THE EFFECT OF FERRIC DOSING OF LAKES ON BENTHIC INVERTEBRATES : FIRST YEAR REPORT

- N. RADFORD

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CONTENTS

INTRODUCTION: Time scales of the first year work.

From November 1990 to January 1991 I undertook a literature survey. This investigated two major areas; firstly, the methods of phosphorus inactivation, their reported success and their repercussions; and secondly, the uptake of heavy metals and their toxicity to benthic invertebrates. The resulting literature review is presented in Chapter 1 of this report.

In early February 1991, I visited the University of Amsterdam for a three month study period, as part of the Erasmus scheme for exchange of students between European Universities. The aim of this trip was to gain experience of working with benthic organisms and metal toxicity. The Ecotoxicology working group at the University of Amsterdam have been prominant in this area, and a project was put forward that, both combined with their current research interests and enabled me to fulfill my aims. The first section of Chapter 2 reports on the findings of this project and the second section deals with other work carried out during the visit. The current status of my Ph.D. project is reported in the third section of Chapter 2 and the proposed direction of the project is discussed in Chapter 3.

Chapter 1- Literature survey

Phosphorus inactivation as a method of lake restoration: Effectiveness and potential effect on benthic invertebrates.

ABSTRACT

Rutland Water in Leicestershire is a large water supply reservoir with high public amenity value. The reservoir is classified as eutrophic, and in recent years nuisance algal blooms have caused problems for water supply and in extreme cases, concern for public health. In order to combat the algal blooms and to allow continued use of the reservoir as an amenity, Anglian Water have instigated the dosing of Rutland Water with ferric sulphate solution. This is designed to remove available phosphate from the water and thus limit algal blooms. In this literature review I shall explore some of the background to this problem and research concerning lake restoration methods of this type. In addition, I shall review the literature on the subject of heavy metal toxicity to benthic invertebrates, particularly chironomids.

PHOSPHORUS CYCLING AND EUTROPHICATION

In the natural situation phosphorus is available to freshwater ecosystems only in very low quantities, usually in the range $\mu g\ g^{-1}$ (Trudinger and Swaine, 1979). It is recognised that phosphorus is very often the limiting resource in aquatic systems. The form of phosphate that is most readily available to organisms is dissolved phosphate. In natural waters and some soils dissolved phosphate is in the form of ortho-phosphate (HPO_4^{2-} and $H2PO_4^-$; depending on [H]) and this is the most important phosphate species for living organisms. The main natural source of ortho-phosphate is the weathering and erosion of phosphate minerals. Through the processes of living organisms, leaching from soils, decomposition of dead organisms and precipitation with metals the ultimate sink of phosphate is marine sediment. It must be noted however, that the phosphate locked in living organisms is much greater than the level of dissolved phosphate, and, that the exchange rate between them is much greater than the net deposition of undissolved phosphate or leaching from soils. On a global scale the phosphate cycle is completed by the diagenesis of phosphate containing sediments into phosphate rock. The turnover rate of the cycle is regulated by the rate of this step, a process that takes between 0.1 and 1 Gy (Fenchel and Blackburn, 1979). Thus, one global cycle will take greater than 1 Gy. Therefore, in the main, the phosphorus cycle can be considered as unidirectional from phosphate rock to sea and freshwater sediments.

In natural freshwater ecosystems therefore, we have low available phosphate levels, limiting production. Increasingly however, man's influence has caused very large increments in the level of available phosphates to these systems. Modern farming methods use fertilizers rich in nutrients, including phosphorus. Also soil erosion and waterlogging cause greater phosphate solubility resulting in increased concentrations in run-off. In addition, intensive animal units provide point sources of nutrient enrichment in run-off. The largest enhancement of phosphorus availability however, comes from human waste. The treatment of sewage produces oxidised soluble compounds of carbon, nitrogen and phosphorus which run out in the works effluent. Approximately, half of the phosphorus going into effluent is from detergents, most of the rest is from human wastes. Phosphorus is also input into systems from industrial and urban run-off.

All these sources of phosphate enrichment can find their way onto freshwater ecosystems. The result of this is that phosphorus is no longer limiting to production in these systems. Ortho-phosphate can be very quickly utilised by organisms, the turnover time in conjunction with high biological activity can be as little as two minutes. Even with low biological activity and unusually high ortho-phosphate levels, turnover time is 100 hours (Fenchel and Blackburn, 1979). Bacteria, algae and higher plants take up ortho-phosphate from the water for combination with ADP to produce ATP. Some bacteria and phytoplankton take up phosphate in excess of immediate needs and store it prior to increases in population. Protozoa graze bacteria increasing phosphate cycling. Larger zooplankton graze algal and detrital particles, recycling in dissolved from approximately 50% of ingested phosphate. Phosphate is, therefore passed round the aquatic system with the sediments acting as a phosphate trap due to the mineralisation of sedimented dead organic matter and the precipitation of metallic phosphates, especially ferric phosphate. This cycling is summarised in fig.1.

One of the adverse effects of high nutrient enrichment in the aquatic environment is the tendancy for very large algal blooms to proliferate. Young *et al.* (1988) reported that such nuisance blooms had caused water extraction from Foxcote Reservoir to be ceased, due to strong odours and tastes and high toxin levels resulting in water intended for supply. In addition, large algal masses caused blockages during treatment. At Rutland Water similar problems have also been encountered. On one occasion the reservoir had to be closed due to deaths of sheep and dogs which had drunk water containing toxins produced by massive blooms of cyanobacteria. These problems are intrinsically linked to eutrophication and are likely to occur wherever nutrient enrichment is a problem. It has been necessary therefore, to search for a solution to phosphate enrichment in order to combat nuisance algal blooms. The methods which have been employed and the extent of their success are discussed in the next section.

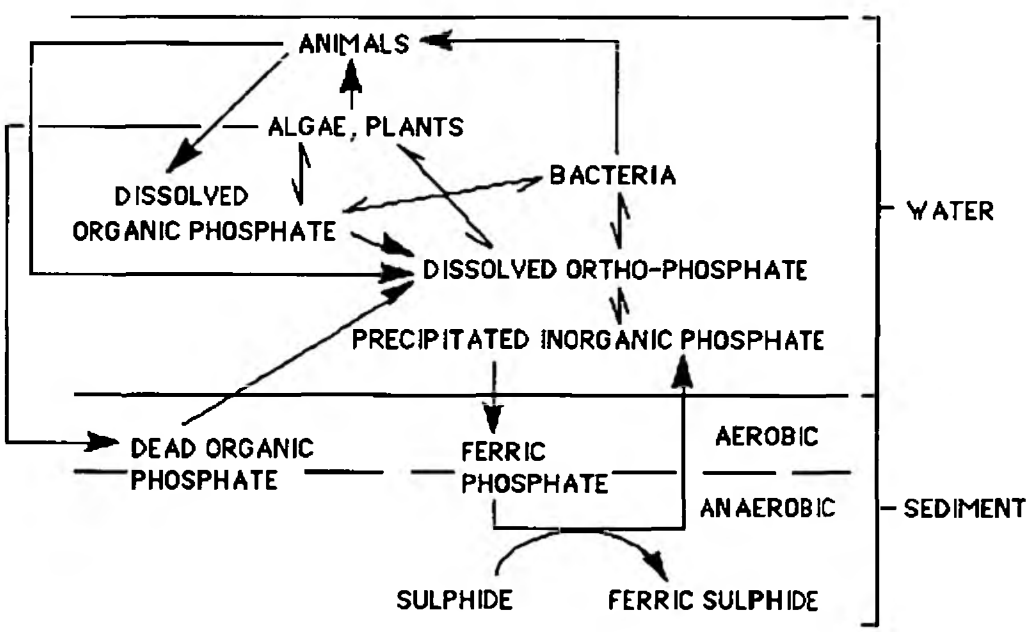

Fig.1. Summary of the Phosphorus cycle in the aquatic environment. (after Fenchel and Blackburn, 1979.)

METHODS OF PHOSPHORUS INACTIVATION IN LAKES AND THEIR INLETS

Ideally, the best way to reduce amounts of phosphate allowed into freshwater systems is to eliminate all the sources of enrichment. This however, would prove an almost impossible task as there are often a great many sources polluting just one system. In the long term elimination of these sources is desirable and should be investigated, however more immediate measures have been looked at to allow continued use of lakes and reservoirs for water supply and public amenity.

The most favoured methods seem to be those involving the use of compounds which inactivate available phosphate. The basic mechanism of phosphorus inactivation is that the river or inlet water is dosed with compounds which bind ortho-phosphate in the water. This forms a preciptate which sediments out as a layer on the surface of the sediment. This layer is then designed to 'mop up' phosphorus released from the sediment. A range of substances have been investigated and used for this purpose, for example, ferric sulphate, ferric chloride, ferric aluminium sulphate, sodium aluminate, lanthanum rare earth chloride and zirconium tetra-chloride. Peterson *et al.* (1976) performed studies into the effectiveness of some potential phosphorus inactivants, and listed the criteria that they felt were required of such substances *in situ*. Eutrophic lakes cover a broad spectrum of pH over which treatment must be effective, toxicity and uptake by aquatic organisms should be considered, long phosphorus-inactivation complex retention times in the sediment-water interface and the capability to withstand alternate anaerobic-aerobic conditions in the hypolimnion, are also required.

In terms of ability to reduce phosphate levels available to algae in the short term, most of the substances that have been used seem to work well. In the study by Peterson *et al.* (1976), sodium aluminate was found to give 90% removal at pH7 when a ratio of 5.7 : 1, Al : PO4-P was used; Lanthanum rare earth chloride removed essentially all phosphate from the test media at an inactivant : phosphate ratio of just 0.9 : 1; and zirconium tetra-chloride was required in a ratio of 2 : 1 for 90% phosphate reduction in pond water in the pH range 3-8. Total phosphorus and chlorophyll a were significantly reduced after addition of ferric chloride in Lake Breukelveen in the Loosdrecht Lake System, The Netherlands although, this was not the case in two other lakes, L.Vuntus and L. Loosdrecht in the same lake system (Van Liere *et al.*, 1990). Phosphate levels in discharge to the River Ant and Barton Broad in Norfolk showed a reduction of 90% after the use of ferric sulphate to chemically precipitate phosphorus in sewage effluents, the insoluble floc formed was allowed to sediment out in the final sedimentation tanks of the sewage process (Phillips & Jackson, 1990). Foy (1985) recorded a decrease of 92% in levels of phosphorus release from sediments of a eutrophic lake dosed with ferric aluminium sulphate in 1980 compared to 1979, it was noted however that phosphorus accumulation in sediments was at a rate sufficient to allow recovery of phosphate release. It certainly appears that in the short term phosphorus inactivation does reduce phosphate levels available to organisms, but what is the long term effectiveness of these methods? What problems have been encountered? And what effects on lake flora and fauna have been seen?

Phosphorus inactivation : long-term effectiveness

Sanville *et al.*(1982) used an experimental pond divided in two, dosing one side with zirconium tetra-chloride. This caused rapid production of a white floc which settled out. Phosphate levels had been declining prior to the treatment but after treatment these leveled off in the control side whilst continuing to decline in the experimental side. Total phosphorus levels were greater in the control than the treated side during the following summer, probably due to direct precipitation of dissolved phosphate and phosphate-associated particulate matter. It was found that internal loading from the sediment was not eliminated and the potential for sediment phosphorus release was greater in the inactivated side. Also it was found that zirconium tetra-chloride addition lowered the surface pH compared to the control side. To combat this sodium hydroxide was added. By the next year enough zirconium had moved into the control side to inhibit sediment-water phosphate exchange and total phosphate levels dropped in the control. In later years, no big algal blooms occured and vascular plants increased in proportion.

At Foxcote Reservoir, a water supply reservoir and Site of Special Scientific Interest (SSSI) near Milton Keynes, inlet water from the Great Ouse was dosed with feric sulphate solution in order to reduce ortho-phosphate levels. The aim was to produce a layer of floc of depth between 3 and 16 mm. It was found however, that the floc was rapidly dissipated and so regular dressings were applied. Young *et al.* (1988) reported that chlorophyll a levels were reduced in the first seven months after dosing but then large algal blooms occured, indicating that available phosphate levels were again high. They suggested that the reason for this was resolubilisation of organic matter deposited on the sediment. Decay of this matter would lead to a reduction of oxygen in the sediment causing the release of bound nutrients. Internal cycling renewed algal developement for three years, until finally becoming less significant. At this time planktonic algal numbers were very low and a change in flora to a predominance of filamentous species and rooted macrophytes was seen. This new floral community caused almost as many problems in water treatment as the algal blooms had done previously, causing tastes and odours in final treated water, elevated coagulant residuals, interference with coagulation processes and filter blockage. Also, ferric residuals increased in treated reservoir water. Since the change in flora, however, the duration that the reservoir was out of use was less than previously.

White Lough, Northern Ireland received doses of ferric aluminium sulphate over a 34 month period from 1980. Aluminium has a low redox value compared to ferric, therefore, phosphate adsorbed onto aluminium floc should not be released from sediments in anoxia. In addition, above pH9, ferric phosphate rapidly increases solubility and thus releases phosphate. Aluminium phosphate has a similar response to pH but at least 1 pH point higher (Foy, 1985). Within three years of the cessation of dosing, a rapid return to pre-treatment phosphate levels was seen. Foy (1985) cites an inability to lower external phosphate inputs as the cause. Therefore, the effectiveness of sedimented aluminium floc for inactivation of incoming phosphate remains uncertain. Also, epilimnion phosphate concentrations showed little response to reductions of hypolimnetic phosphorus release and overwintering total phosphorus levels. Continued phosphate release to the epilimnion from littoral areas maintained the lake's eutrophic condition. Thus, reduction of hypolimnetic phosphate release appears to be an inefficient process for reduction of phosphorus in the epilimnion.

From these studies it can be seen that one major problem associated with phosphate inactivation particularly involving ferric compounds, is the failure of the flocculant layer to remove internal phosphate cycling. In their study Young *et al.*(1988) calculated that a flocculant layer of 100mm in depth would be required to prevent nutrient cycling in the long term. As they point out, this measure would prove too expensive and the environmental damage would be too high for it to be feasible. In general, in oxidising conditions lake sediments bind phosphorus and in reducing conditions sediments release phosphorus (Mortimer 1941-42). This release occurs due to the reduction of ions which bind phosphorus, of these the most important are ferric (3^+) and to a lesser extent manganese (4^+). In reducing conditions these ions are reduced to Fe^{2+} and Mn^{2+} respectively, thus releasing associated phosphate into solution in sediment pore water. If reduced sediment is overlain by anoxic water, which is a frequent occurance in the hypolimnion of deep stratified lakes, then diffusion of dissolved phosphate occurs into the overlying water. The sediment becomes reduced when the oxygen supply from the overlying water is insufficient for mineralisation processes in the sediment. As a result of this oxygen depletion, anaerobic respiration takes over requiring electron acceptors, therefore, reducing the environment (Phillips & Jackson 1990). If hydrogen sulphide is present in sediments it can precipitate ferric as ferrous sulphide, also causing the release of phosphate.

Transfer of soluble phosphate from sediment pore water is assisted by any turbulence, for example from water currents, resuspension of sediment and gas bubbling, and bioturbation by benthic organisms. In Barton Broad (Phillips & Jackson, 1990) the highest phosphate release was found to occur in June which coincides with the presence of large numbers of fourth instar larvae of *Chironomus plumosus*. Lower numbers of these larvae in July, due to emergence, resulted in low phosphate release. This indicated, that the burrowing activity of benthic organisms into anaerobic sediment layers increases movement of soluble phosphate into overlying water.

An oxidised layer can be present between anaerobic sediment and aerobic water, but it may only be a few millimetres thick. This layer is destroyed by high temperatures, as the microbial oxygen demand increases at these times, but where it is present the ratio of iron to phosphorus is the determining factor in phosphate release (Phillips & Jackson 1990). In an aerobic layer, oxidation of ferrous to ferric should precipitate phosphate, but if the atomic ratio of iron to phosphorus is less than 1.8 then phosphate release results. Thus, phosphate can not only be lost from anaerobic layers but also in certain conditions from aerobic layers. Phosphate is released from sediment particles into pore water by desorption from inorganic complexes e.g. with iron, manganese and aluminium hydroxides and by mineralisation of organic phosphates. The latter is more rapid in aerobic conditions than anaerobic. Any turbulance, as mentioned above will assist the diffusion of phosphates from pore water to the hypolimnion. Sediments with high interstitial iron : phosphorus ratios are unlikely to release phosphate.

Phosphate inactivant addition would seem to be fairly effective at removing phosphates from the water column, but their efficiency in the long term appears in doubt. The method of their action increases the amount of phosphate in the sediment, thus making it unavailable to algae, but for how long? In the summer months when the hypolimnion and sediments are most likely to be anoxic, especially in deep stratified lakes, it is in doubt whether any flocculant layer would reduce sediment phosphate release. It appears likely that large pulses of phosphate release could occur, allowing the renewal of algal blooms.

Phosphorus inactivation : Interactions of phosphorus inactivants with lake flora and fauna.

The use of phosphorus inactivants has been brought about as a method of combating nuisance algal blooms, and thus changes in lake floral community is part of the desired effect. Indeed, eventual decline of planktonic species and the return of filamentous and macrophytic species has been mentioned in several studies (Sanville *et al.*, 1982; Young *et al.*, 1988). Little literature is available concerning further effects of inactivants on lake flora.

Levels of phosphorus inactivants being input into lake systems are likely to be well above natural levels. We may therefore, consider phosphorus inactivants as contaminants. Due to the mechanism of phosphate removal by sedimentation to produce a flocculant layer, sediment dwelling species may be considered among those most likely to be stressed by inactivant addition. Field and laboratory toxicity tests with a variety of contaminants on benthic organisms have been widely carried out and will be discussed in a later section. Data on toxicity to lake fauna in conjunction to studies of phosphorus inactivation are however, less abundant.

Gulati (1990), reported that zooplankton communities did not reveal any significant changes following considerable reduction in external phosphate loading in the Loosdrecht Lake system, the Netherlands. Ferric chloride was used for phosphate reduction in inlet water from the Amsterdam-Rhine Canal. A decrease in mean size of crustacean populations was recorded, linked to an absence of large bodied forms. This was explained by an increase of fish predation rather than toxic effects of inactivants.

Sanville *et al.* (1982), noted a cladoceran kill soon after addition of zirconium tetra-chloride in their experimental pond. Benthic invertebrates collected five days after inactivation showed no dead or distressed organisms. However, zirconium deposits were found on the outer surfaces of plankton, benthos and macrophytes. Also, there was some evidence of ingestion of settled floc by plankton and benthos. Long-term toxic effects on benthos were said to minimal.

Peterson *et al.* (1976), as part of their study looked at the toxicity of three potential phosphate inactivants to salmonid fish and to *Daphnia magna.* Both fish and water fleas survived concentrations of sodium aluminate ($NaAlO3$) upto 40mg Al l^{-1} for 96 hours. Aluminium compounds have been shown to be very toxic in conditions of low pH, although not in this test. Salmon fingerlings exposed to greater than 1.0mgl^{-1} lanthanum rare earth chloride died in the first 24 hours of testing and high toxicity of this substance to *Daphnia,* was also shown. No mortalities of fish at 96 hour exposure to concentrations upto 10mg Zr l^{-1} were recorded and when the test was continued upto 240 hours, only two deaths occured, one of which was in the control (0.0mg Zr l^{-1}). Zirconium tetra-chloride was also concluded to be relatively non-toxic to *Daphnia* in 96 hour tests. In tests on *Daphnia* lasting 9 weeks however, each suceeding generation may have experienced some cummulative effects. For example, the 1-week TL_m (concentration at which 50% of the test organisms survive over a set period) for the 3-week age class was considerably lower than for the 1-week age class.

The concensus of these studies seems to be that acute toxicity due to the addition of phosphorus inactivants is unlikely, however the evidence seems inconclusive. Also, little work has been done concerning chronic effects of such addition. If potentially large amounts of heavy metal compounds (particularly ferric compounds) are to be added seasonally over a number of years, then questions as to the long-term effect of such measures warrant investigation. Most phosphorus inactivants are intended to settle to a flocculant layer on the sediment surface, thus, some effects to benthic dwelling organisms are likely. Even sublethal effects on benthic invertebrates, such as chironomids, could have damaging consequences for species, both invertebrate and vertebrate, who feed on them. Whilst, little investigation as to the effects of ferric compounds used in phosphate inactivation on benthic species has been performed, much information is available concerning the toxicity of other heavy metals to these species. The uptake of heavy metal contamination and its effects on benthic invertebrates is discussed in the next section.

Summary

With the impracticality of removing all the external inputs of phosphate, and the need for immediate action to combat nuisance cyanobacterial blooms, the use of dosing with phosphate inactivants has been instigated in several lakes and rivers. The short-term effectiveness of these measures appears promising. Water levels of available phosphate do drop after the instigation of dosing. The efficiency of the inactivant substances in reducing internal loading from the sediment, and thus, their long-term effectiveness, does however seem in doubt. Strongly reducing conditions in sediments, particularly in early summer, are likely to allow the release of phosphate from complexes with inactivants e.g. ferric. An internal input of this kind is likely to become available to algal blooms. The problem may be compounded rather than eliminated. Prolonged dosing with larger amounts of inactivants may be a solution, but what of the effect of such action on freshwater life? Research into this area of phosphate inactivation is limited, work done in conjunction wth dosing studies show few problems in the short-term. The cummulative effect of dosing over several years could however, cause sub-lethal effects to many freshwater species. Further work to investigate these detrimental effects is required.

UPTAKE OF HEAVY METALS AND THEIR TOXICITY TO BENTHIC INVERTEBRATES

Uptake of heavy metals by benthic invertebrates

When considering the uptake of heavy metals by organisms it is important to know which species of the metal are present in the system and to what extent they are bioavailable; that is the chemical state taken up by an organism which can react with the metabolic machinery (Timmermans, 1991). There are in general, four fractions that can be defined.

(i) exchangeable cations - loosely bound.

(ii) reducible metals - often co-precipitated with iron, manganese or carbonate-oxides.

(iii) organically bound metals.

(iv) residual metals

Of these (i) to (iii) are all at least partly bioavailable.

The use of ferric compounds for phosphate inactivation means the introduction of large amounts of one of these bioavailable fractions, reducible iron. In marine sediments sulphate is in continuous supply from the surface waters to the sediment and thus, all reducible iron is transformed to iron sulphide. In freshwater the sulphate concentration is much lower (Salomens *et al.*, 1987). Only partial transformation of ferric hydroxide to ferrous sulphide occurs. If the reduction of sulphur (methanogenesis) has occured available reducible iron is converted to siderite (iron carbonate). Reducible iron is also involved in acting as a buffer against decreasing pH. Therefore, several iron species may be present in freshwater sediments dependant on conditions. Bioavailability and toxicity effects will depend on the prevailing conditions and metal species present.

Experiments looking at copper uptake by *Chironomus tentans* from water have shown that uptake of free cupric ions and copper hydroxy complexes occurs. No uptake was observed when copper-glycine [Cu(Gly2)] and copper-NTA complexes [$CuNTA^-$] were the dominant species (Dodge & Theis, 1979). Uptake was concluded to be largely passive due to interactions between copper and sorption sites at the surface or interior of the organism. Namminga & Wilhm (1977), found higher levels of copper, lead and zinc in unspecified chironomids than in sediments. This indicated uptake of these metals by the organisms. Chromium levels however, were greater in sediments than in chironomids. Zinc accumulation was said to be principally by adsorption and cation exchange. Accumulation of zinc and copper in chironomids has been investigated by other authors (Krantsberg & Stokes, 1989; Timmermans,1991). Aquatic organisms appear to be able to regulate these essential metals.Iron ,nickel and to some extent manganese can also be regulated (Krantsberg & Stokes, 1989).

Rapid uptake of cadmium and lead by various species of the genus *Chironomus* has been recorded by several authors (Rathore *et al.*, 1979; Yamaura *et al.*, 1982; Timmermans, 1991).These are non-essential metals and aquatic organisms are unable to regulate them (Krantsberg & Stokes, 1989; Timmermans, 1991). Timmermans (1991), concluded that adsorption to the outside of *Chironomus riparius* larvae accounted for only a minor portion of trace metal accumulation by the larvae, also that active uptake was highly unlikely. It is more likely that accumulation is associated with nutrient uptake in the digestive tract or with diffusion over the thin body covering of chironomids. The production of a flocculant layer of high ferric content on the sediment surface would make the ingestion of ferric by benthic species seem a very likely scenario.

Sensitivity of benthic organisms to heavy metal contamination

Certain species of chironomids, e.g *Chironomus riparius* and *Chironomus tentans*, have been cultured successfully under laboratory conditions and this has lead to an abundance of tests involving their sensitivity to heavy metals and other contaminant groups. The number of chironomid species that are known to be easily cultured are few. With other species, difficulties arise often due to the space needed for successful swarming and mating behaviour (Timmermans, 1991). Culturing of test organisms allows tests on different life-cycle stages, and greater freedom with timing of experiments.

Despite being limited in the information they can provide, acute toxicity testing does provide indications about interactions between organisms and contaminant substances. Rathore *et al.* (1979), looked at acute toxicity of fourth instar larvae of *Chironomus tentans* to cadmium chloride and lead nitrate of concentrations in the range 10 -10,000 ppm. Time to death of all larvae was measured. LD_{100} values were 70 ppm for lead and 20 ppm for cadmium. For the same species, 48 hour acute static bioassays on third instar larvae found that metals tested came in the following order of decreasing toxicity:

Ag , Hg , Cu , As , Cd , Zn , Cr , Pb , Co , Ni.

Compared with nickel, silver was 6683x more toxic, and mercury 2397x (Khangarot & Ray, 1989). These results show that metal contamination can be toxic to chironmid larvae to varying degrees. It is also interesting to see whether different life stages have varied toxicity to metals.

Gauss *et al.* (1985), performed acute toxicity tests of copper on first and fourth larval instars of *Chironomus tentans*, varying concentration and the hardness of the water used. Fourth instars were found to be between 12 and 27x more resistant than first instars. Eggs too were tested in this study and were found to be more resistant than either larval stage. Toxicity seemed to be reduced in harder water, percentage hatch was significantly reduced in soft water compared to medium and hard water. These results were backed up in studies by Williams *et al.* (1986). Their acute tests with cadmium on all four instar stages of *Chironomus riparius*, showed fourth instars to have LC50 values approximately 950x greater than first instars which were the most sensitive stage. Lower calcium hardness also led to increased toxicity. First instars of *C. riparius*, were also found to be more sensitive to contamination by nickel than second instars, though increased tolerance to nickel was given with increased water hardness (Powlesland & George, 1986). Thus, first instars of the species tested, appear to be the most sensitive to a range of metal contaminants. Metal toxicity is lessened by increased water hardness.

Also using acute toxicity, Kosalwat & Knight (1987a), compared toxicity of copper supplied in water or in food and substrate to fourth instar larvae of *C. decorus*. Copper was found to be more toxic in water. Tubificids have also been shown to accumulate metals from water rather than sediment, but when relative concentrations in sediments and water are examined sediment often becomes the dominant input (Reynoldson, 1987).

Acute tests tend to concentrate on contaminants causing death , however death is a rather crude measure of stress. Other, often sublethal criteria, are important indicators of stress to organisms from contamination. Longer term tests involving partial life-cycles are a way of investigating these other criteria.

Chronic toxicity tests have confirmed that first instars of chironomids are the most sensitive to metal toxicity and that mortality of this life stage is relected in the numbers of emerging adults (Pascoe *et al.*, 1989; Timmermans, 1991). Wentsel, McIntosh and McCafferty (1978), took chironomids of unknown species from two sites; one uncontaminated the other contaminated by cadmium, chromium and zinc at 1030ppm, 1640ppm and 17300ppm respectively. Emergence of adults from the uncontaminated site reared in the contaminated sediment was reduced by greater than 3x and delayed for two days. Timmermans (1991), found that first instar mortality to cadmium was reflected by reduced emergence, but that survivors developed faster than in controls and emerged earlier. This was probably due to no reduction in food in the cultures. In the same study, no emergence occurred in cultures contaminated with zinc.

Larval growth is considered one of the best indicators of toxicity. Powlesland and George (1986) recorded significant reductions in larval growth of *C.riparius* at 2.5mg l^{-1} Ni. Cadmium levels of 0.15mg l^{-1} Cd caused significant reductions in larval development, survival and production (Pascoe *et al* .,1989). Larvae of *C. decorus* reared in spiked food-substrate have also been shown to suffer reduced growth (Kosalwat & Knight, 1987b). These results are backed up in Timmermans' study (Timmermans, 1991), cadmium concentrations of 0.061mg Cd l^{-1} impaired *C.riparius* larval growth and development and levels as low as 0.007mg Cd l^{-1} delayed development of early larval stages. In the same study zinc, lead and copper contamination all impaired larval growth. Anderson *et al*. (1980) also looked at toxicity of these four heavy metals to *Tanytarsus dissimilis* from embryogenesis through to second or third instar stages. Unfortunately, the study used mortality as its criteria for toxicity. *T. dissimilus* was found to be very sensitive to heavy metals and was most sensitive to cadmium, then copper, zinc and lead in decending order.

Other sublethal effects of metal contamination have been seen. For example, Kosalwat & Knight (1987b) recorded epipharyngeal plate deformities linked to their other findings of reduced larval growth and delayed emergence of *C. decorus* in conditions contaminated with higher than 1800mg/Kg copper. Williams *et al*. (1987) found that eggs of *C.riparius* were tolerant of cadmium contamination using percentage hatching as a measure. Egg-ropes are laid in a protective gelatanous coating which expands on contact with water. When this coating was removed and eggs were placed in cadmium contaminated water hatching was reduced to 60%. These results refer to eggs which have been laid in clean water and transfered to contaminated water. It was found however, that viabilities of eggs laid directly into contaminated water were greatly reduced. This causes some doubt as to the tolerance of eggs laid in toxic conditions. The study also found that female *C.riparius* laid fewer eggs in water of high cadmium concentration (100 & 300mg l^{-1} Cd) than in lower concentration or clean water. Chemo-receptors in the antennae may be used to detect suitable sites for oviposition.

In addition , there is some evidence that populations of chironomids from long-term metal contaminated sites develop tolerance. Wentsel, McIntosh and Atchinson (1978), took specimens of *C.tentans* from a site contaminated with cadmium, chromium and zinc and from a uncontaminated site. 45.5% survival of the population from the uncontaminated site was recorded when reared in contaminated sediments, compared to 75% survival of the contaminated population in the same sediment. Yamaura *et al.* (1982) found rapid uptake of cadmium by *Chironomus yoshimatsui* larvae, bound in the organisms to high molecular weight proteins. With continued exposure however, low molecular weight proteins were slowly induced in the larvae. This protein was found to be a mix of four isoprotein subunits, showing similar characteristics to metallothionein, a protein which has been linked to tolerance of metal contamination in other marine and freshwater organisms. In this study, the induction of metallothionein type protein could not explain the high tolerance of *C. yoshimatsui* to cadmium contamination.

In summary, with potentially high levels of ferric compounds used as phosphorus inactivants what effects on populations of chironomids might we see? Accumulation of iron principally by ingestion with food could lead to several effects. Mortality, especially of early instar stages, could occur, reflected in reduced adult emergence. Larval growth may be reduced and delayed leading to late emergence. Eggs laid into contaminated water may have greatly reduced viability. Populations living long term in contaminated water might become tolerant, but alterations in genetic structure or aberrations in genetic expression could lead to physical abnormalities, e.g. asymmetrical mouthparts. Reproductive success could also be impaired. In addition, chironomids could be important in the movement of contaminants around the system, for example, biomagnification has been shown for *Hygrobates* (water mite) predation of *Stictochironomus histrio* and *Chironomus muratensis*; water mites had twice as much zinc accumulated than the midge larvae, on which they had been fed, and 4-5x as much cadmium (Timmermans, 1989).

None of the studies in which these effects have been recorded, have directly involved ferric. Ferric is an important natural constituent of freshwater sediments and may have been overlooked for this reason. With its use in phosphate inactivation however, ferric may be considered a contaminant and as such, investigation of possible detrimental effects on freshwater species is necessary. Benthic invertebrates are likely to come into contact with large concentrations of ferric in sediments as a result of dosing. Lethal and sub-lethal effects, similar to those described, may occur with potentially devastating consequences for benthic invertebrate populations.

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Chapter 2- Current status of project: work achieved

Macro/meio-benthic interactions of littoral species: *Chironomus riparius* (Chironomidae; Diptera) and *Chydorus piger* (Chydoridae; Cladocera), and the effect of copper contaminant on these two species.

ABSTRACT

In order to learn ecotoxicological techniques and gain experience in their use, an investigation concerning the effects of copper toxicant on benthic invertebrates and their interactions was made during study in collaboration with the Aquatic Ecology department of the University of Amsterdam. Some evidence was found suggesting that two benthic littoral species; *Chironomus riparius* and *Chydorus piger* exhibit partial overlap in the food they utilize. Under certain conditions however, both species can gain mutual benefit from each other's presence. *Chironomus riparius* suffers high mortality at copper concentrations of 500ppb and above. This sensitivity is variable within the population, some individuals appear tolerent of the levels mentioned. *Chydorus piger* was shown to be less sensitive to copper, but at concentrations of 500 ppb or above may suffer reduced or slowed reproduction.

INTRODUCTION

At the Aquatic Ecology section of the University of Amsterdam current projects include two areas of study; (i) the ecology of the benthic organisms of the littoral zone of Lake Maarsseveen I, and (ii) the effects of heavy metals on benthic organisms. In combination with both of these project areas my study allowed me to learn about the ecology of benthic organisms, use laboratory cultures, and work with benthic invertebrates in experimental set ups involving metal toxicity.

The Maarsseveen Lakes are located in the centre of the Netherlands, near the city of Utrecht, approximately 35 km south-east of Amsterdam. The area of the lakes was formerly swamp which was utilized for the digging of peat. The method of this digging resulted in a series of small canals ('petgaten') with narrow strips of land ('legakkers') in between. These were used for drying the peat. Thus, longitudinal alternating strips of water and land ('zodden') was created. With time, the 'legakkers' became eroded by wind and wave action, resulting in the formation of shallow lakes. In 1960 and 1967 dredging for sand formed two lakes, Lake Maarsseveen I and II. Rain water falling on pleistocene dunes in the east supply Lake I via seepage through the bottom. Lake I has oligo-mesotrophic status as a consequence. Lake II, however is fed by the River Vecht and is polluted and eutrophic.

The benthos of the littoral zone of Lake Maarsseveen I has been investigated at the Aquatic Ecology section of the University of Amsterdam for several years. Chironomids are the dominating macrobenthic group, *Cladotanytarsus mancus* and *Stictochironomus sticticus* are the main components to a depth of 4m. Below this depth *Tanytarsus bathophillus* is dominant. Water mites (particularly *Hygrobates nigromaculatus* and *H. trigonicus*) are very important predators of chironomid larvae in the lake. In addition, cyprinid fish, bream (*Abramis brama* L.) predate upon chironomid larvae larger than 4.5 mm in length (Ten Winkel 1987). The chironomids themselves feed on algae and detritus. The meiobenthos is dominated by chydorids (benthic cladocerans) which rely on similar food resources to the chironomids (van de Bund 1991). Chydorids are predated by young fish; tanypod larvae and other invertebrate predators also utilize them for food (Robertson 1988; Robertson 1990). Even over apparently uniform habitat chydorids are found to have a clumped or contagious distribution (Davids, Stolp *et al.* 1987; Whiteside 1974). This gregarious behaviour is thought, most likely, to be an evolutionary response to food or predation. The latter certainly, is reported to have a marked effect on the reproductive strategy of chydorids. Chydorids have a maximum of two eggs per brood. The young are large, relative to their size at maturity and they mature early, this is typical of small planktonic cladocerans. Growth is curtailed however, at the onset of reproduction, a stratey used by large planktonic cladocerans. The chydorid strategy appears therefore, to be half way between those of small and large planktonic cladocerans. Littoral habitats have a high abundance of invertebrate predators, therefore, the production of relatively large fast maturing young is an advantage. The curtailment of growth at the onset of reproduction may help to protect against visual predators such as young fish. Also, Cladocera associate with aquatic plants which provide a degree of protection from predation and buffering from wave action which may be size dependant, selecting for smaller cladocerans (Robertson 1988).

Information concerning the ecology of individual groups in relation to feeding, reproduction and predation abound; but, the nature and importance of food competition interactions between macro- and meio-benthos in freshwater systems is poorly understood. A series of laboratory experiments has been performed to investigate interactions between chironomids and chydorids (van de Bund 1991). Individual growth of food limited early instar *Chironomus riparius* larvae was negatively influenced by the presence of *Chydorus piger*. *C. piger* population growth was however, promoted by the presence of both early and late instar *C. riparius* larvae. These results suggest that the food spectra of *C. piger* and *C. riparius* do, at least partly, overlap. This would explain the effect on the chironomids. Furthermore, the presence of *C.riparius* must make some food resource better available to *C. piger*. It is recognized that chironomid activity can stimulate bacterial production, so it is possible that the chydorids are taking advantage of this bioturbation effect (van de Bund 1991).

The effect of trace metals on benthic organisms has also been a major concern of the Aquatic Ecology section. The uptake of and response to cadmium, zinc, lead and copper by chironomid larvae and other freshwater benthic macro-invertebrates has received much attention (Timmermans, van Hattum *et al.* 1989, Timmermans 1991). Details of the results and conclusions of these studies can be found in the literature review (Chapter 1). Currently, work is focused on cadmium and copper toxicity to freshwater mussels, (*Dreissena polymorpha*), from contaminated and uncontaminated sites in the Netherlands.

In combination with these two areas of interest my study's aim was to further investigate the interactions between *Chironomus riparius* and *Chydorus piger* and the effects of copper on growth of the two species and their interactions. With a study time of just three months, the intention was that the learning of techniques should be a major concern during my stay in Amsterdam.

METHODS

The first part of the project was to further investigate the interactions between *Chydorus piger* and *Chironomus riparius*. 10 chydorids and 5 chironomids were used in each of three replicate vials. Control situations with just one species were set up for comparison. In initial experiments, the organisms were placed in conditions of food stress, however it became apparent that the stress was not equal for both species. The necessity for the addition of chironomid food was recognized. An experiment varying the amount of this food (1 drop or 3 drops) was combined with the interaction set up, in order to discover the amount of food to use. Experiments concerning the sensitivity of both species to copper contaminated sediment were also performed. 10 chydorids and 5 chironomids were separately exposed to a range of 0 -1000 ppb copper, again three replicates of each treatment were used. A repeat of the chironomid experiment was also performed.

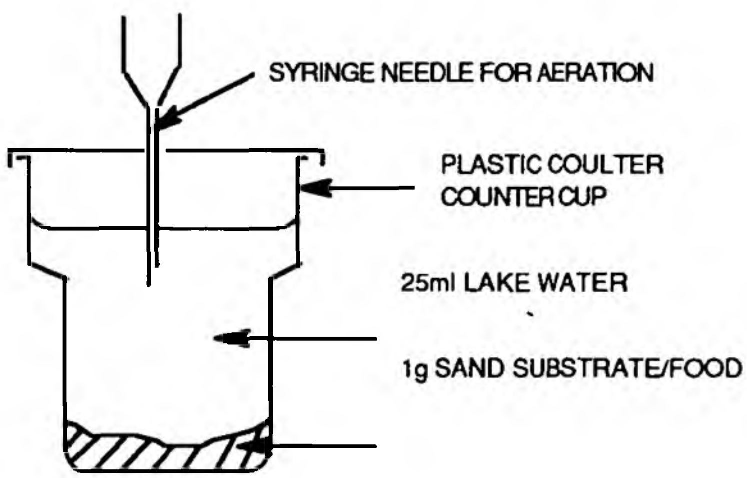


Fig.1. Diagram of basic experimental set up.

A diagram of the basic experimental set up is shown in Fig.1. Its purpose is to provide the organisms with a micro version of the sandy littoral habitat, where they can interact with each other and be convieniently monitered. Coulter counter cups were used as the vessels for the experiments. These were acid rinsed and in those experiments involving copper were saturated overnight with copper solution of the concentration to be used. 1 g of dried sand from Lake Maarsseveen I was used as substrate, food source, and in toxicity experiments to administer copper to the organisms. The sand was shown to adsorb greater than 50% of copper supplied in water over a period of 48 hours (Fig.2.). The slight increase over time in the copper concentration in the water only vials was due to evaporation. The sand can therefore, be used to administer copper to organisms utilizing it as a substrate/food source. 25ml of filtered water from Lake Maarsseveen I was supplied in each vial. When used, copper solutions were made up in the lake water to 25ml from a 10ppm standard. After, copper addition to the water, a period of at least, 3-4 days was allowed for adsorption by the substrate. Generally, the running time of the experiments was two weeks. This period only differed in order to fit the experiments into the short time available. In most experiments aeration was given to each vial for 1/4 hr in every hour. It became apparent however, that the animals could survive the experimental time without any aeration. Aeration of the vials was therefore abandoned as it increases evaporation, altering water copper concentrations, and causes greater disturbance to the animals.

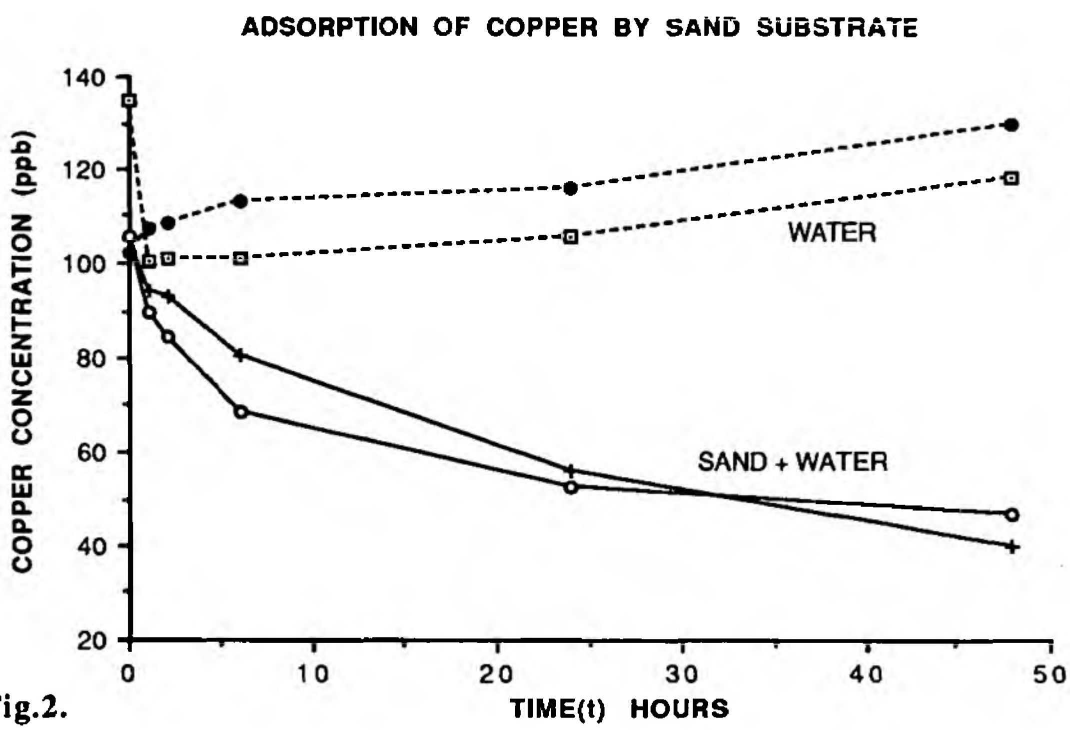
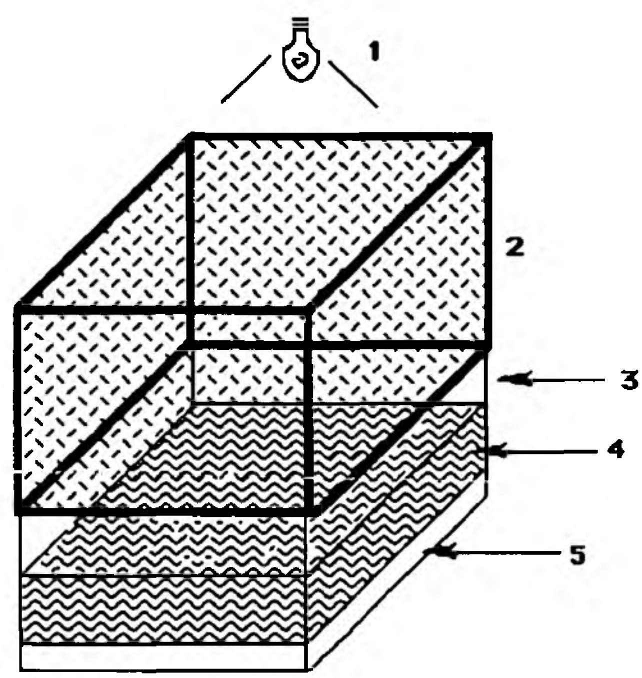


Fig.2.

Specimens of both *Chironomus riparius* and *Chydorus piger* were taken from lab cultures. *Chironomus riparius* were cultured in glass aquaria (50x30x15 cm) topped with gauze cages, kept at 20^{o}C, aerated and given a light regime of 16 hours on, 8 hours off. A 1-2cm deep substrate of cellulose fibres was provided and approx. 5ml of ground trout starter pellet solution (Trouvit and Tetraphyl mixture) was given as food each week (Fig.3.). *Chydorus piger* were reared in aerated conical flasks and provided with sand as substrate/food. Egg-ropes of *Chironomus riparius* were gathered from culture, placed in a small container of aerated lake water and left to hatch for three to four days. Initially, no food was provided during this period. Although, this did not seem to give unhealthy larvae, high mortality in some experiments suggested otherwise. As a result, 1 drop of chironomid food was supplied. This method gives a large number of early instar chironomids all the same age and size.



1 : Light source; 2 : Gauze cage; 3 : Glass aquaria;

4 : Lake Maarseveen I Water; 5 : Cellulose fibres.

Fig.3. Laboratory set up of *Chironomus riparius* cultures

Large mature specimens of *Chydorus piger* were selected from lab cultures. The numbers of such specimens available were low and this was often a limitation on the number of treatments and replicates that could be used in an experiment. Also, not all selected specimens were of exactly the same age or stage of developement. This factor resulted in increased variation between chydorid specimens compared to chironomid specimens.

Measurements

In copper experiments, 3ml samples of water were taken and added to 20µl of concentrated nitric acid in small Eppendorf tubes. In this way the samples can be kept stable until numbers are sufficient for analysis. Duplicate samples were taken at three times (i) after the addition of copper, (ii) before the addition of animals, and (iii) at the end of the experiment. Analysis of copper samples was acheived using flame mode atomic absorption spectrophotometry.

Population growth was used to measure responses by the cladoceran *Chydorus piger*. Numbers counted at the end of the experiment were compared to numbers placed in the vials at the beginning. The two weeks running period allowed time for the chydorids to reproduce. Chironomid larval growth was indicated by comparing the dry weight of larvae taken out of the experiment with the average dry weight of larvae into the experiment, taken from weights of similar larvae from the same egg-rope.

RESULTS

Experiments in this study have looked at the interactions of macro- and meio- benthic organisms (*Chironomus riparius* and *Chydorus piger*) and at the toxicity of copper to these animals. The results of the interaction experiments will be addressed first.

Interaction experiments - Chironomid growth

During preliminary experiments, it became apparent that even with food addition in pre-treatment chironomid survival was uncertain over the two week running time with just sand as food source. High mortality resulted in some experiments. One illustration of this can be seen in Table.1. These results indicate that the sand used provides a very limited food source for early instar chironomid larvae. Also, the total mortality of chironomid larvae in the presence of chydorids, indicates that some interspecific food competition has occurred. It may be suggested then, that there is, partial

TREATMENT	% SURVIVAL OF CHIRONOMID LARVAE
5 CHIRONOMID LARVAE	40
10 CHYDORIDS	_
5 CHIRONOMIDS +10 CHYDORIDS	0

Table.1. Results of preliminary interactions experiment.

overlap of food utilized by *Chironomus riparius* and *Chydorus piger*. It was decided that the chironomid larvae required extra food. In order, to decide what quantity of chironomid food to add a similar interactions experiment was performed with the addition of either one drop or three drops of food. No aeration was used in this experiment. Fig.4(a-c) illustrate the results gained. Individual bars within different treatments in Fig.4(a &b) represent the individual replicates, giving some indication of variation.

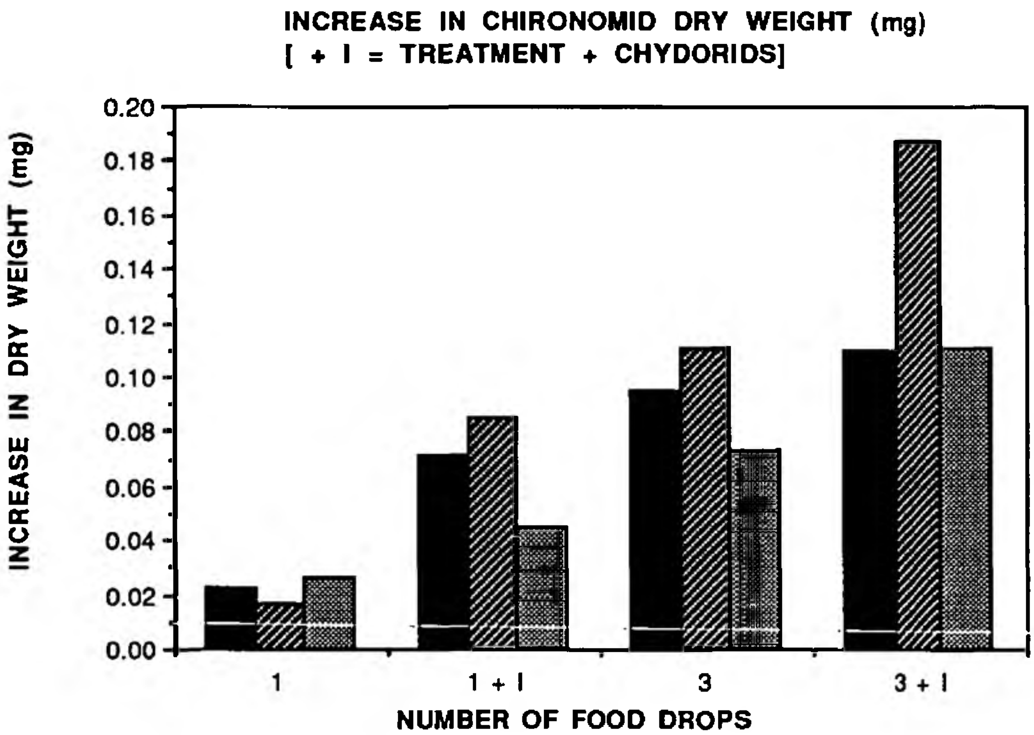


Fig.4(a) Chironomid growth.

It can be seen from Fig.4(a) that chironomid larval growth increases when more food is given, 3 drops as opposed to 1. This, of course, was expected. There is also however, a greater increase in larval dry weight in the treatment with 1 drop of food plus chydorid presence than with just 1 drop of food. This, relationship may also be present when comparing 3 drops + chydorids with 3 drops + no chydorids. It appears therefore, that greater chironomid larval growth occurs when chydorids are present.

Chydorid growth

In this experiment, chydorid growth was very restricted, numbers only increased in a third of the vials (the horizontal lines on Fig.4(b) & (c) represent numbers into the experiment) and more mortality than normal was seen. The variation of the chydorid results is larger, but it is possible to see a relationship between chironomid larval presence and greater chydorid numbers for treatments

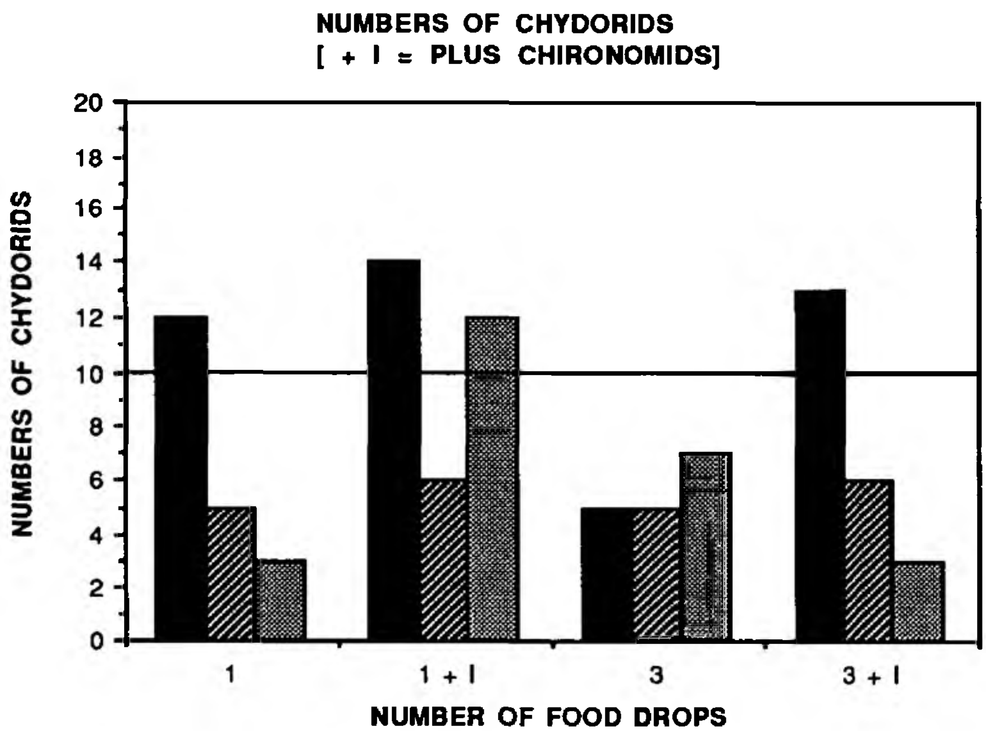
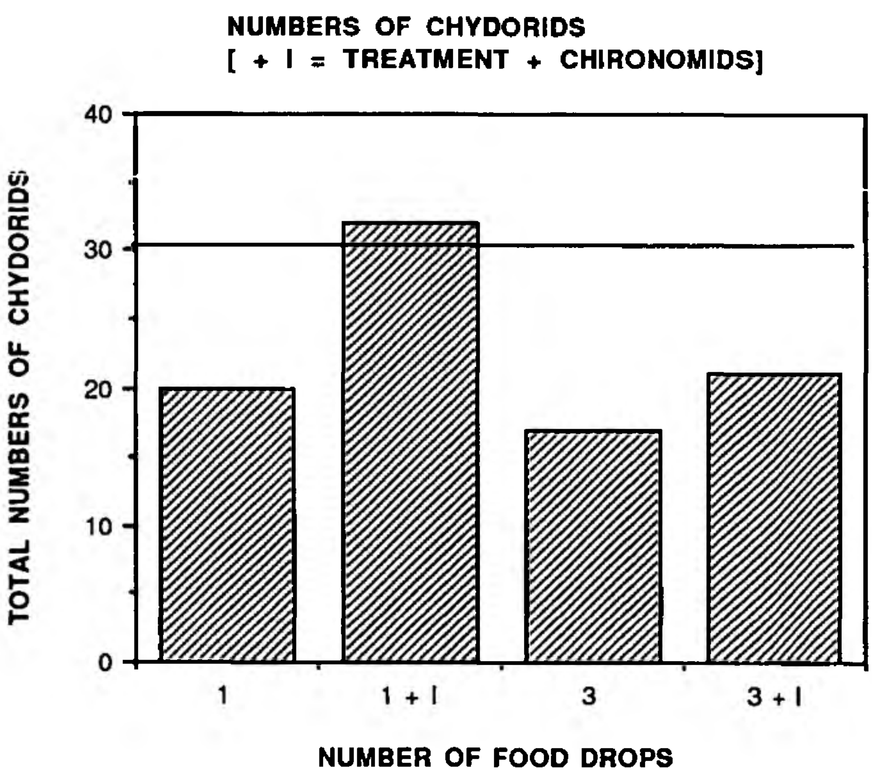


Fig.4(b) & (c) Chydorid growth.



(c)

with 1 drop of chironomid food (Fig.4(b)). This is amplified by Fig.4(c), which shows that the treatment with 1 drop of food + chironomid larvae was the only treatment in which chydorid numbers increased. This would suggest that the chydorids gain benefit from the presence of chironomid larvae.

The low numbers of chydorids achieved are unlikely to be due to the lack of aeration. The small size of the vials means that low oxygen levels would be unlikely to occur. A more likely cause, is that the running time of this experiment was 10 days instead of two weeks. This shorter period does not give enough time for the chydorids to reproduce to the normal extent. Despite, the high variation, that is inevitable with the use of small numbers of animals and replicates, these results suggest that *Chydorus piger* and early instar *Chironomus riparius* larvae gain mutual benefit from each others presence. It shows perhaps, that both species make some food source better available to the other species.

Copper sensitivity experiments

The toxicity of copper to *Chydorus piger* and early instar *Chironomus riparius* was also investigated. In initial experiments, groups of 4 chironomid larvae and seperately, groups of 6 chydorids were exposed in vials to copper concentrations of 100; 500 and 1000 ppb. Control situations (0 ppb Cu) were provided by one of the interactions experiments. Unfortunately, due to high mortality of chironomid larvae in that experiment no control information was obtained for chironomids. Fig.5(a). illustrates the sensitivity of *C. riparius* larvae to copper. The increase in larval dry weight over the running time was used as a measure of growth. Larval mortality was included in this calculation as negative growth. For example, in a treatment in which no chironomid larvae survived the increase in growth would be the negative of the dry weight of larvae into the treatment. Fig.5(a) shows that early instar *C. riparius* larvae exhibit high sensitivity to copper concentrations of 500 ppb or greater. At 1000 ppb mortality out-weighed any growth by surviving animals. Good growth was seen at 100 ppb copper.

The toxicity of copper to chydorids is illustrated in Fig.5(b). *Chydorus piger* appears less sensitive to copper than the chironomids, even at 500 ppb numbers are close to those in the control treatment and at 100 ppb high growth levels are seen. However, at 1000 ppb chydorid numbers are less than control, indicating, that at high copper levels chydorids may suffer reduced or at least, slowed reproduction.

In further experiments involving chironomid sensitivity to copper concentrations of 0; 100; 250; 500 and 1000 ppb, high mortality was seen in all treatments bar the control. Total mortality occured in the 1000 ppb copper treatment. Despite this, there was high larval growth in many of the treatments (Fig.6.). It was observed, that those larvae that survived grow larger than those in treatments with greater survival. This is probably due to a reduction of intraspecific competition, after sensitive individuals have died and increased food provided by their decomposition. This indicates that the sensitivity of individuals of *Chironomus riparius* to toxicity by copper is variable.

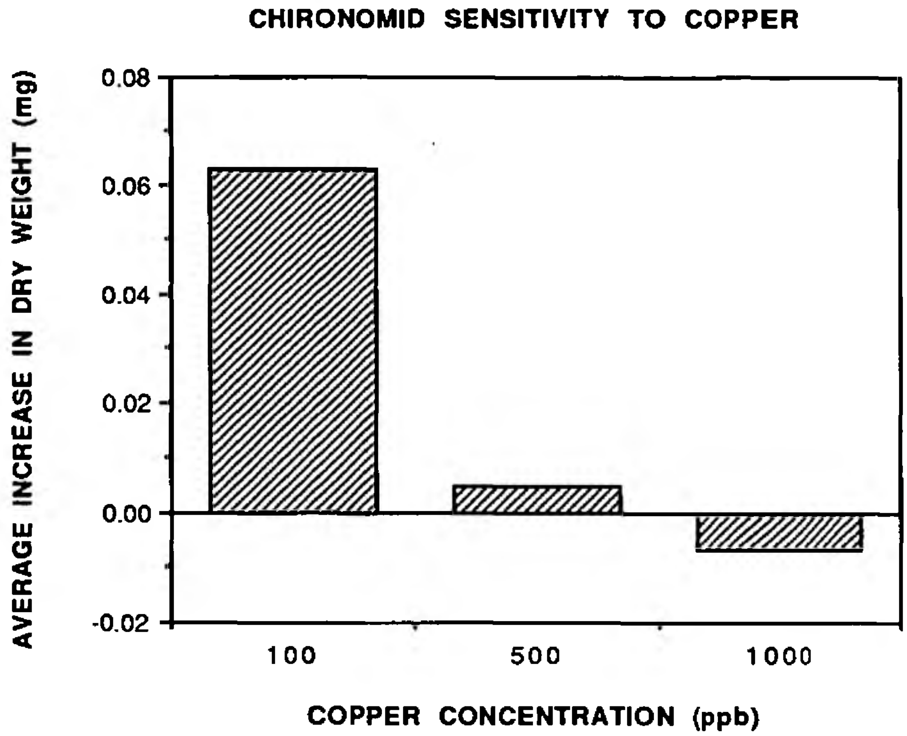

CHIRONOMID SENSITIVITY TO COPPER
AVERAGE INCREASE IN DRY WEIGHT (mg)
0.08
0.06
0.04
0.02
0.00
-0.02
100
500
1000
COPPER CONCENTRATION (ppb)

Fig.5(a)

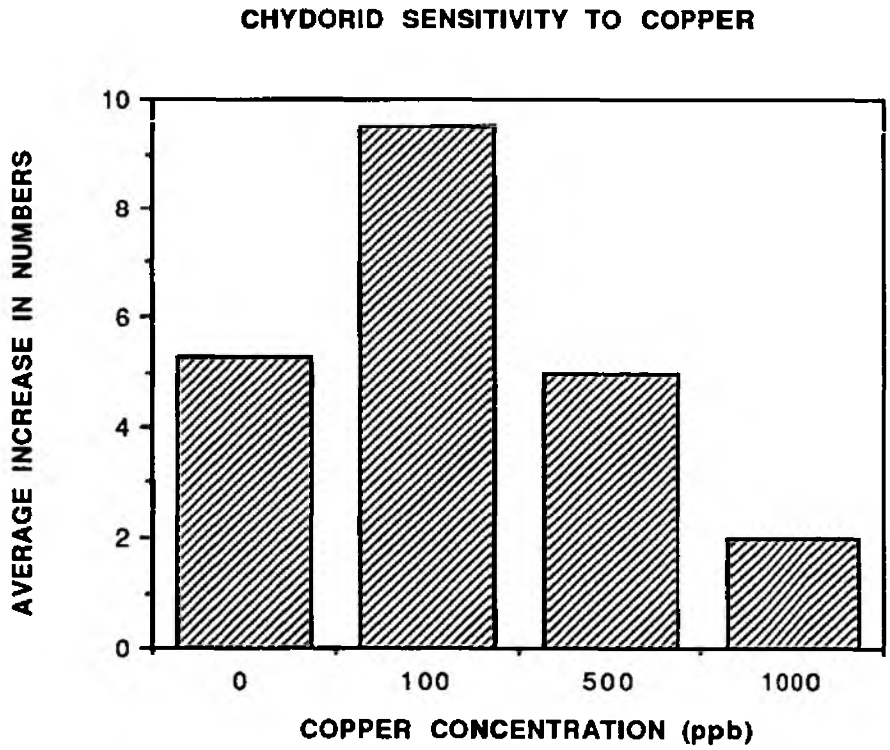

CHYDORID SENSITIVITY TO COPPER
AVERAGE INCREASE IN NUMBERS
10
8
6
4
2
0
0
100
500
1000
COPPER CONCENTRATION (ppb)

Fig.5(b)

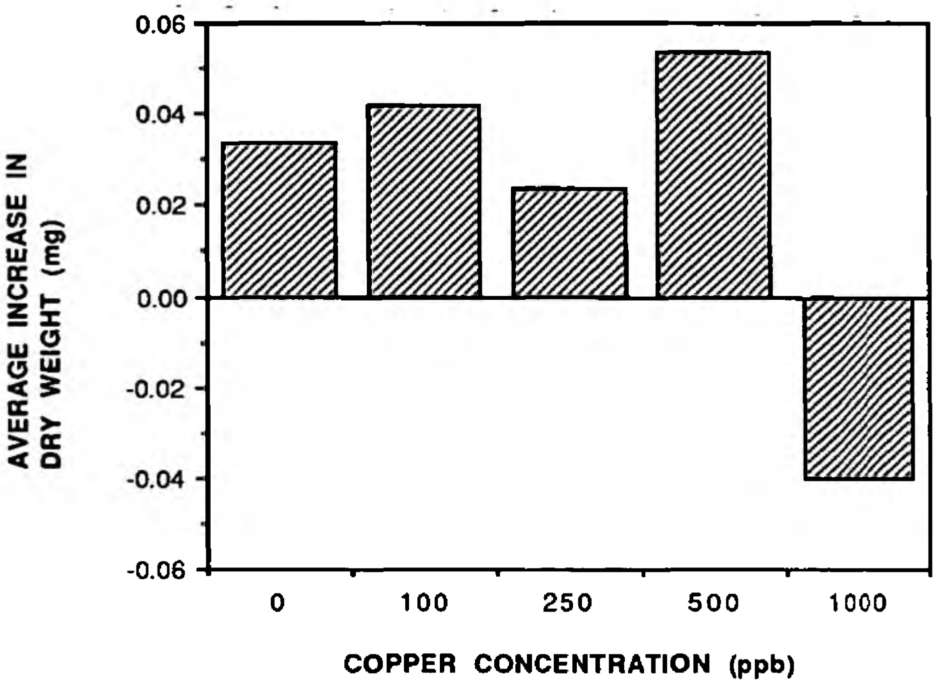


Fig.6.Sensitivity of early instar *Chironomus riparius* larvae to copper.

DISCUSSION

This study has examined the interactions between a macro-benthic species, *Chironomus riparius* and a meio-benthic species, *Chydorus piger* and the effect of copper toxicant on the growth of these two species. The experimental set up used is simple and convienient. Small numbers of organisms are placed in small vessels, allowed time for growth and, in the case of chydorids, reproduction. These factors are then used to quantify between treatments. The major drawback of this set up is the high variation caused by the small number of animals and replicate treatments used. For statistical analysis to be worthwhile many replicates of these experiments would be required. The data obtained however, gives some indication of the interactions and copper sensitivity of the two species.

Toxicants are often administered to organisms via solution. Adsorption of toxicant to the substrate results in the need for continuous flow techniques or renewal of solutions in static tests. In this study the substrate/food source was used to administer the organisms with copper. This utilized the adsorptive ability of the substrate and allowed the use of a static without renewal procedure. It has been reported that the most likely methods of uptake of metal toxicants by chironomids are nutrient uptake in the digestive tract and/or diffusion through the thin chironomid body covering (Timmermans 1991). The effect of a toxicant in solution has been said to be greater than when associated with sediment (Kosalwat and Knight 1987a), but it has also been stated that when relative concentrations of toxicant associated with sediment and water are examined, sediment is often the dominant input (Reynoldson 1987). These statements and the results of this study indicate that the use of substrate/food to administer toxicants to certain organisms is viable.

The high mortality of *C. riparius* larvae which occured in some experiments, indictated that the sand substrate provided could not sustain the larvae for the duration of the test. The food stress that resulted was compounded by the presence of *C. piger* and even lower survival of *C. riparius* occured. This competition effect, indicates that partial overlap of the food spectra of the two species exists. This agrees with the results of similar experiments by van de Bund (van de Bund 1991). Chydorids are known to utilize large particles, such as the sand used here, whilst, chironomids favour smaller particles which they can manage with their mouthparts.

Van de Bund's experiments (van de Bund 1991) suggested that *C.piger* gained benefit from the presence of *C. riparius* larvae. *C. riparius* larval growth was however, reduced by chydorid presence. In the current study, both species appeared to gain benefit from the other's presence. In both studies it is suggested that the chironomid larvae make some food resource better available to the chydorids. In addition, results of the present study indicate that the reverse also occurs. The activity of chironomid larvae is believed to stimulate bacterial production and it is possible that the chydorids take advantage of this bioturbation effect.

This may offer an explaination for chydorid benefit from chironomid presence, but it does not explain the occurence of mutual benefit. A similar case is provided by McLachlan *et al.* (1979) in their paper examining macro/meio-benthos interactions in peat bogs. Peat particles entered the bog lake due to wave erosion of the shore. During settling of the peat from suspension, its calorific and protein content has increased by 23% and 200% repectively. This is due to colonization by micro-organisms. The particles are eaten by the dominant midge, *Chironomus lugubris*. After passage through the gut of the chironomid larvae, the rate of oxygen consumption of the peat (now in the form of faecal pellets) has risen by greater than 87%. This change, presumably due to a further increase in microbial activity, suggests that the faecal pellets are potentially valuble as food. Despite their being a richer food source, *C. lugubris* showed no interest in the faecal pellets, except for tube building. The pellets proved too large to be eaten whole and too hard to be broken down by weak mandibles. Additionally, a change in dominant micro-organisms, from bacteria on the peat particles, to fungi on the pellets, may be a contributing factor. The chydorid, *Chydorus sphaericus* is common in the lake and was shown to depend on the faecal pellets of *C. lugubris* for food. *C. sphaericus* clasp the faecal pellets just inside the valve of the carapace and rotate it, whilst grazing from its surface. This feeding action results in the fragmentation of the pellets. These fragments are greater in nutritional value than the whole pellets, indicated by a rise in bacterial numbers of about

300%. The broken pellets could then provide a rich food source for the *C. lugubris* larvae. The interaction between the two species may therefore, be mutually beneficial.

In this study, tests concerning the effect of copper on *Chironomus riparius* have concentrated on early instar larvae. It is widely reported that the first instars of chironomid species are the most sensitive life stage (Gauss, Woods *et al.* 1985 , Powlesland and George 1986 , Timmermans 1991), and thus, their use provides a good indication of whether stress is occuring. Larval growth has been widely used as a measure of toxicity to chironomids. In the present study, early instar *C. riparius* larvae have been shown to suffer reduced larval growth and high mortality at copper levels of 500 ppb or greater. From literature, larval growth has been reported impaired by copper concentrations of 0.09 mg/l, 1.0 mg/l and 0.64 mg/l for *C.riparius* , *C. decorus* and *Paratanytarsus parthenogeneticus* repectively (Timmermans 1991 , Kosalwat and Knight 1987a , Hatakeyama and Yasuno 1981). Much work concerning the toxicity of a variety of metals to chironomid species has been done. Details of this work is reviewed in Chapter 1.

The higher concentrations of copper used resulted in mortality of chironomid larvae, but those individuals that survived increased in dry weight more than larvae in treatments where low mortality occured. The most likely explaination is that some individuals are more tolerant of copper contamination. These individuals often being the sole survivors in a treatment have no competition for the food source and can grow faster. This variation, even between larvae from the same egg-rope, would suggest that a population exposed to a metal contaminant over several generations would become tolerant. Evidence of this has been recorded for a *C. tentans* population exposed to sediments contaminated with cadmium, chromium and zinc (Wentsel, McIntosh *et al.* 1978).

The sensitivity of chydorid species to metal contaminants has recieved little attention. In the present study, *Chydorus piger* appears to be more tolerant of copper than *Chironomus riparius*, but at copper concentrations of 500 ppb or greater may suffer reduced or slowed reproduction. Casual observation, showed that chydorids in the higher concentration treatments appeared to increase their numbers later than those in lower concentrations, but caught up by the end of the experimental period. Survival of young chydorids born into copper contaminated vials appears not to be reduced.

Experiments examining the effect of copper contamination on the interactions of *Chydorus piger* and *Chironomus riparius* , and whether these effects can be predicted from the stress of copper on the individual species, are planned by Wouter van de Bund, University of Amsterdam.

SUMMARY OF CONCLUSIONS

- Substrate will adsorb copper from solution, and can therefore be used to administer the contaminant to benthic species.
- Partial overlap of the food spectra of *Chydorus piger* and *Chironomus riparius* has been indicated.
- Under certain conditions however, both species can gain mutual benefit from each others presence.
- Early instar *C. riparius* larvae show high sensitivity to 500 ppb copper or greater, suffering high mortality. Some individuals appear tolerant.
- *C. piger* appear less sensitve to copper, but at 500 ppb or greater may suffer reduced or slowed reproduction.

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Ferric chloride dosing for phosphate inactivation, at two lakes in the Netherlands.

ABSTRACT

In addition, to the research study detailed in the previous section, two ferric dosed lakes in the Netherlands were visited and some sampling of sediments was carried out. The mechanisms of ferric dosing at each lake has been described. Some indication that high levels of ferric associated with sediments can have a profound effect on benthic invertebrate communities was obtained.

INTRODUCTION

High phosphate levels present in eutrophic lakes and rivers have resulted in nuisance algal blooms. These cause difficulties in supply water treatment and in extreme cases, toxins produced by algal blooms can be a danger to lake/river users. In order to combat nuisance blooms, lakes and rivers have been dosed with substances which remove available phosphates from the water. This action, is intended to limit the growth of the algae. Ferric compounds, e.g. ferric chloride and ferric sulphate, are often used as phosphate inactivants. Some discussion of the use and effectiveness of phosphate inactivation, using ferric compounds and other inactivants, can be found in Chapter 1. The lakes visited have both been treated with ferric chloride *via* their inlet waters. The reported success of these dosing schemes and the results of sediment sampling will be discussed in this section.

Both lakes are part of a series of lake systems situated between Amsterdam and Utrecht in the centre of the Netherlands (Fig.1.). Lake Botshol is part of the Vinkeveense plassen west of the Amsterdam-Rhine canal. The Loosdrecht Lakes are to the east of this canal.

LAKE BOTSHOL

Lake Botshol is a nature reserve run by a water company, which carry out regular water chemistry tests on the lake. From April to September 1990, the lake was dosed with ferric chloride for 131 days *via* the inlet water. The average quantity of inactivant entering the lake was 7.7 g $FeCl_3$ m^{-3} inlet water. The ferric/phosphate floc, formed as a reult of the dosing, is allowed to sediment out prior to reaching the main lake. The sedimentation area is made up by the alternating strips of land and water ('zodden') formed by peat digging and by artificial barriers put in by the water company. The result is a convoluted water channel maximising the length of water between the inlet and the lake (see Fig.2.). The sedimentation area is open to the main lake.

Prior to dosing, Botshol had a salinity gradient from brackish to freshwater. Since dosing the water is brackish over the whole area. This is probably due to the increse of chloride ions from dosing. The water company seem satisfied that dosing with ferric has significantly reduced the amount of phosphate in the lake. Fig.3. is a graph of ortho-phosphate measurements taken by the water company. A decline in o-P levels can be seen after the end of ferric dosing. Information concerning the long term effectiveness of this procedure is not yet available.

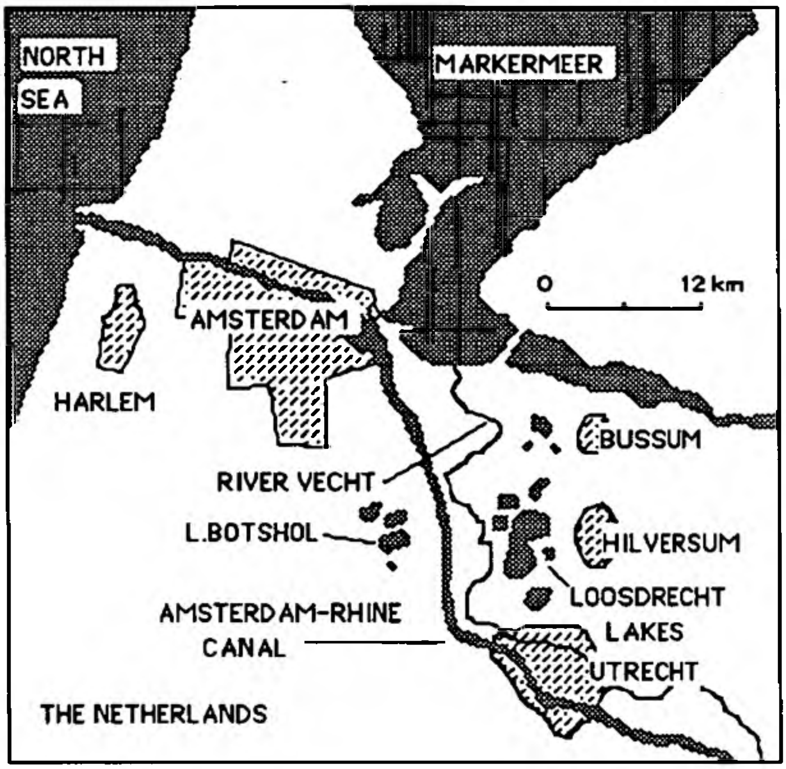

Fig.1. Map showing the position of Lake Botshol and the Loosdrecht Lakes.

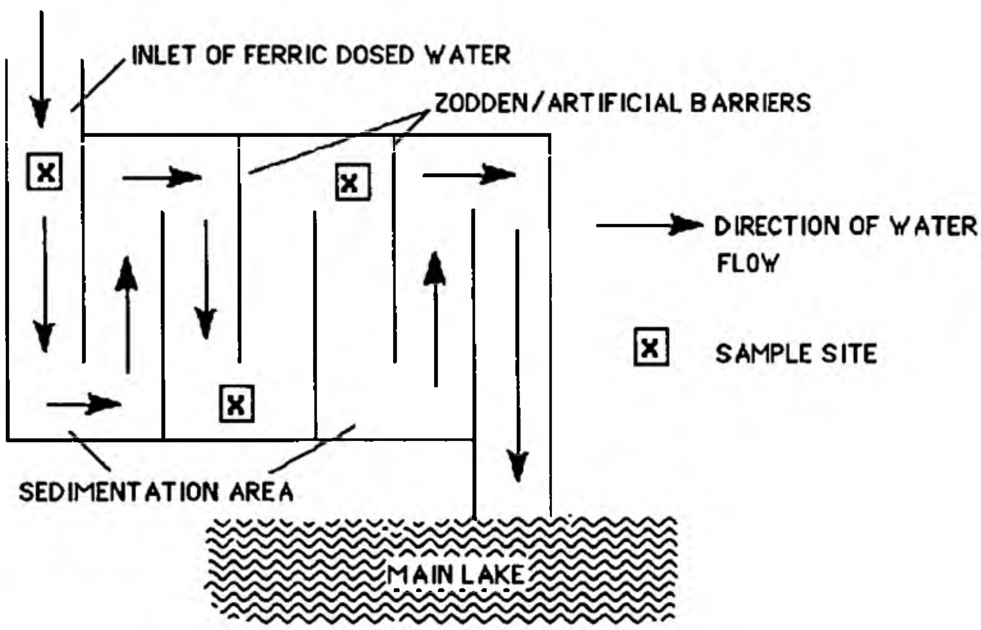

Fig.2. Schematic diagram showing the mechanism of ferric dosing at Lake Botshol and the approximate postion of grab sample sites.

Three sample sites were chosen; one close to the inlet of ferric dosed water, one close to the lake and one intermediate site. Five grabs were taken from each site. Samples were taken back to the lab, where animals were removed by sieving and salt preparations, killed in formaldehide and identified. Just five specimens (2 *Tanypus kraatzi* ; 1 *Procladius sp.*; 1 oligochaete and 1 nemotode) were found from fifteen grab samples. This almost total lack of animals was unexpected. The mud in the samples was very anoxic, but this would not account for so few organisms being present. It is impossible to make any firm conclusions from these results without further sampling and chemical analysis of the water and sediments. The suggestion is however, that the use of ferric chloride and its linked salinity changes is having a profound effect on benthic organisms, at least within the sedimentation area.

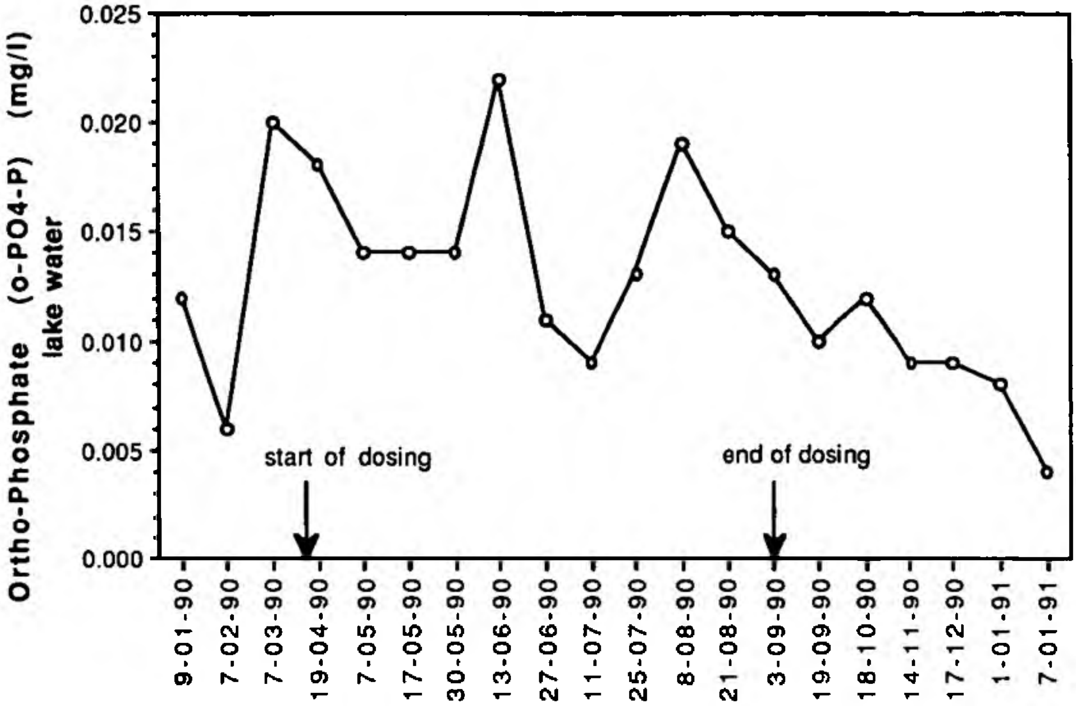

Fig.3. Graph of fluctuations in ortho-phosphate levels at Lake Botshol (Data supplied by K.Everards).

THE LOOSDRECHT LAKES

The Loosdrecht Lakes have a negative water budget due to evaporative water loss exceeding precipitation and net seepage loss to surrounding polder country. To compensate for this deficit, water from the polluted River Vecht was drawn into the lake in the summer. In mid-1984 the inflow from the river was replaced by $FeCl_3$-treated water from the Amsterdam-Rhine canal. This has led to a decrease in the annual external phosphate loading to 0.3 g m^{-2} $year^{-1}$, mainly in the summer (Gulati 1990). This reduction may not have been as successful as possible, due to not all the sources of phosphate having been eliminated.

At Loosdrecht, inlets from the polder and the Amsterdam-Rhine canal are dosed with ferric chloride. Information concerning the period of dosing and the quantities of inactivant in use, are unavailable to me. Fig.4. illustrates the mechanisms of dosing employed at Loosdrecht. Water from the polder is dosed with ferric chloride at its inlet into a sedimentation pond. From there it is drawn into a further sedimentation pond and from there drawn into the lake system. Water from the Amsterdam-Rhine canal is pumped into a convoluted sedimentation area very similar to that at Botshol. Both sedimentation areas have water drawn from them into the lakes and are thus, closed to the lakes. This is unlike the open system at Botshol.

The approximate positions of sample sites are shown in Fig.4. Three grab samples were taken at each site. Analysis of the samples was similar to that used for the Botshol samples.

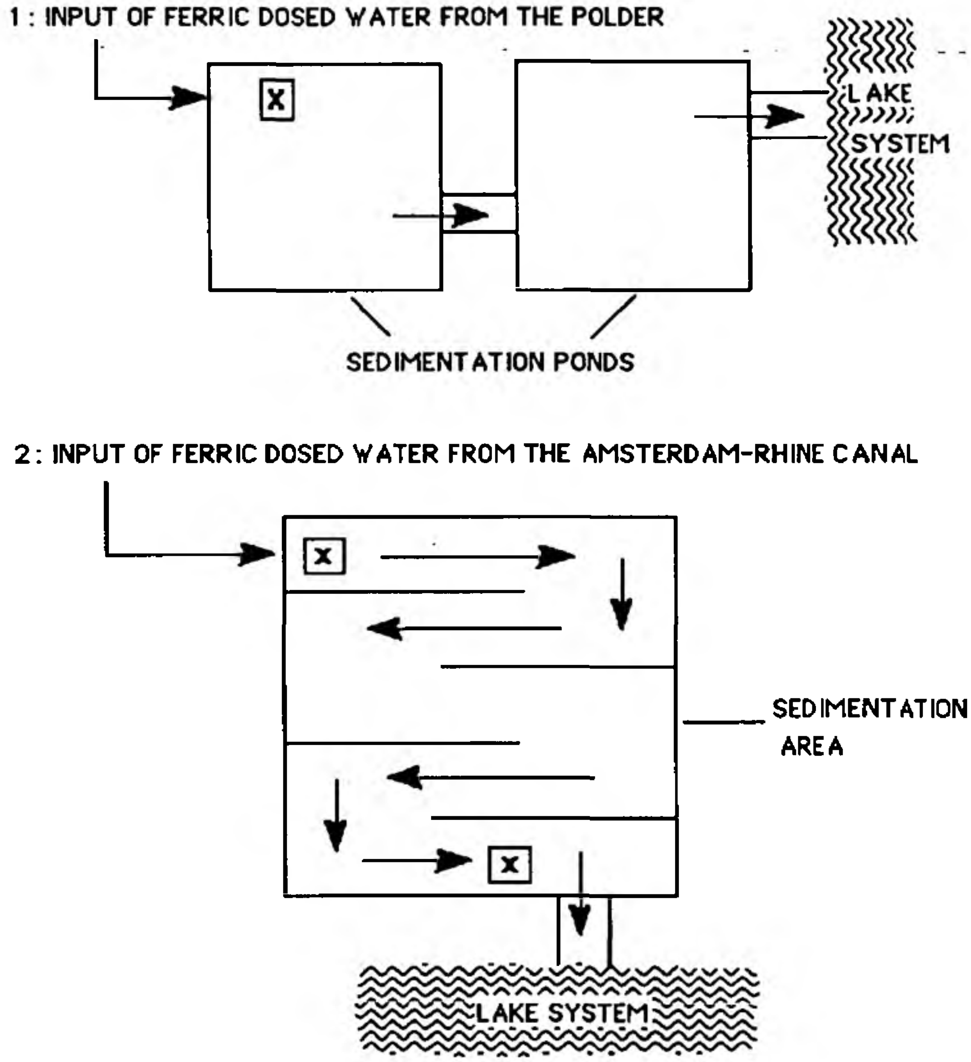

Fig.4. Schematic diagrams showing the mechanism of ferric dosing at the Loosdrecht Lakes and the approximate positions of sample sites.

Orange ferric deposits could clearly be seen in the first sedimentation pond for water from the polder. The mud underlying these deposits was black and anoxic. The samples from this site yielded many Cyclopoids and one *Clinotanypus nervosus* larvae. The former are more likely to be associated with the water rather than the sediment. The second sample site was situated close to the inlet of water from the Amsterdam-Rhine canal. This, like site 1, was picked as a ferric polluted site. Black mud and leaf litter overlay sand substrate at this site. The benthic organisms found mirror the different substrates present. Table 1. lists the genera found, the numbers found and the substrate type they prefer.

The third site, near the outlet of water from the sedimentation area to the lakes was mainly of soft sediment type and the organisms present confirm this. The genera found and the numbers present are listed here: Oligochaetes (8), *Chironomus* (1), *Polypedilum* (2), *Macropelopia* (1), *Tanypus* (2). As at Botshol, it is pointless to base conclusions on just one set of samples. It is noticable however, that the yield of animals was small at all the sites. There were practically zero animals found at site 1. Whilst, it can not be concluded that the very high, visible, content of ferric in the surface sediment of site 1 is the cause of this deficiency, it must be a strong possibility. The high sedimentation present in such a small area may also be a factor inhibiting growth at this site. No clear difference can be seen, between the genera found at sites 2 & 3 that can not be explained by differences in substrate.

Table 1. Benthic genera occuring at site 2.

GENERA	NUMBERS	PREFERED SUBSTRATE
Oligochaetes	12	soft sediments
Chironomus	9	" "
Polypedilum	5	" "
Tanytarsus	2	variable
Orthocladius	2	"
Cryptochironomus	1	variable (particularly sandy)
Endochironomus	1	aufwuchs, vegetation
Glycotendipes	1	" "
TOTAL	33	

SUMMARY

Both of the lakes visited have been dosed with ferric chloride to reduce ortho-phosphate levels and thus, combat nuisance algal blooms. Dosed water is passed through sedimentation areas, which allow ferric/phosphate flocs to settle out prior to entering the main lake. At Botshol these areas are open to the lake, at Loosdrecht water is drawn from sedimentation areas into the lakes. In both cases a reduction in external loading of phosphate has been seen.

The sediment in the sedimentation areas can be expected to have high ferric content. In one case orange deposits were clearly visible. Samples from these areas revealed that the numbers of animals present is often much smaller than would be expected. Whilst firm conclusions can not be made, the addition of ferric would seem the most likely cause of these effects. Changes in salinity and high levels of sedimentation, linked to the dosing, may also be factors. This may have serious implications where dosed inlets enter directly into lakes. It is clear, that further study of the effects of ferric dosing on benthic organisms is needed.

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Laboratory Cultures of the midge *Chironomus riparius*, Meigen at Leicester.

Laboratory cultures of *Chironomus riparius* have been set up along similar lines to the cultures used during my study period in Amsterdam (see Fig.3., page 24). The egg-ropes used to start the cultures were provided by Dr. David Pascoe, from existing cultures at the University of Wales, Cardiff. *C.riparius* is a littoral/riverine species and is among a few chironomid species that can be cultured. Adults of other species often require large volumes for swarming behaviours essential to mating success, and are thus impractical to culture in the laboratory. Chironomid cultures have been used extensively in both acute and chronic toxicity testing, e.g. (Kosalwat and Knight 1987a; Pascoe, Williams *et al.* 1989; Timmermans 1991). The cultures are easy to maintain, have a fast generation time (*C.riparius*; 25 days at 20°C) and can provide large numbers of chironomids at any required life stage. Chironomids provided by such a method are therefore, very convienient for experimental use.

I have set up cultures of *C. riparius* for use in acute and chronic tests concerning ferric toxicity. I hope to gain some insight into the probable effects of ferric dosing (as used on Rutland Water) on chironomids as representatives of lake benthos. Whilst, the use of a related species can be useful to gauge the effects of a contaminant, it is also intended that cultures should in time be set up of chironomid species that are common as part of the benthic fauna of Rutland Water. The success of such cultures will of course, depend on the practicalities mentioned above.

Practical details

The cultures are set up in plastic aquaria (approx. 46x26x25 cm). A muslin cage is fitted to the top of the aquaria, this allows adult midges some area in which to fly without letting them escape to the surrounding room. Light is provided to a 16 hour on, 8 hour off regime. Cultures are kept in a constant temperature room at 20°C. Filtered lake water (from Rutland Water) is provided to a depth of approx. 10cm and is constantly aerated. Torn up kitchen paper is used as substrate, the procedure is as follows: Rinse with acetone, thoroughly rinse with distilled water, boil for four hours, rinse with distilled water, bake for $1^1/_2$ hours at 90°C, the last two steps are repeated three times. The resulting mulch is placed in the tanks to a depth of 2-3 cm. The method described above is time consuming and difficult. In the future, a shorter, more convienient method is being considered. At the University College Cardiff, filter paper (Whatman No.1) is used, this is made into a mulch using a food blender, this is then directly supplied as substrate for the cultures. For food, 5 ml of a ground suspension of *Xenopus* food is provided, once a week to each culture.

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Chapter 3 - Proposed ideas

Proposed ideas for the study of the effects of ferric dosing on benthic invertebrates.

INTRODUCTION

Rutland Water in Leicestershire has in recent years been dosed with ferric sulphate, in order to inactivate phosphate and thus reduce nuisance blooms of cyanobacteria. In conjunction with other studies, run by Leicester University and the National Rivers Authority, this project is concerned with monitoring the effects of the dosing scheme. In particular, my project concerns the effect of ferric on the benthic invertebrate fauna.

Dosing with ferric compounds produces a floc with ortho-phosphate, which settles out to the lake sediment. The benthic organisms associated with the sediment are therefore likely to come into contact with large amounts of ferric floc. Uptake of ferric by these organisms is likely to occur, potentially resulting in profound effects. Ferric is an important natural constituent of lake sediments and is involved with the transfer of phosphate and other substances to and from the sediment. It is probable that for this reason it has seldom been looked at in terms of its toxic effect to benthic organisms. The toxicity of other metals (e.g. cadmium, zinc, lead and copper) to benthic species, particularly chironomids, has however, been well recorded (see Chapter 1). With the onset of ferric dosing, it is now necessary to view excess ferric as a contaminant and to research possible detrimental effects from its addition. The plans I have for research into this area will be detailed in this chapter.

PROJECT IDEAS

Toxicity testing

Different benthic species will have different sensitivities to contamination by excess ferric. A variety of lethal and sub-lethal effects may therefore, be expected. The quantities and the methods by which different species come into contact with the ferric will influence this. Initially, it has been decided to concentrate on chironomids. These represent one of the dominant benthic groups. Chironomids are closely associated with sediments, many species are detritivores and utilize the sediments for food, some are predatory and eat other sediment dwellers.

As has been mentioned in Chapter 1, much work on the toxicity of metals to chironomids has been done. In brief summary, this work has shown that the first instar chironomid larvae are the most sensitive life stage to metal contamination, often suffering large mortalities, reduced larval growth and delayed development (Kosalwat and Knight 1987; Pascoe, Williams *et al.* 1989; Powlesland and George 1986; Timmermans 1991; Wentsel, McIntosh *et al.* 1978). Lethal and sub-lethal effects on these early instar larvae are reflected in reduced adult emergence (Timmermans 1991). Under acute toxicity conditions lethal effects can be seen in any of the life stages if the concentration is high enough. With lower concentrations, in longer term chronic tests, a range of sub-lethal effects have been recorded. Egg-ropes laid into contamination appear to have reduced hatching success (Williams, Green *et al.* 1987). Larvae with abnormal mouthparts have been reported (Kosalwat and Knight 1987). Larval growth is reduced and development delayed even at seemingly low concentrations. The percentage of adult midges emerging is reduced with partial life cycle exposure (Timmermans 1991). Behaviour may also be affected. Adult females apparently show a preference for uncontaminated sites for egg laying (Williams, Green *et al.* 1987). It is clear, that much information can be gleaned by the use of acute and chronic toxicity testing, particularly the use of partial life-cycle assays.

The studies mentioned above, have concentrated on metal contaminants other than ferric. It would be of interest, to use procedures similar to those used in these studies, to investigate the effect of ferric contamination on chironomid species. Acute tests on different life stages could measure the sensitivity range of the organisms to ferric. This information combined with the actual ferric levels encountered in the lake situation, can be used to produce worthwhile chronic and partial life-cycle tests. These could be used to yield information of lethal and sub-lethal effects, measuring parameters such as % hatch, early instar mortality, larval growth, developemental time, % emergence, reproductive success and behaviour.

As a tool, for the provision of large numbers of chironomids of whatever life stage, culture set ups are widely used. Cultures of the littoral/riverine species *Chironomus riparius* have been set up for use in this study. *C. riparius* is one of the few species which so far have been cultured. A generation time of just 25 days at 20°C makes this organism ideal for short and longer term metal toxicity studies. To this end it has been used substantially, and its use in this study should give some indication of the effects to chironomids of ferric contamination. In addition, to experiments with *C. riparius*, it is intended that some attempt should be made to investigate ferric dosing in relation to chironomid species present in the fauna of Rutland Water. Ideally, cultures of these species would be set up, allowing a full range of tests to be performed. The complex swarming behaviours of some species make this impractical. If the Rutland species fall into this group then the range of tests may be limited. Also, cultures of profundal species may require lower temperatures and the length of their life cycles may prove inhibitary to partial life-cycle tests.

Benthic sampling

The dosing scheme at Rutland Water has been carried out *via* the inlet into the south arm, or when this has not been possible over the side of a barge, again in the south arm. Any effects in terms of faunal diversity and species composition are likely to be already visible in the south arm. In the north arm, however, the problems are likely to be less pronounced. The north arm may, therefore, prove useful for comparison with the dosed south arm. Both the National Rivers Authority (NRA) and Anglian Water, currently have benthic sampling programmes at Rutland Water. The NRA's programme involves grab samples taken along three transects, one in the south arm, one in the north arm and one across the basin. Some littoral sampling is also undertaken. Identification of the samples is being taken as far as family and the samples have all been kept. The results of this study are not yet in my possession, however, the preserved samples are to be made available to me. It may prove interesting to take the identification of the samples beyond family, to genera or species, in order to more closely compare between the three transects. This may identify species which have suffered from ferric addition. These species could then be concentrated upon in further toxicity testing.

Ferric loading

The actual amounts of ferric entering the reservoir, the duration of dosing and the precise methods used, are secrets presently guarded by Anglian Water. The levels of ferric in water, sediments and organisms can, however, be ascertained using a variety of techniques all culminating in atomic absorption spectrophotometry. In addition, these techniques may provide information concerning the methods by which organisms take up ferric, and whether they can regulate the uptake. Work in this area may prove interesting later in the study.

Summary and proposed timescales.

- Cultures of *Chironomus riparius* set up and maintained (Sept. '91 onwards).
- Toxicity testing of ferric on *C. riparius*, involving acute and chronic tests (Oct. '91- early '92).
- Cultures and toxicity testing of dominant Rutland Water chironomids (Mar./Apr.'92- onwards).
- Further examination of NRA samples, for comparison of dosed and non-dosed sites (Early '92 onwards).
- Ferric content of water, sediments and organisms (Late '91 onwards).

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	Stephan Spaas
	Klass Everards

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