute toxic effects on the individual growth and survival of zebra mussels (10 ug/l). Long term effects of cadmium on population dynamics are not to be expected either, as these are reported at levels of 0.3 - 1.0 ug/l with internal tissue-concentrations of 15 - 40 mg/kg dry weight (Van Urk and Marquenie, 1987, not published). Because zebra mussels do survive present physicochemical conditions along the branches of the river Rhine they can be used in active and passive biomonitoring programmes. Both the exposition in cages and the sampling from river banks are methods currently in use.

Direct sublethal effects of Rhine pollutants on fish were recorded from field surveillances by Slooff (1983) and by Van der Gaag and Kerkhoff (1982), who exposed trout to Rhine water under experimentally controlled conditions. Both references report effects on liver, kidney and reproductive organs which are due to the pollution load of the river Rhine at that time. Now it is tentatively assumed that sublethal effects at the organism level in indigenous fish species will not be as evident as around 1980, at least not as a consequence of the presence of well known priority pollutants such as heavy metals, PCB's and organochlorine pesticides. In fish research too attention focusses on the monitoring of tissue residues of persistent priority pollutants in dominant fish species such as eel and perch (Van der Valk *et al.*, 1989).

As species specific critical levels for several compounds are not exceeded in zebra mussels and eel, we decided to compare tissue residues with ecotoxicologically derived water quality criteria, which were recalculated to tissue concentrations assuming equilibrium partitioning (Stortelder *et al.*, 1989; Van der Kooij *et al.*, 1991). This, together with an evaluation of water and sediment concentrations, enabled us to analyse the trends in concentrations to answer two major questions: firstly, do the tissue residue trends reveal the same information as measurements in other compartments and subsequent assessment using environmental quality criteria? Secondly, do the data on tissue residues indicate whether or not continuous ecotoxicological stress on the river system is to be expected?

With regard to the first question it may be assumed that organisms are in a permanent dynamic equilibrium with their environment. This means that internal concentrations only reflect actual water concentrations when a sufficient time interval has passed to enable equilibrium partitioning. For some metals this might take days to weeks, for very lipophilic compounds equilibrium might be reached in hours or days. Therefore only analyses of watersamples taken at regular short time intervals relative to the variation in concentrations in time are expected to indicate the same quality as integrative tissue concentrations in aquatic organisms. *De facto* concentrations in particulate matter and sediment have an intermediate level of integration over time.

A second argument that contributes to the hypothesis that in a dynamic system internal concentrations cannot accurately be predicted from the EP-theory is that in deriving quality criteria generalised data for bioconcentration and sediment water partitioning are used, which not always represent site and species specific characteristics.

For management purposes, however, the EP method is a useful tool to set priorities in reducing the contaminant input in aquatic ecosystems. The Rhine Action Programme of the International Rhine Commission aims at a reduction of 50 % in the emission of some 40 priority pollutants in 1995 compared to the reference year 1985. Whether or not this goal is acceptable from an ecotoxicological point of view can most easily be read from ecotoxicologically derived quality criteria for the different compartments.
In this study we present trends for some priority pollutants, *id est* mercury, cadmium, lead, PCB-52, PCB-153 and dioxines and coplanar PCB's, in *Dreissena, Anguilla*, sediment and water from the lower Rhine. The questions mentioned above are shortly discussed, but will be worked out in detail elsewhere.

Materials and Methods

The data used in this study were available from the datasets of the monitoring programmes of the Institute for Inland Water Management and Waste Water Treatment, Lelystad (data on mussels, sediment and water, 1977-1988), the Netherlands Institute for Fisheries Research, IJmuiden (data on fish, 1988-1990), and the Working Group on fish pollution of the Agricultural Advisory Committee (LAC) of the Department of Agriculture, Nature Management and Fisheries (data on fish, 1988-1990). From these datasets some characteristic bioaccumulating IRC-priority pollutants have been selected (heavy metals, PCB's, dioxins), using data for the Lower Rhine from Lobith to lake IJsselmeer, including Kampen and lake Ketelmeer, and also some fish-data for the Biesbosch area.
Mercury analyses were performed according to a modified flameless AAS method using a LDC/Milton Roy Mercury Monitor, after digestion of the samples at high pressure and temperature (150 C) in destruction bombs for four hours (Pieters, 1988). The analysis of cadmium and lead was performed with differential puls anodic stripping voltammetry.
The determination of PCB's implied a Soxhlet extraction with pentane/dichloromethane, clean-up over alumina columns, fractionation over silica columns and GC analyses on narrow bore capillary collumns (Boer and Hagel, 1991). Non-ortho (planar) PCB's (nrs. 77, 126, 169) were analysed using a HPLC fractionation over graphitized carbon columns, whereas the Soxhlet extraction has been replaced by a saponification step. For the final analyses GC/MS with negative chemical ionization was used (Boer *et al.*, 1991). Mono-ortho PCB's (nrs. 105, 118, 156) were detected using GC/MS.

Recalculation of criteria to internal concentrations

In Stortelder *et al.* (1989) and Van der Kooij *et al.* (1991) a set of formulas is presented which, according to the equilibrium partioning theory, links concentrations in the different compartments water, sediments and organisms. In Table 1 the relevant data in these documents on bioconcentration (generalised for fish and mussels) and sediment water partitioning are summarised for the contaminants discussed in this study .

Table 1: Generalised data on bioconcentration and sediment water partitioning, and ecotoxicologically derived quality criteria for biota, water and sediment. (from Stortelder et al., 1989; 1: Niimi and Oliver, 1989).

compound	BCF l/kg	Kd (l/g) log Kow	ecotox value biota mg/kg dm	ecotox value water ug/l *	ecotox value sediment mg/kg dm **
mercury	10000	170	0.5	0.005	0.6
cadmium	100	130	0.025	0.025	2.2
lead	45	640	0.585	1.3	533
PCB-52	62950	6.1	80	0.064E-3	0.002
PCB-153	185770	6.57	200	0.054E-3	0.005
dioxines			20 ng/kg***		

*: total, 30 mg/l particulate material; **: corrected for Dutch standard sediment;
***: TCDD equivalents

Results and discussion

In the figures 1-3 the concentration trends for the metals mercury, cadmium and lead are shown. Clearly the effect of measures taken in the late seventies to reduce the emissions of those chemicals can be read from the data on dissolved and total water concentrations. In 1988 water concentrations in lake IJsselmeer of all the three metals are at or below the ecotociologically derived 'acceptable' concentrations.

However, data from 1985 on mercury water concentrations in lake Ketelmeer indicate that a reduction of 50 % does not necessarily lead to concentrations below the quality criterium for water. Here the release of mercury from older sediments may play a role. Due to dynamic sediment conditions in this shallow lake in this area old sediment layers are not completely covered by more recent, cleaner material. Sediment concentrations for mercury in lake Ketelmeer show considerable variation over a period of time, which too can be due to the spatial heterogenicity of the sediment top layer. Nonetheless concentrations in *Dreissena* are all well below the recalculated critical levels for mercury in biota. This discrepancy can be explained by the use of the generalised value for bioconcentration of both organic and anorganic mercury compounds. This generalised value from Stortelder *et al.* (1989) may underestimate the environmental risk of mercury for the ecosystem, especially where organic mercury compounds are available for biouptake.

The bioaccumulation of mercury in eel appears to be somewhat higher (factor 2.5) than in zebra mussels when the data of Lobith 1988 are considered (fig. 1a and 6). This correlates well with the tentative idea that eel has a more direct contact with sediment pore water than zebra mussels have. In 1988 sediment concentrations at Lobith, and presum-

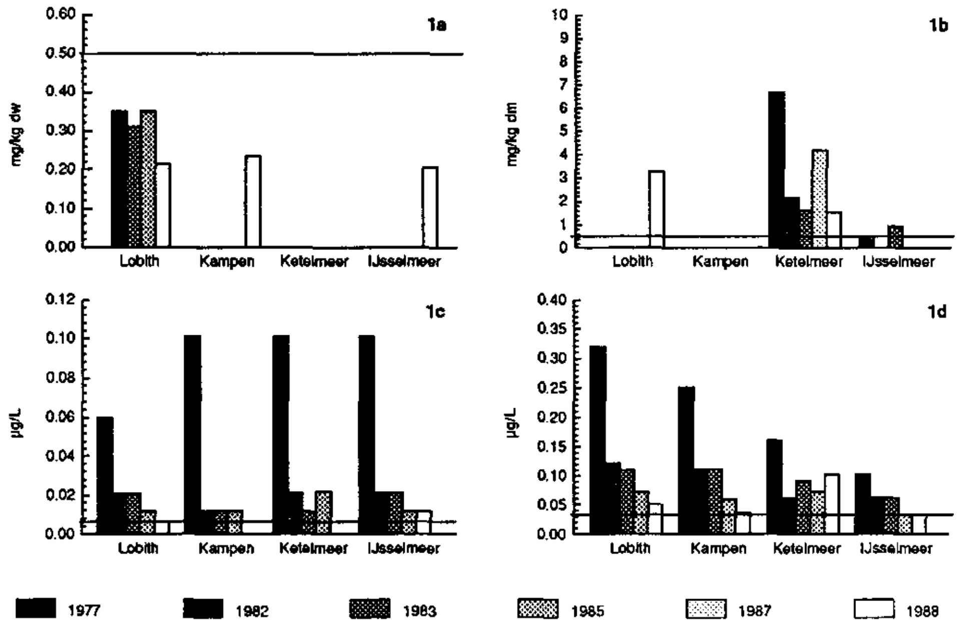

Figure 1: Trends in Mercury concentrations in the Lower Rhine from Lobith to lake IJsselmeer (1a: Dreissena; 1b: sediment (standardised); 1c: water dissolved; 1d: water total (standardised)). The ecotoxicologically derived or recalculated criteria are indicated by horizontal lines.

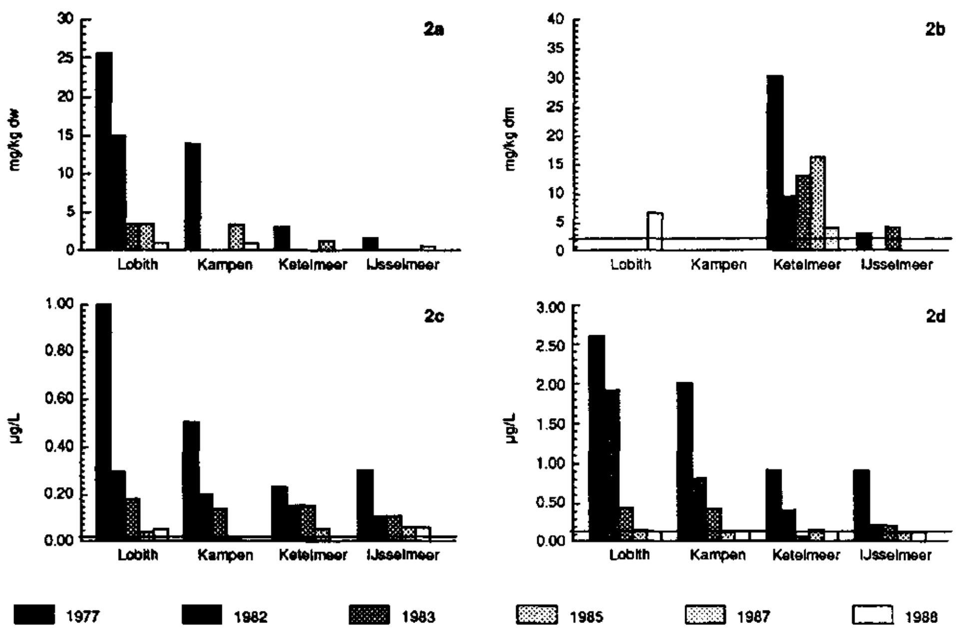

Figure 2: Trends in Cadmium concentrations in the Lower Rhine from Lobith to lake IJsselmeer (2a: Dreissena; 2b: sediment (standardised); 2c: water dissolved; 2d: water total (standardised)). The ecotoxiocologically derived or recalculated criteria are indicated by horizontal lines.

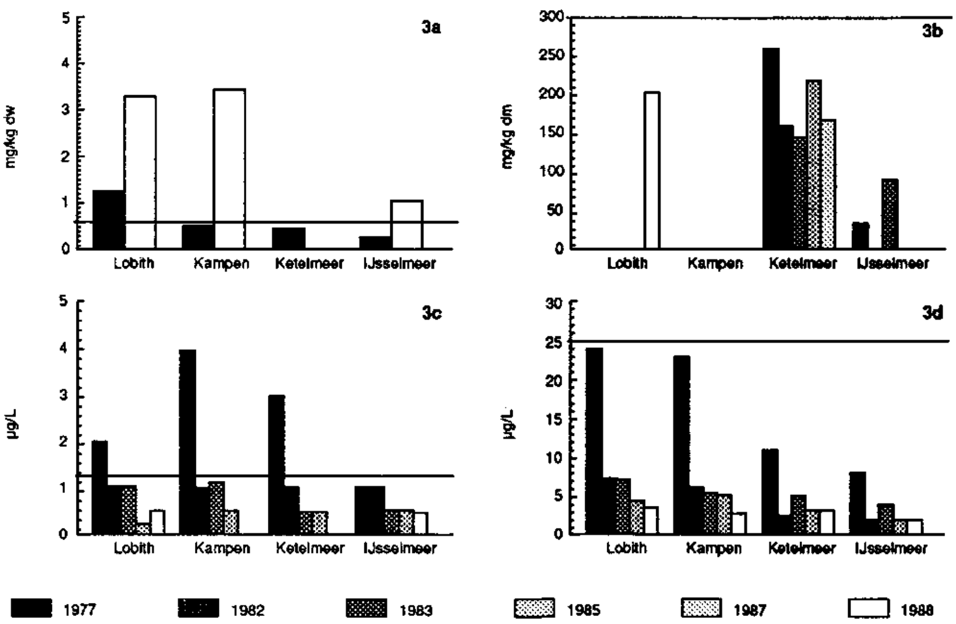

Figure 3: Trends in Lead concentrations in the Lower Rhine from Lobith to lake IJsselmeer (3a: Dreissena; 3b: sediment (standardised); 3c: water dissolved; 3d: water total (standardised)). The ecotoxicologically derived or recalculated criteria are indicated by horizontal lines.

ably pore water concentrations as well, were much higher than the quality criterium level, which was not the case for water concentration levels. Apparently no equilibrium between water and sediment has been reached here, for cadmium concentrations in the sediment are still above the ecotoxicologically derived criterium (fig. 1b). Although data are scarce, the trend in time in sediment concentrations seems to slowly follow the observed trend in water concentrations. Tissue concentrations in zebra mussels also show the same decreasing trend in time. However, they do still indicate a continuous stress on biota, because concentrations are up to 40 times as high as the recalculated criterium (fig. 2a). On the other hand concentrations in eel seem not tot be that critical, as can be read from the data of 1988-1990 in figure 6. Species specific metabolic capacities are known to contribute to the differences in accumulation levels of cadmium observed in different species (Rand and Petrocelli, 1985).
Concentration trends for lead have resulted in levels well below the criteria for water and sediment, but not for biota. Here an increase in bioaccumulation was even observed in *Dreissena* between 1977 and 1988. Tissue levels in fish, however, are below the ecotoxicologically recalculated criterium for lead (RIVO dataset). Here also species specific bioconcentration factors might explain the discrepancies observed.

Time trends of PCB congeners nr. 52 and 153 in yellow eel from the Rhine at Lobith and Ketelmeer are given in figures 4 and 5, respectively. The contents of PCB's have been recalculated for 18 % lipid concentration in eel tissue (standard eel). In the same figures the ecotoxicological values for the two PCB congeners are indicated. These

have been derived from the ecotoxicological values for water and equilibrium partitioning constants (Stortelder *et al.*, 1989), and subsequent recalculation for 18 % lipid.
From both pictures it is obvious that after the initial rapid decrease stopped in the late seventies the PCB contents in yellow eel have been constant from 1980 untill now, disregarding annual fluctuations (Van der Valk *et al.*, 1989). For the PCB congener 52 a weak decrease can be thought to exist still, especially for the Ketelmeer, despite the small increase shown in 1990. Compared with the ecotoxicological values, the contents

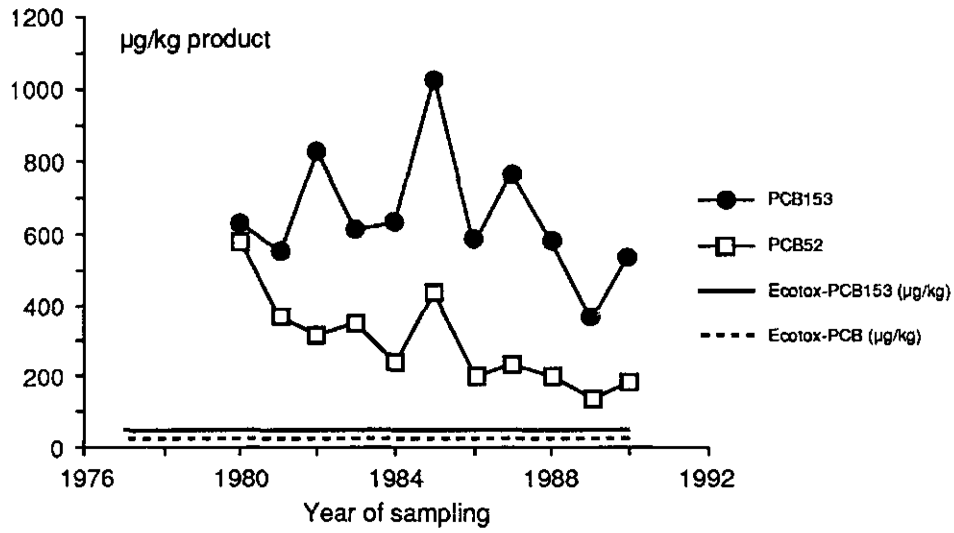

Figure 4: Time trends of PCB 52 and 153 in standard yellow eel of 18 % fat for the Rhine at Lobith. The ecotoxicological values have been drawn as horizontal lines.

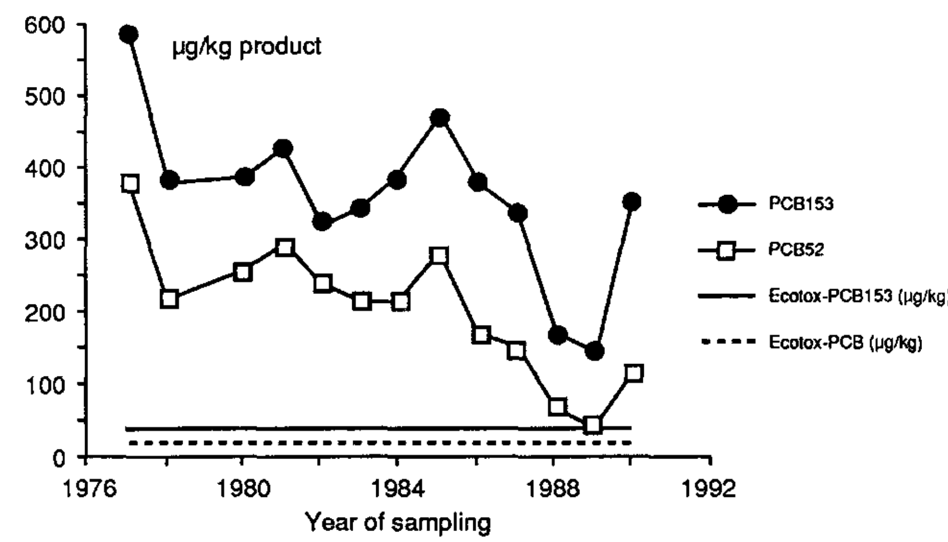

Figure 5: Time trends of PCB 52 and 153 in standard yellow eel of 18 % fat for lake Ketelmeer. The ecotoxicological values have been drawn as horizontal lines.

of PCB's in eel tissue are still considerably higher than these values measured during the last decade, as are tissue levels in zebra mussels from 1988 (RIZA dataset). Sediment concentrations in the Lower Rhine are known to exceed ecotoxicologically derived quality criteria as well. All these data indicate persistent ecotoxicological pressure on the ecosystem.

In figure 6 a comparison is made between the contents of a number of priority pollutants (mercury, cadmium, PCB's and dioxines plus toxic PCB's) in yellow eel and the recalculated ecotoxicological values for biota. For the metals and PCB's the recalculation is outlined above.
For the total content of dioxines plus toxic PCB's (TEQ value) the calculation method, including the usage of TCDD equivalent factors (TEF's), is outlined in table 2. For Yellow eel from the Rhine at Lobith each PCB congener and dioxine compound has been converted with their TEF value into a concentration expressed in ng/kg TCDD equivalents. TCDD is the most toxic dioxine with a TEF value of 1. For yellow eel from the Ketelmeer and the Biesbosch area approximations for the total content of dioxines plus toxic PCB's have been made assuming a linear correlation of the toxic PCB's and ΣPCB's.

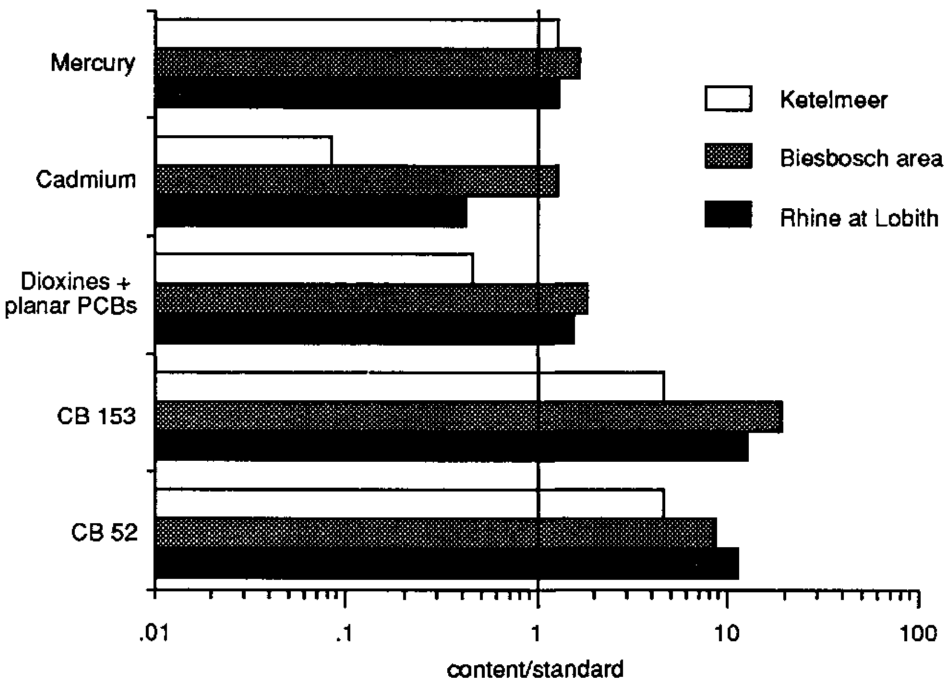

Figure 6: Comparing the contents of a number of priority pollutants in yellow eel (1988-1990) with their ecotoxicological values recalculated for yellow eel. Dioxines plus planar PCB's are compared with the Canadian standard for dioxines. See also table 1.

Table 2: Calculation of the TEQ value (TCDD equivalents) in ng/kg product for dioxines and toxic PCB's in yellow eel from the Rhine at Lobith.

CB congener nr.	content in ng/kg product	TEF recalculation factor	TCDD equivalents ng/kg product
77	94	0.01	0.94
126	112	0.1	11
169	70	0.005	0.03
105	27000	0.0001	2.7
118	110000	0.00005	5.5
156	8000	0.0005	4
dioxines (PCDDs + PCDFs)			3.6
	total content of TCDD eq.		27.8

The total content in yellow eel on a product basis has been compared with the Canadian standard for dioxines (20 ng/kg TCDD equivalents; Niimi and Oliver, 1989).
From figure 6 it can easily be read that mercury contents in yellow eel are at the same level as the ecotoxicological value for all the three locations investigated, whereas contents of cadmium are approaching the ecotoxicological value only in the Biesbosch area. The total content of dioxines plus toxic PCB's in yellow eel from the Lower Rhine is at the same level as the Canadian standard for dioxines. In the case of PCB congeners 52 and 153 the ecotoxicologically 'acceptable' levels for yellow eel are clearly exceeded by a factor 10 or more.

Concluding remarks

All the selected IRC-priority pollutants show concentration trends decreasing in time in water and suspended solids. In 1988 sediment concentrations did not always reflect this decreasing load, partly due to sediment dynamics resulting in spatial heterogenicity of the sediment top layer, which can consist of either eroded, older and more contaminated sediments from the seventies or of newly deposited, relatively cleaner particulate matter. When recalculated ecotoxicological criteria are used to assess tissue residue levels in zebra mussels and eel information revealed can differ from the assessment based on sediment or water analyses and related quality criteria. This is most evident with respect to the bioaccumulation of heavy metals. The use of generalised bioconcentrationfactors and sediment-water partition coëfficients highly contributes to the discrepancies observed.

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Impaired breeding succes of some Cormorant populations in the Netherlands: the net tightens around compounds with a dioxin-like effect

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Abstract

The breeding succes of some cormorant populations in polluted area's in the Netherlands was lower than in less affected biotopes. An integrated ecological and toxicological study has demonstrated that contamination with dioxin-type compounds is one of the majors causes of the lower reproductive succes. In the offspring of cormorants, various types of toxic effects have been observed that are caused by dioxins (PC- DD) and structurally related compounds such as dibenzofurans (PCDF) and mono-ortho polychlorinated biphenyls (MO-PCB). The toxicity of these groups of chemical substances is additive, and can be expressed as 2,3,7,8 TCDD Toxicity Equivalence Factor (TCDD-TEF), relating it to the toxicity of the most toxic congener amongst the group. A significant correlation was found between the TCDD-TEF concentrations in the chicks and the toxic effects that contribute to the lower survival. The paper presented in this book is no more than an extended abstract of the final summarizing publication of this study, as a number of analyses and correlation studies of the material are still in progress.

Introduction

A toxic time-bomb in sedimentation area's

The flow of the rivers Rhine and Meuse carries large quantities of suspended solids, which settle down in the Delta region of the south-western Netherlands. Micropollutants which are bound to suspended particles are also trapped in these sedimentation area's. Over the past decennia, micropollutants from one of Europe's largest and most industrialized hydrological bassins geo-concentrated in a very small area, thus forming a huge 'reservoir' of toxic substances that threatens the surrounding ecosystems. Because of their binding properties and their persistence many of the micropollutants in sediment can potentially accumulate in food chains. The tons of heavy metals and organic micropollutants (DDT and other organochlorinated pesticides, chlorobenzenes, PCB's, dioxins, etc.) geo-accumulated over the years in this region therefore are a potential toxic time bomb for the ecosystem.

In the early eighties, cormorant populations started to recover from the critically low numbers that had been caused by hunting and pollution. New colonies developed, especially along the different branches of the Rhine and Meuse delta. In contrast to other sites along the rivers, where the colonies developed rapidly, the population of a colony in the Dordtse Biesbosch remained small. Being a predator at the top of the food chain, the cormorant is particularly vulnerable for the bioaccumulating compounds from the sediments. As the Dordtse Biesbosch is located very near to the most eastern sedimentation area's of the Rhine delta, the possibility of an intoxication by bioaccumulated compounds was rapidly raised as one of the possible causes. Almost twenty years ago, Koeman and coworkers (1972, 1973) had already drawn the attention to the effects of bioaccumulating orgonochlorine compounds on the reproduction of fish eating birds, and in particular demonstrated that PCB-mixtures affected the breeding succes of cormorants in the laboratory.

Unsuitable habitat or toxic contaminants, who did it?

Toxic substances are not the only factor that can affect the reproductive performance of free living animals. Physical conditions as for instance the circumstances at the colony site, the availabitlity of foraging locations at a reasonable distance, etc. can exert a great influence. A link with pollution as causative agent is suggested in many situations where wild populations show a decline, but is often difficult to prove. The lower hatching success and the higher chick mortality observed in a cormorant colony located in a polluted habitat with good potentials for wildlife development, formed the starting point of a series of investigations aimed at pinning down the real cause of the phenomenon. Over a period of three years, the ecology of the cormorant colony in the Dordtsche Biesbosch was monitored, and compared with other colonies of a similar size in less polluted biotopes. Both ecological and toxicological aspects of breeding success were investigated. A first ecological orientation in the Biesbosch colony in 1987 was followed by a large scale comparison of six colonies in the Netherlands, which included also the analysis of contaminants (Dirksen et al., 1992). The final study in 1989 focussed on the possible role of some specific organochlorine contaminants. The toxicological part of the study aimed at quantifying a cluster of effects which are specific for the toxicity of dioxins (PCDD's), dibenzofu- rans (PCDF's) and planar PCB's: lower vitamin A and thyroïd hormone level in blood plasma and increased activity of ethoxyresorufin-o-de thylase (EROD) in the liver (van den Berg et al., 1992). The investigation was carried out in the Dordtsche Biesbosch and at the less polluted site of Oude Venen, in the north of the Netherlands. Ecological, physiological, biochemical and toxicological parameters were measured, as much as possible in the same animals and nests, to provide a large interrelated data set for correlation studies.

Methods

Field studies

The data and material were gathered in the colonies of the Dordtsche Biesbosch in the eastern part of the Rhine Delta, and of the Oude Venen, a wildlife area in Friesland. In a previous comparative studie, these colonies showed a low (Biesbosch) respectively high (Oude Venen) breeding success (Dirksen et al., 1992). As intensive ecological studies had been carried out in previous years, and had shown a consistent pattern, the ecological investigations in 1989 were focussed on gathering data related to the assessment of the breeding success. Both colonies were surveyed every second week throughout the breeding season according to the procedures described by Dirksen et al. (1992). In the Dordsche Biesbosch, forty five nests were inspected in the same area of the colony which had been selected for previous studies. Thirty of these nests were intensively surveyed. Fifteen nests situated in very tall trees were only subject of a global investigation, restricted to assessing the presence of breeding adults, eggs and offspring. Seventy-eight nests were inspected in the Oude Venen colony. In the selected area, all trees with nests were marked, and the height of each nest was estimated. The presence of eggs or chicks was checked at each inspection in order to determine the lenght of the incubation period. The eggs were marked, weighed to the nearest 0.1 g. Length and width were measured with an accuracy of 0.1 mm. At each visit, the young were measured (lenght to the nearest mm of wing, tarsus and total head, bill length and height, with an accuracy of 0.1 mm) and weighed. The area underneath each nest was searched for eggshells and dead young. Regurgitated fish was gathered for analysis on contaminants. Eggshell thickness was measured to the nearest 0.001 mm after removal of the membranes.

Laboratory study

Thirteen eggs from the Biesbosch colony and twenty six from the Oude Venen were gathered for laboratory studies. The eggs were removed from the nest and immediately transfered to an incubator in the laboratory in an insulated box, thermostated at 37 °C. During the incubation, the egg-conductance was measured at regular intervals. The 'in ovo' metabolism of the developing embryos was monitored (oxygen consumption, carbon dioxide production). Within 24 hours after hatching, the blood of the chicks was collected and stored as plasma at -20 °C until further processing. Then the chicks were sacrificed and examined for external lesions and weighed. The size of the head and tarsus was measured in the same way as in the field study. Eggshell, liver, yolk sac, bursa and thymus were collected and weighed. The liver was immediately homogenized and split up in two sub-samples. One part was stored at -20 °C for the analysis of vitamin A, thyroïd hormone and retinol according to the method from Brouwer et al. (1988). The remaining homogenate was used to prepare microsomal suspensions for the analysis of activities from total cytochrome P-450, and from the specific related systems, the pentoxy- and ethoxy-resorufin de thylase (PROD, respect. EROD), as described by van den Berg et al., 1992). The yolk sac was processed for the congener-specific analysis of PCB's, PCDD's and PCDF's (van den Berg et al., 1992).

Results

Lower reproductive performance in the Biesbosch

The overall breeding success in both colonies is consistent with the results from the previous studies: with only 0.7 young per clutch leaving the nest, the Biesbosch colony was significantly less successfull than the cormorants in the Oude Venen (1.5 young per clutch, table 1). This difference is mainly caused by the poor survival of embryo's and chicks. The clutch size of Biesbosch nests is only slightly smaller than that in Oude Venen. In contrast to preceeding years, this difference was not significant in 1989. The number of eggs that hatch is almost 50% lower in the Biesbosch, whilst the survival of the young is also impaired.

Table 1: Reproductive performances of the Cormorant colonies in the Dordtse Biesbosch and Oude Venen. The number of young that finally leaves the nest is more than 50% lower in the polluted Biesbcsch area than in the Oude Venen. The higher mortality from eggs and young has the greatest impact on this poor reproductive performance.

	year	clutch size	hatchling/nest	surviving young/nest
Dordtse Biesbosch	1989	3.21	0.9-1.1	0.7
	1987-'89	3.23	1.0	0.6
Oude Venen	1989	3.29	1.9	1.5
	1988-'89	3.44	2.0	1.6

Table 2: Physiological and biochemical measurements in eggs and new-born chicks (from: van den Berg et al., 1992)

	Dordtsche Biesbosch	Oude Venen
Metabolism		
Oxygen consumption	4668 ml	3778 ml
Carbon dioxide production	3401 ml	2529 ml
Thyroïd hormone and vitamin A in plasma		
free thyroxin (pmol/l)	1.94	3.65
total thyroxin (nmol/l)	4.7	8.8
tot. triiodothyronin (nmol/l)	0.65	1.34
retinol (vitamin A)	0.108	0.110
Cytochrome P-450 related enzyme activity		
cyt. P-450 (nmol/mg prot.)	0.18	0.12
EROD (nmol/mg proteïn)	0.70	0.48
PROD (nmol/mg proteïn)	0.034	0.046

Biochemical fingerprint

Various physiological and biochemical parameters in the birds from the Biesbosch area were significantly different from the Oude Venen group. The metabolism in the eggs was elevated by 25-30%, and thyroïd hormone levels were low (table 2). Although the cytochrome P-450 and EROD-activities 40-50% higher in the Biesbosch group, this increase was not found to be significant, mainly due to the high individual variability within each group. The PROD activity was low in both groups, being lowest in the Biesbosch.

Homing in on compounds with a dioxin-type effect

The first correlations studies between a number of parameters measured in the individual birds confirm the role of compounds with a dioxin-type mode of action. The individual correlation studies provide for a better insight than a simple analysis of the differences occurring between both colonies, because of the high variability in contaminant burdens between individuals within a colony. Significant correlations have been found between the thyroxin level in plasma and the EROD activity in the liver on one side, and the sum of dioxin-toxicity equivalents from dioxins, dibenzofurans and PCB's on the other side (figures 1 and 2). The dioxin-toxicity is also significantly correlated with the increased metabolism that was observed in the eggs (results not shown).

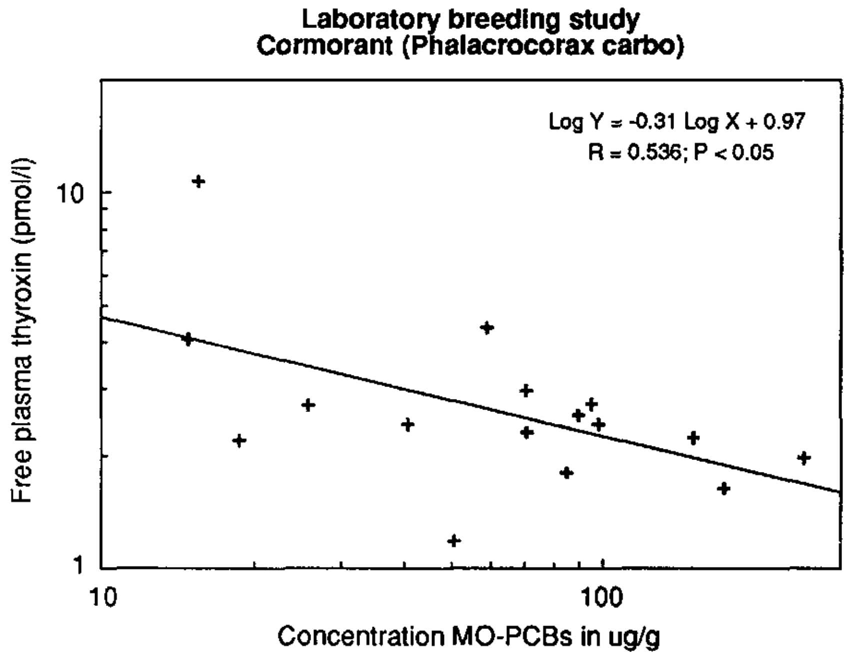

Figure 1: Correlation between the free tyroxine level in the blood plasma and the dioxin-toxicity equivalents in the yolk sac. As the concentration of dioxin-type contaminants increases, the blood plasma levels are found to be lower in the freshly hatched cormorant chicks.

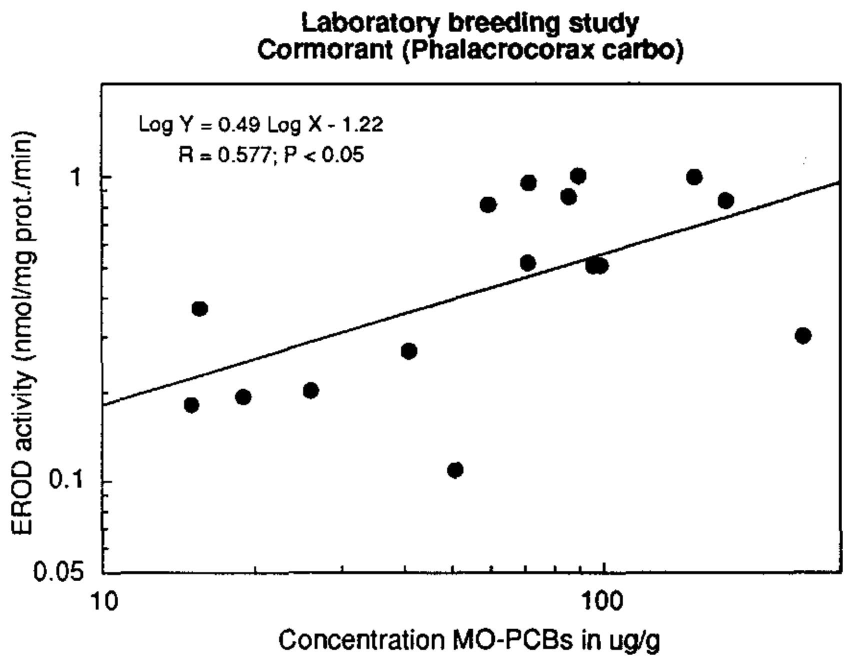

Figure 2: Correlation between the EROD activity in the liver and the dioxin-toxicity equivalents in the yolk sac. The direct relation between both parameters points to a toxic effect of compounds related to dioxins in the cormorant chicks.

Finalizing the study

Final conclusions on this study could not be drawn at this moment, because not all data were available. Further analysis of the data will focus more on the correlations between the measured ecological and toxicological effects, in order to attempt a further quantification of the contribution of contaminants to the reproductive success of cormorants in contaminated habitats.

Acknowledgements

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