Ecotoxicological Impact of Ferric Sulphate on Chironomid Cultures and Profundal Reservoir Communities

N P Radford
University of Leicester

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Kingfisher House
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Orton Goldhay
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PE2 5ZR

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N P Radford
School of Biological Sciences
University of Leicester
University Road
Leicester
LE1 7RH

NRA Project Leader
The NRA Project Leader Anglian Region OI Project 534 was:

Dr Bill Brierley - NRA Anglian Region, Northern Area

Further Information:
Further information relating to this document may be obtained from the Anglian Region R&D Coordinator in our Peterborough Office.

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EXECUTIVE SUMMARY

- Information concerning the potential toxicity of iron pollution in the aquatic environment is limited. Deleterious effects of deposited iron precipitates are particularly poorly studied.
- The use of in-reservoir ferric sulphate dosing as a phosphate inactivation measure introduces large amounts of iron to the system. Most of this iron accumulates in precipitate form overlying the natural reservoir sediment.
- Appraisal of the effects of prolonged exposure to deposited iron precipitates and whether effects are due to direct toxicity or indirect physical impedance were the principal aims of the study.
- The study has concentrated on changes in chironomid community struacture and on laboratory toxicity tests exposing *Chironomus riparius* to iron precipitates.
- The abundance and diversity of chironomid communities was reduced in areas coincident with the presence of deposited iron precipitates in Rutland Water. The Tanytarsini tribe and *Procladius* sp., dominant elsewhere in Rutland Water, were largely absent from sites affected by iron deposition.
- In the laboratory study deposited iron precipitates did not effect mortality of *C. riparius* at target iron concentrations ≤ 75 mg litre^{-1} at circumneutral pH.
- The presence of deposited iron precipitates significantly retarded larval growth and development at target iron concentrations ≥ 30 mg litre^{-1}.
- This was reflected by delays in adult emergence of 6 days at 30 mg Fe litre^{-1} and 18 days at 50 mg Fe litre^{-1} within a 25 – 30 day life cycle. Such delays represent major deviations from normal life history patterns.
- Total iron content of *C. riparius* larvae increased significantly with increasing target iron concentration.
- Iron associated with gut contents accounted for the majority of the total larval iron content, indicating that iron uptake by larvae was principally by ingestion of precipitates.
- It seems probable that ingestion of precipitates leads to reduced energy intake per unit time and so leads to developmental retardation.
- Such a mechanism could potentially apply to any organism that lives in contact with deposited iron precipitates and is able to ingest them. Predatory organisms may be affected indirectly via loss of prey species.
- Deposition of iron precipitates is therefore likely to have a deleterious effect on benthic invertebrate communities.
- The dosing regime used and chemical and physical factors of each dosed reservoir will affect the amount of precipitate formed and the amount reservoir sediment overlain by precipitates. It is difficult therefore to identify a universally 'safe' dosant concentration. The only simple recommendation is that deposition of iron be avoided in lakes and reservoirs.
- The environmental impact of iron precipitate deposition is clear from this study but the potential impact of toxic impurities should also be investigated before the use of in-reservoir ferric sulphate dosing is considered for other eutrophic reservoirs.

Keywords: Ferric sulphate dosing; Deposited iron precipitates; Toxicity; Iron uptake; Chironomids; *Chironomus riparius*; In-reservoir eutrophication control.

ECOTOXICOLOGICAL IMPACT OF FERRIC SULPHATE ON CHIRONOMID CULTURES AND PROFUNDAL RESERVOIR COMMUNITIES

1. GENERAL INTRODUCTION

Eutrophication (nutrient enrichment of aquatic ecosystems) has escalated in recent years, principally due to the use of fertilisers in modern farming methods and the discharge of sewage works effluent. The usual response to enhanced nutrient levels in lotic ecosystems is elevated phytoplankton growth which can lead to shading out of aquatic plants, oxygen depletion of hypolimnia and severe problems for the treatment of water for supply (Hayes and Greene, 1984; Moss, 1988; Harper, 1992). It is generally recognised that long-term control of eutrophication requires the limitation of nutrient supply. Phosphorus, rather than nitrogen, is deemed easier to limit and so most schemes for eutrophication control aim to reduce phosphate input from external sources or remove internal phosphorus loadings. The former are necessary as an initial step but recovery of the system can be delayed by recycling of phosphates previously accumulated. Sediments are of particular importance in this respect (Osborne and Phillips, 1978; Phillips and Jackson, 1990). Chemical treatment to remove available phosphates from the water column and/or to prevent internal loading from the sediment is the basis of most schemes. Iron salts are most commonly used for this purpose.

In aquatic systems the cycling of iron affects the accumulation of nutrients to and their release from the sediments. The oxidised iron III form is present in oxic conditions and precipitates readily binding organic acids and inorganic anions such as phosphates and silicates. In anoxic conditions iron III is reduced to the iron II form and releases bound compounds (Mortimer, 1941; Mortimer, 1942; Davison and Tipping, 1984).

Iron occurs most commonly as a contaminant in drainage from disused mine workings. Where these discharges are acidic, toxicity from low pH and dissolved iron can occur (Mance and Campbell, 1988). Where neutral, the presence of suspended or sedimented ferric precipitates coincides with reduced macroinvertebrate abundance and diversity (Letterman and Mitsch, 1978; Scullion and Edwards, 1980b). Fish biomass is also low at these sites possibly due to the reduction of invertebrate food (Letterman and Mitsch, 1978; Scullion and Edwards, 1980a). An Environmental Quality Standard (EQS) for the protection of freshwater life of 2 mg total iron $litre^{-1}$ is recommended by the Water Research Centre. Avoidance of iron deposition is also suggested in the report (Mance and Campbell, 1988).

The concern of this study is that the use of large quantities of ferric salts for in-reservoir phosphate inactivation may have a serious detrimental impact on the receiving system. The study has concentrated on changes in chironomid community structure and on toxicity tests exposing laboratory cultures of *Chironomus riparius* (Chironomidae) to iron precipitates. Chironomids are burrowing detritivores and are liable to come into direct contact with the ferric floc which results from ferric dosing. Appraisal of the effects of prolonged exposure to ferric precipitates in terms of mortality and sub-lethal parameters and whether effects are due to direct toxicity or indirect physical impedance are the principal aims of the study.

2. FIELD STUDY OF THE IMPACT OF FERRIC DOSING AT RUTLAND WATER, LEICESTERSHIRE ON THE DISTRIBUTION OF CHIRONOMIDS.

2.1 Introduction

Rutland Water is a lowland pump storage reservoir of high amenity value. The occurrence of large nuisance blooms of algae, particularly toxic cyanobacteria, are a consequence of the eutrophic status of the reservoir. In order to combat nuisance algal blooms Anglian Water Services have employed the use of ferric sulphate as a coagulant for phosphate precipitation to the sediments (since June 1990). Dosing of ferric sulphate is made direct to the reservoir via its submerged inlet.

Field monitoring of the impact of the dosing scheme has been carried out by the National Rivers Authority since the summer of 1990 (Champion, *et al.*, 1991; Extence, *et al.*, 1992). Preserved samples of chironomid larvae from bi-monthly profundal benthic grid surveys (Dec. '91 – Nov. '92) were sent to the author for identification beyond family level.

2.2 Procedure

The positions of the National Rivers Authority grid survey sites at Rutland Water are shown in Figure 1. Samples from the December 1991 grid survey were identified to genus. The clearing and mounting procedures used are described by Extence *et al.* (1992) and Radford (1994). Chironomid larvae from subsequent grid surveys (Feb. '92 – Nov. '92) were separated into four easily distinguished groups; *Chironomus*, *Procladius*, Tribe Tanytarsini and 'others'. This scheme avoids time-consuming preparation of specimens, whilst allowing identification of the dominant chironomid taxa.

The similarity between sites on each sampling date was examined using the Bray-Curtis Coefficient (Bray and Curtis, 1957). Calculation of coefficients and cluster analysis of the resulting matrices were performed using a suite of IBM PC programs called PRIMER (Plymouth Routines in Multivariate Ecological Research).

2.3 Results and Discussion

Densities of chironomid genera found in the December 1991 grid survey are presented by Extence *et al.* (1992) and Radford (1994). Densities of chironomid taxa in subsequent grid surveys are shown in Figure 2. The abundance and diversity of chironomid larvae at the Slipway and Inlet sites were lower than elsewhere in Rutland Water from all the grid surveys examined. Chironomid communities were dominated by *Procladius* sp. and the Tanytarsini tribe at the majority of sample sites. These groups appeared to be largely absent from sites close to the inlet of ferric dosed water. Cluster analysis (a dendrogram of the November 1992 grid survey is presented in Figure 3) indicates that the inlet site and sites along the Slipway transect were significantly dissimilar ($p \leq 0.002$, ANOSIM Global R test statistic) from the other sample sites at Rutland Water. Close to the inlet the dominant chironomid was *Chironomus* sp., though its abundance was no greater at these sites than elsewhere in the reservoir. Impoverished chironomid communities corresponded well with high iron in sediment levels recorded by the NRA and in particular with the presence of amorphous iron precipitates (Extence, et al. , 1992).

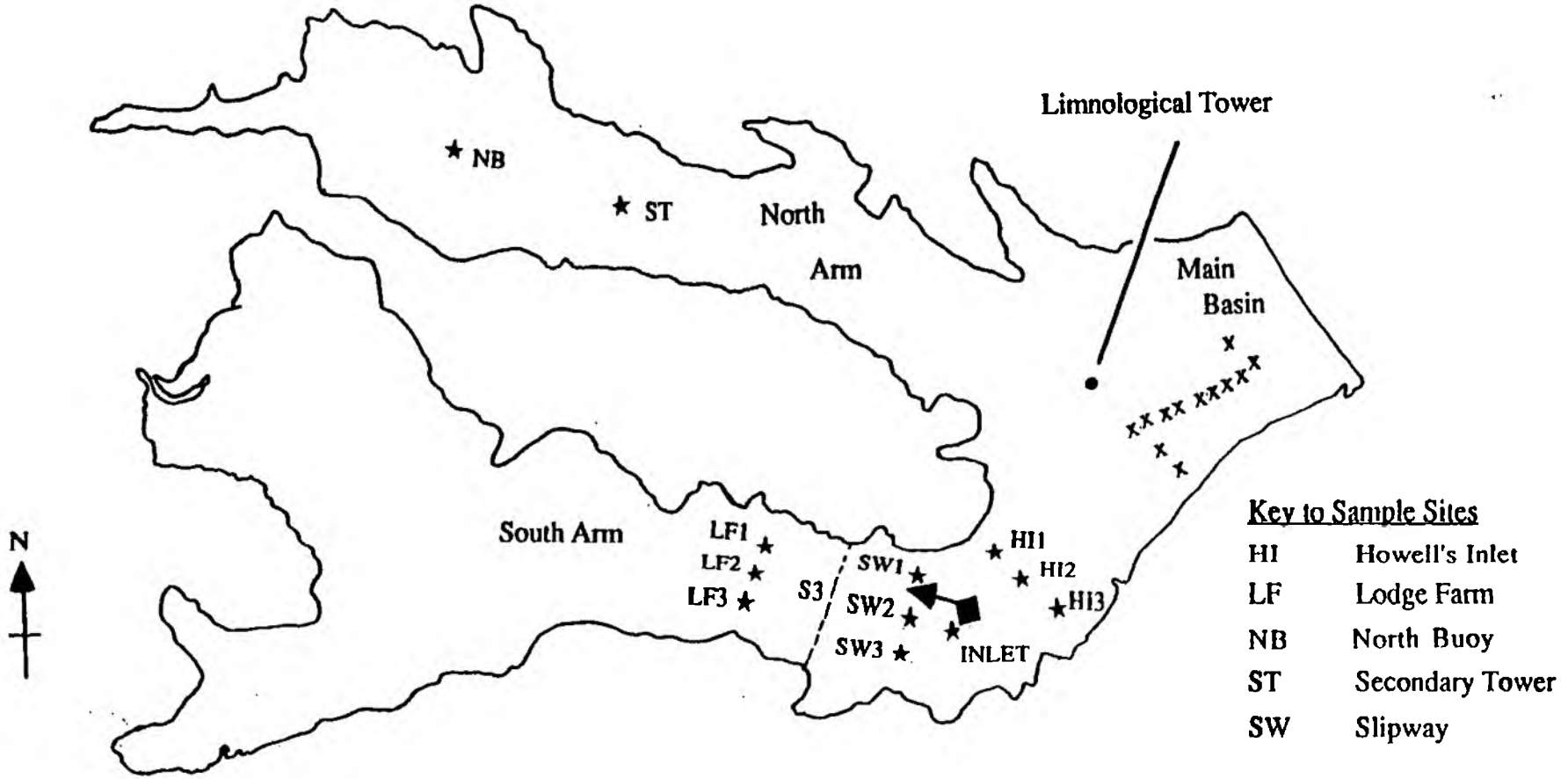

Figure 1 Outline map of Rutland Water, Leicestershire showing the positions of the inlet jet, limnological (draw-off) tower. 'helixor' destratification cylinders (x) (Harper, 1982), National Rivers Authority grid sampling sites (★), and the S3 transect. Modified from Extence, et al., (1992).

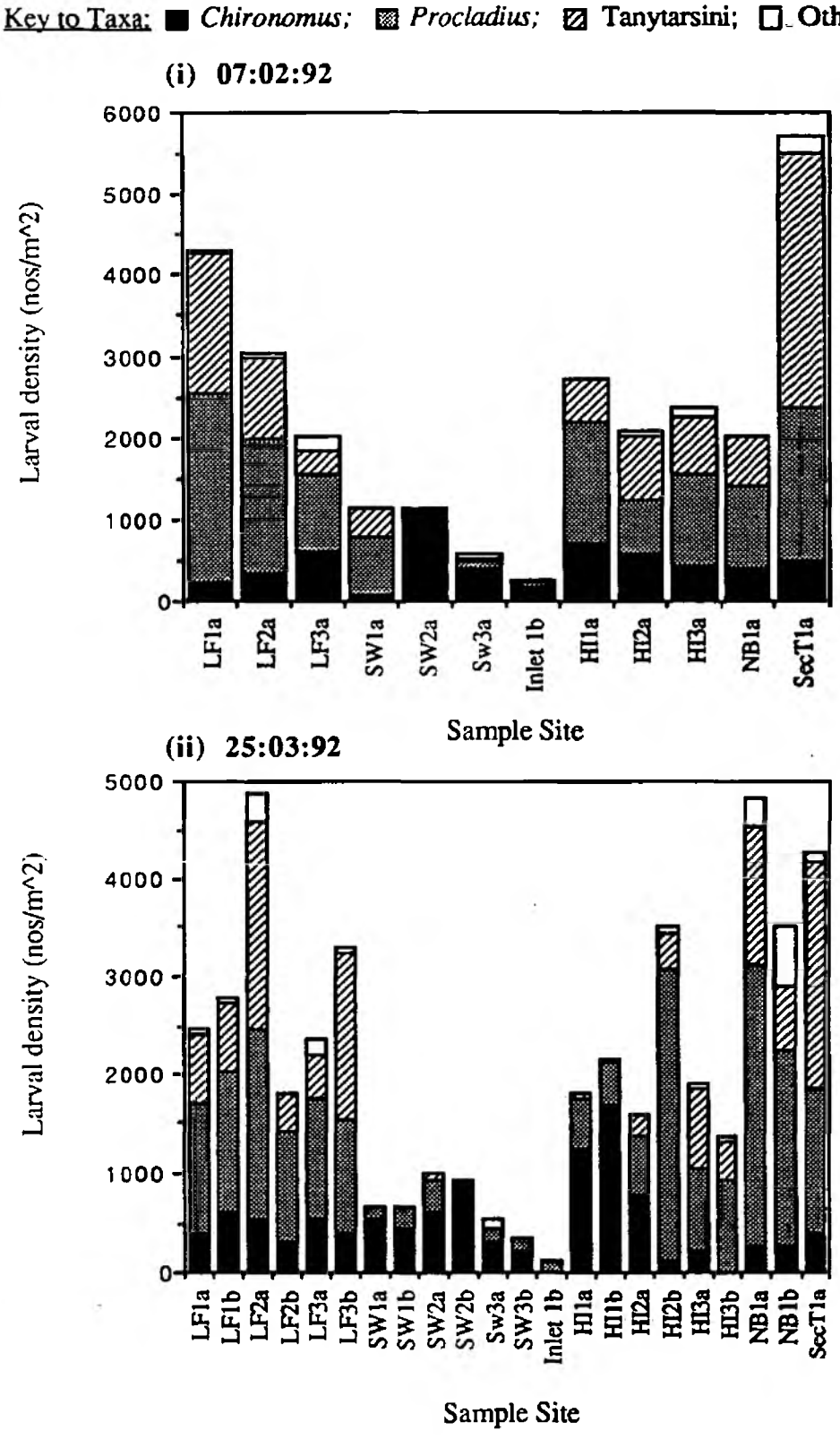

Figure 2 Larval density of four chironomid taxa from benthic grid surveys at Rutland Water. (i) February 1992, (ii) March 1992. Key to sites:HI = Howell's Inlet, LF = Lodge Farm, NB = North Buoy, SecT = Secondary Tower and SW = Slipway

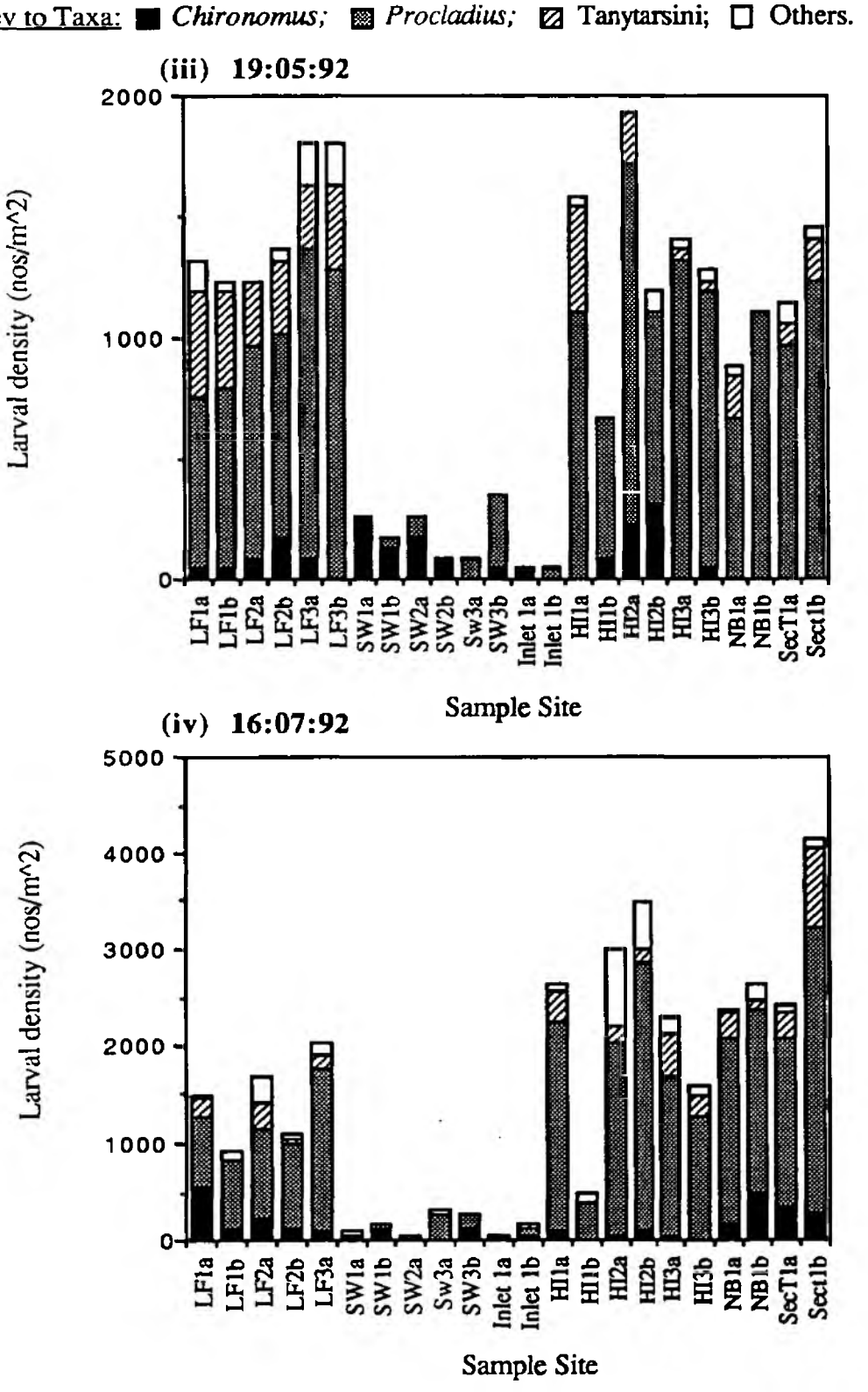

Figure 2 Larval density of four chironomid taxa from benthic grid surveys at Rutland Water. (iii) May 1992, (iv) July 1992. Key to sites: HI = Howell's Inlet, LF = Lodge Farm, NB = North Buoy, SecT = Secondary Tower and SW = Slipway.

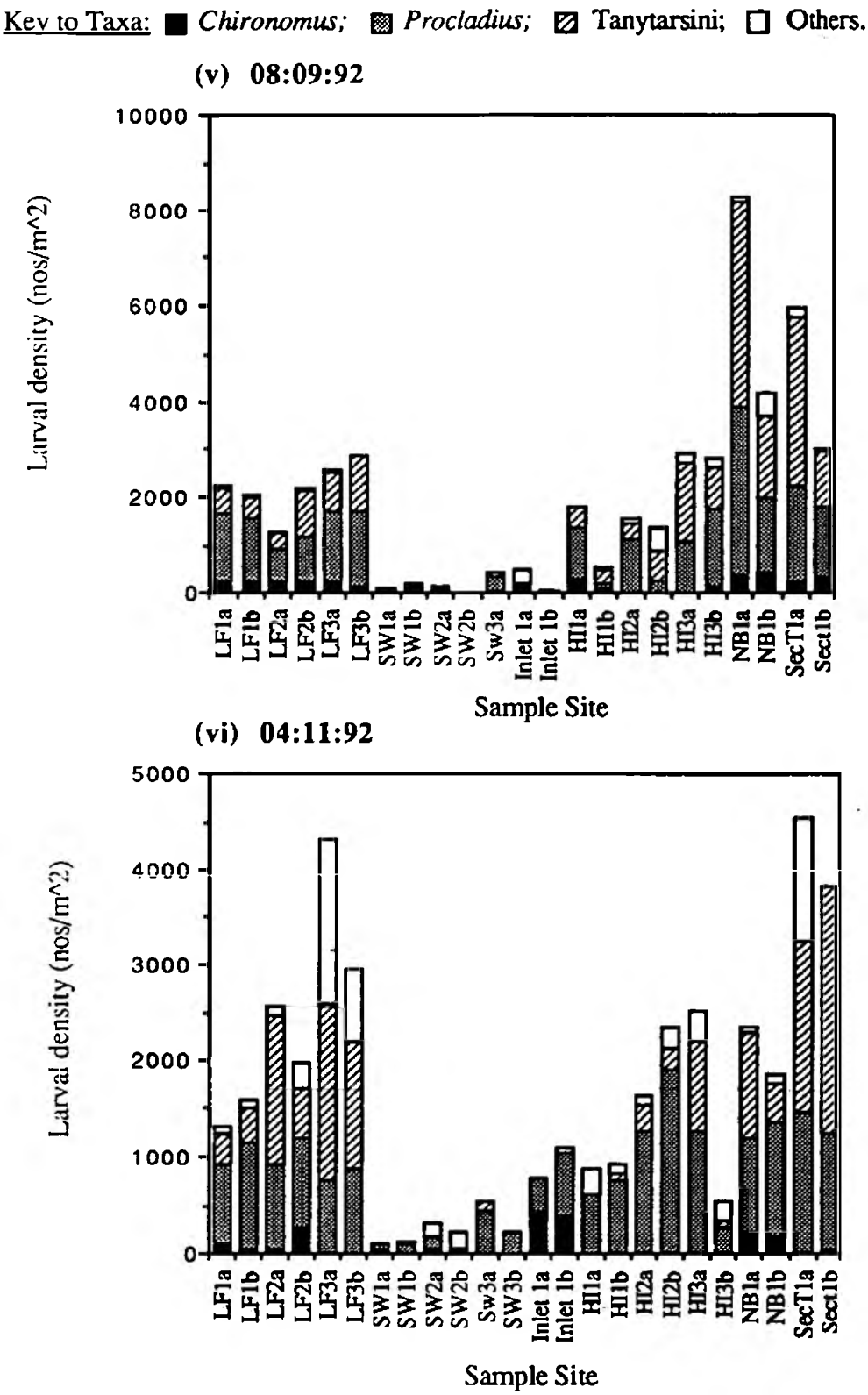

Figure 2 Larval density of four chironomid taxa from benthic grid surveys at Rutland Water. (v) September 1992, (vi) November 1992. Key to sites: HI = Howell's Inlet, LF = Lodge Farm, NB = North Buoy, SecT = Secondary Tower and SW = Slipway.

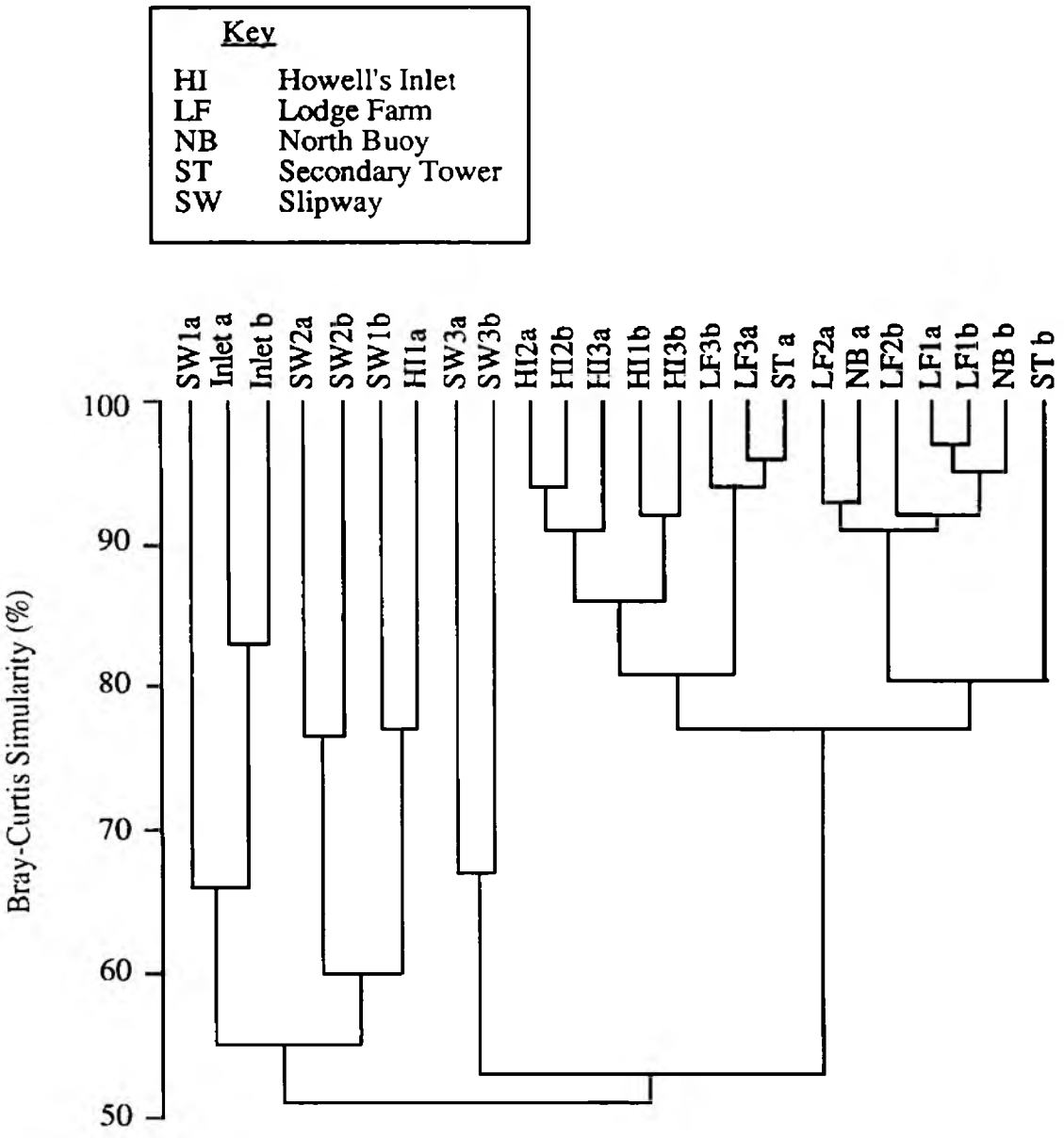

Figure 3 Dendrogram of Bray-Curtis similarity coefficients from the NRA November 1992 grid survey.

Whether the response of chironomid communities is to toxicity of iron or to a physical effect of its presence is unclear from this data. The laboratory based project explained in the following sections has aimed to clarify this situation and to quantify the response of a chironomid species, *Chironomus riparius*, to the addition of ferric sulphate.

3. IMPACT OF FERRIC PRECIPITATES ON GROWTH AND DEVELOPMENT OF *CHIRONOMUS RIPARIUS* (MEIGEN)

3.1 Test organism, *Chironomus riparius*

Larvae of the non-biting midge *Chironomus riparius* inhabit both lotic and littoral lentic habitats and are particularly associated with organic pollution (Learner and Edwards, 1966; Gower and Buckland, 1978; Rasmussen, 1984). It is one of a few chironomid species that can easily be cultured in the laboratory (Pascoe and Edwards, 1989). The culture technique is continuous and relatively simple (Credland, 1973; Batac-Catalan and White, 1982; McCahon and Pascoe, 1988; Radford, 1994), providing a constant supply of all life stages of the midge. *C. riparius* is a burrowing detritivore which indiscriminately ingests sediment with detritus (Smock, 1983a). Thus it is ideal for testing of sediment bound contaminants (Pascoe and Edwards, 1989) or, as in this study, testing of inorganic precipitates present as a layer on top of the sediments.

3.2 Experimental Procedure

A simple static-with-replacement system was chosen. Normally preferred flow-through systems seemed inappropriate for use with a floculent test substance which is neither dissolved nor strongly bound to the substrate. In addition, first instars of *C. riparius* are very small in size (approximately 500–600 µm total body length) and have a greater tendency to be planktonic than later instar stages, this stage is less likely to be lost from a static system than from a flow-through system. A test duration of 25-days allowed exposure of *Chironomus* from hatching to emergence. This is of value since different life stages can vary in their sensitivity to a contaminant (Gauss, *et al.*, 1985; Powlesland and George, 1986; Williams, *et al.*, 1986).

Plastic aquaria measuring approximately 280 x 160 x 90 mm (length x width x depth) were used as experimental chambers. These allowed several hundred larvae to be reared within one treatment. An appropriate amount of aerated, 53 µm filtered reservoir water was placed into each tank. Cellulose mulch was added to give an approximate depth of 1 cm of artificial substrate. Fresh 500 mg Fe litre^{-1} stock solution was applied evenly over the surface of the medium to provide the required target concentration and bring the total volume of medium to 2 litres. Preliminary experiments revealed that depression of pH was associated with addition of ferric sulphate (Radford, 1994). High mortality of larvae was associated with low pH. It was noted, however, that pH depression was temporary. This is probably due to the high pH (8.0–8.5) and calcium carbonate levels of the filtered reservoir water. The problems of pH depression were avoided by allowing the tanks to stand for 14 days after the addition of ferric sulphate, prior to the addition of test organisms. Aeration of tanks was constant during this period. 5 ml of 50 g litre^{-1} food suspension was added to each tank.

The number of eggs in eggmasses collected within 18 hours of oviposition were counted. A single eggmass was allocated to each tank. Eggs were left for four days to hatch, numbers of unhatched eggs were counted at this time. Once hatched the progress of larval development was monitored using larval dry weight and larval instar stage. Samples of larvae and substrate were made on days 6, 14, 20 and 25 of the experimental period. The instar stage of each larva was identified from head capsule widths (Radford, 1994). Larvae were weighed individually. Substrate samples were also taken on day 0 for initial tanks and day 14 for replacement tanks (see below). The medium of each tank was sampled on days 0, 6, 14, 20 and 25.

Replacement of tanks was carried out on day 14 of each test after the removal of the day 14 samples. Replacement tanks were prepared using the procedure outlined above and also allowed 14 days for pH to equilibrate. Larvae from each initial tank were carefully sorted from medium and substrate and counted on transfer to the appropriate replacement tank. Thus

providing an accurate census of the number of surviving larvae. A similar census was carried out at the end of the test (day 25). Any emergence of adult flies was noted towards the end of each test.

All experiments were performed in a 20 ± 0.5 °C constant temperature room. Light was provided on a 16-hour on, 8-hour off basis. All glassware and plastics were rinsed after use, first in 1% nitric acid and then in deionised water prior to air drying covered by laboratory paper.

Samples of test medium and particulate matter were prepared for atomic absorption spectrophotometry using methods based on those used by the National Rivers Authority (1991). Weighed larval samples were digested in the same manner as particulate matter. Regular monitoring of pH was made throughout the tests.

Three replicate experiments were performed using the following target iron treatments in each: 15, 30, 40, 50 and 75 mg Fe litre^{-1}. A control tank to which no ferric sulphate addition was made was included in each replicate experiment.

3.3 Results

Table 1 provides a list of the initial (day 0) and replacement (day 14) pHs for all 25 day experiments. Circumneutral conditions prevailed in the majority of treatments during the exposure period. Iron levels in dissolved/suspended and particulate matter forms are illustrated in Figure 4. Dissolved plus suspended iron measurements exhibited no significant difference between treatments (Kruskal-Wallis H-statistic, 5.188; $p > 0.05$). In contrast, particulate iron levels between treatments were significantly different (H statistic, 85.829; $p < 0.001$) and differed significantly from the control in all iron treatments above 15 mg Fe litre^{-1}.

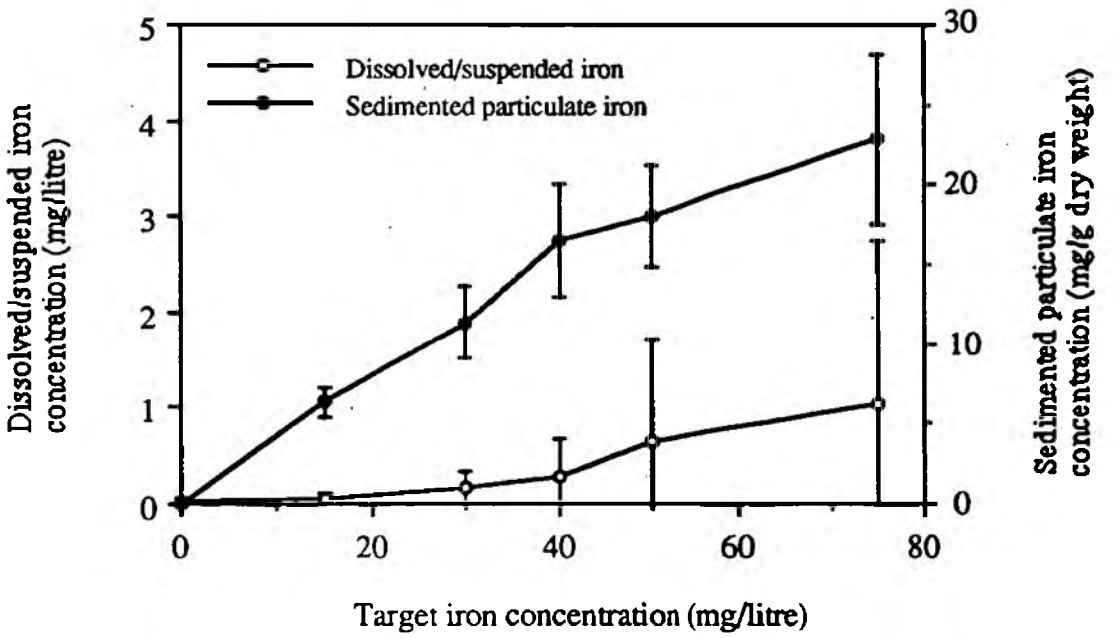

Figure 4 Dissolved/suspended and particulate iron concentrations from target iron and control treatments. Error bars represent 95% confidence intervals from the Student's t-distribution.

Table 1 Initial (day 0) and replacement (day 14) pH's for each replicate ferric precipitate experiment.

Replicate	A		B		C	
Treatment (mg Fe litre^{-1})	Initial pH	Replacement pH	Initial pH	Replacement pH	Initial pH	Replacement pH
Control	8.38	8.45	8.44	8.57	7.96	8.45
15	8.19	8.23	8.13	8.35	7.75	8.31
30	8.07	7.97	7.61	8.19	7.29	8.07
40	7.86	7.58	5.25	7.79	6.79	7.83
50	7.04	6.04	6.73	7.38	5.39	7.30
75	5.67	3.67	4.15	5.15	4.67	4.74

Mortality was split into four time periods; egg non-viability, larval mortality prior to leaving the eggmass (LD1), larval mortality after leaving the eggmass but before the first larval census (LD2) and larval mortality between the first census and the final census. In preliminary experiments high larval mortality, particularly LD1 and LD3 were linked to temporary low pHs (Radford 1994). Figure 5 illustrates mortality in experiments where pH was allowed to reach circumneutral levels prior to the addition of animals. No relationship between target iron concentration and mortality is apparent. No LD1 mortality occurred in these experiments.

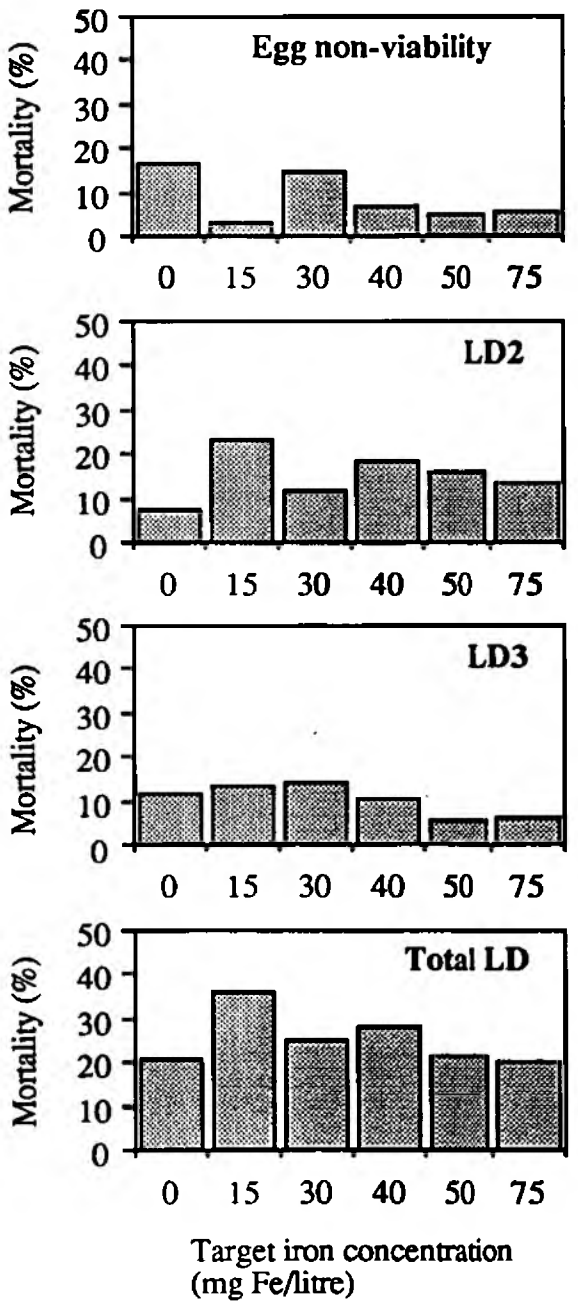

Figure 5 Egg and larval mortality from ferric precipitate experiments. LD2 equals larval mortality after leaving the eggmass but before larval census I; LD3 equals the mortality of larvae between census I and census II and Total LD is the total larval mortality.

A significant trend of decreasing larval dry weight was recorded with increasing target iron concentration (Figure 6). Using Kruskal-Wallis one way analysis of variance significant differences between the treatments were found on all sampling days ($p < 0.001$).

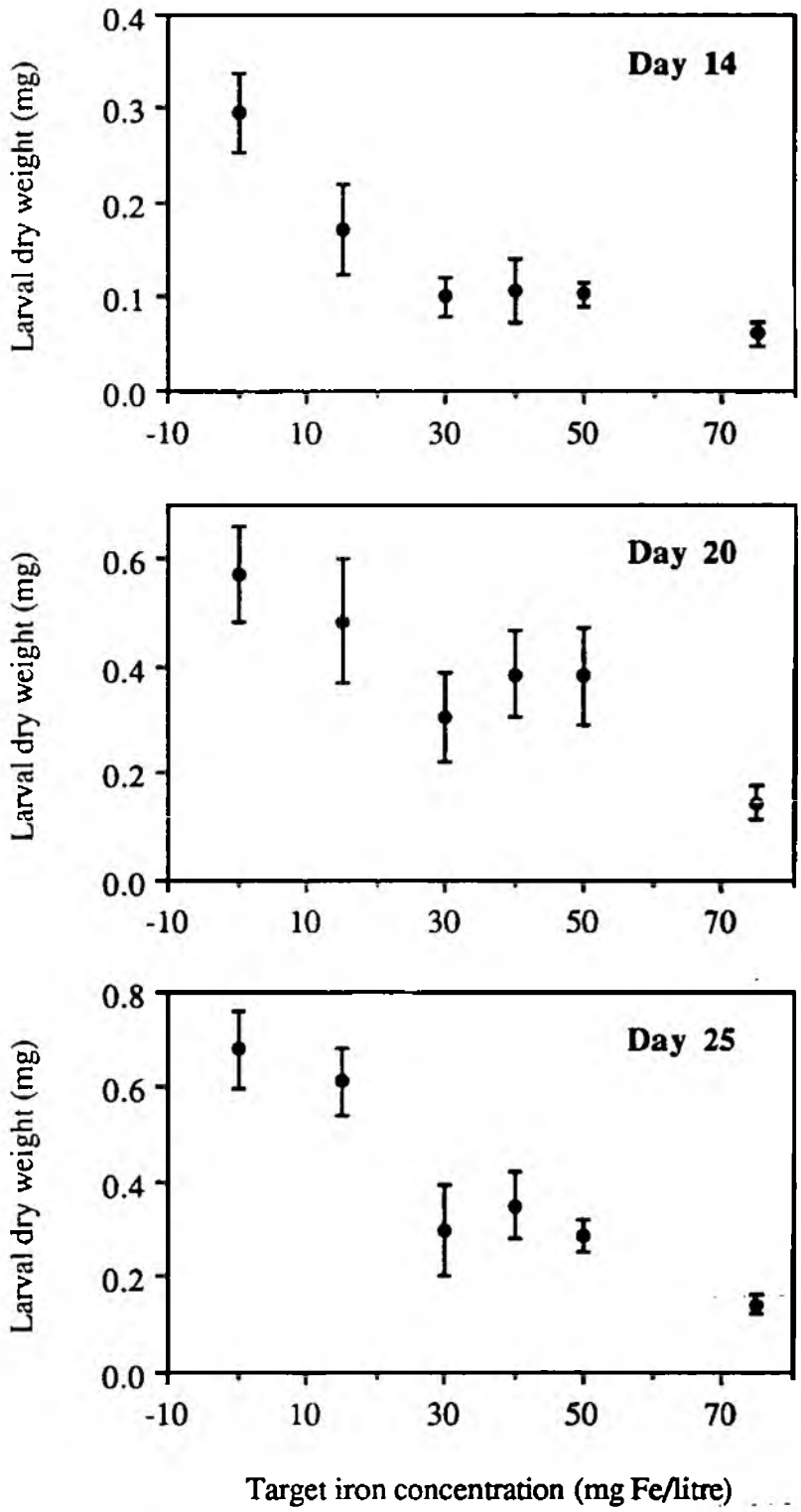

Figure 6 Effect of target iron concentration on larval dry weight. Error bars represent 95% confidence intervals from the Student's t-distribution.

From identification of instar stages retardation of larval development was observed. It was most noticeable from the day 14 samples where the proportion of final instars decreased with increasing target iron concentration from over 85% in the control to 0% in the highest iron treatment (Figure 7). Using the G-statistic for independence (Sokal and Rohlf, 1981) significant dependence of developmental stage on target iron concentration occurred on sampling days 14, 20 and 25 ($p < 0.001$). Developmental stage and iron concentration were independent on day 6.

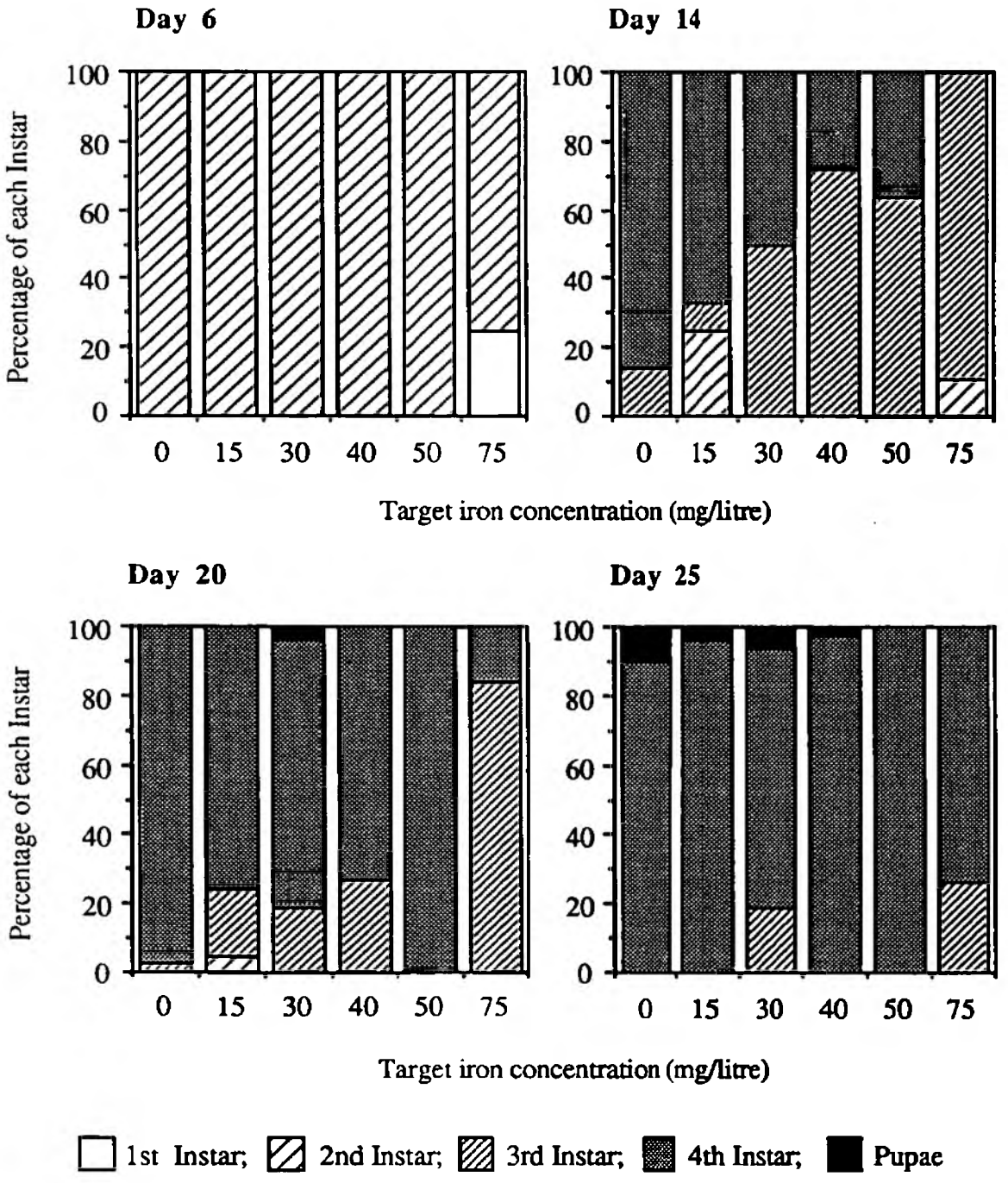

Figure 7 Effect of target iron concentration on *C. riparius* larval development.

The impact of iron precipitates on adult emergence was unclear from these experiments since termination of the experiment precedes the bulk of adult emergence.

Total iron content of larvae exhibited a significant trend of increasing iron content with increasing target iron concentration (Kruskal-Wallis H-statistic, 20.551; $p < 0.001$). This is illustrated in Figure 8. Observation of larvae from even the lowest iron treatment revealed orange precipitates within the guts. It is likely that ingestion of iron precipitates by *C. riparius* larvae has is the major uptake mechanism of iron.

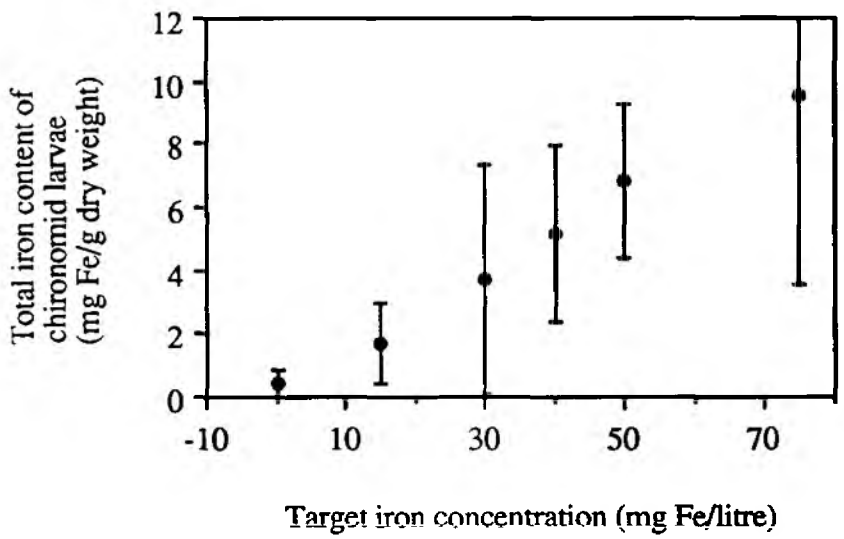

Figure 8 Effect of target iron concentration on total iron content of chironomid larvae. Error bars represent 95% confidence intervals of the Student's t-distribution.

3.4 Discussion

Iron precipitated from solution rapidly and almost completely after addition. Levels of iron in dissolved/suspended fractions remained low throughout the experiments, suggesting that no net re-solubilisation of iron occurred. It can be concluded that retardation of larval growth and development of *C. riparius* at target iron concentrations ≥ 30 mg litre^{-1} are due to the presence of iron precipitates.

Few previous studies have exposed test organisms to iron precipitates. Fifty percent mortality of coupled *Gammarus minus* adults exposed to suspended ferrous sulphate occurred at 7.2 mg litre^{-1} (Sykora, *et al.*, 1972a). Gerhardt (1992) reported that motility and feeding of the detritivorous mayfly, *Leptophlebia marginata* were reduced at 10 mg Fe litre^{-1} at pH 4.5. The animals were constipated. The source of iron used was ferrous sulphate solution. Ferrous iron was the dominant dissolved iron form but some precipitation of iron, presumably ferric, was observed. Reduced growth of fish species e.g. brook trout (*Salvelinus fontinalis*) and coho salmon (*Oncorhynchus kisutch*) in the presence of suspended ferric precipitates was ascribed to impaired visibility effecting food consumption (Smith and Sykora, 1976), but once settled ferric precipitates appear to have little direct effect on fish (Abram and Collins, 1981). To the authors knowledge only the current study has demonstrated a range of effects caused exclusively by the presence of sedimented ferric iron precipitates.

The mode of action of the iron is not clear from the experiments described thus far, but it is apparent that *C. riparius* larvae ingest iron precipitates and that the total iron content of larvae increases with increased target iron concentration.

4. IMPACT OF FERRIC PRECIPITATES ON EMERGENCE OF *CHIRONOMUS RIPARIUS* (MEIGEN)

4.1 Experimental procedure

Experimental conditions were similar to previous tests, i.e. 20 °C, 16-hour on 8-hour off light regime, constantly aerated filtered reservoir water, artificial substrate and 5 ml of 50 g litre^{-1} food suspension. Addition of ferric sulphate stock solution was as before and all treatments (including replacement tanks) were left for 14 days for pH to rise naturally to circumneutral pH. Four days prior to the start of the experiment 4 – 5 eggmasses, oviposited within 18 hours, were isolated in separate culture. After four days incubation 50 first instar larvae were allocated to each of twelve experimental chambers. Four treatments were used; a control to which no ferric sulphate was added and three target iron treatments; 15, 30 and 50 mg Fe litre^{-1}. Three replicate tanks of each treatment were used.

Test duration was forty-six days to allow the majority of larvae to emerge. The number and sex of emerged adults was ascertained for each tank on a daily basis. After counting, emerged adults and pupal exuviae were removed from the tanks. Replacement of tanks was carried out on two occasions during the test, days 13 and 25. On these occasions replacement was carried out after adults and exuviae had been removed. Surviving larvae were then counted and transferred to the replacement tanks.

To minimise disturbance, measurements of pH, dissolved/suspended iron and iron in particulate matter were only made on days 0, 13 and 25 of the test.

4.2 Results

Approximately neutral pH conditions prevailed throughout the exposure period in the majority of treatment tanks. Some of the second replacement tanks of the 50 mg Fe litre^{-1} treatment did not reach neutrality but in none of these was the pH below the tolerance level of late instar larvae (pH 3) (Radford, 1994). Levels of dissolved/suspended iron rarely exceeded the detection limit (< 0.056 mg Fe litre^{-1}) of the atomic absorption spectrophotometer. Iron in particulate matter levels were below the detection limit in the control but increased significantly with increasing target iron concentration (H statistic, 28.365, $p < 0.001$).

High mortality occurred in all treatments including the control, increasing with target iron concentration. These differences between treatments were, however, not significant (H statistic, 5.821, $p > 0.05$).

Cumulative emergence of males and females is illustrated in Figure 9, confidence intervals are omitted for clarity. For both sexes emergence decreases with increasing target iron concentration. Delayed emergence was also observed in the two highest iron treatments. Commencement of emergence at 30 mg Fe litre^{-1} was approximately 6 days later than in the control and an 18 day delay was observed at 50 mg Fe litre^{-1}. Female emergence started 4 – 5 days behind that of males in all treatments except 50 mg Fe litre^{-1}.

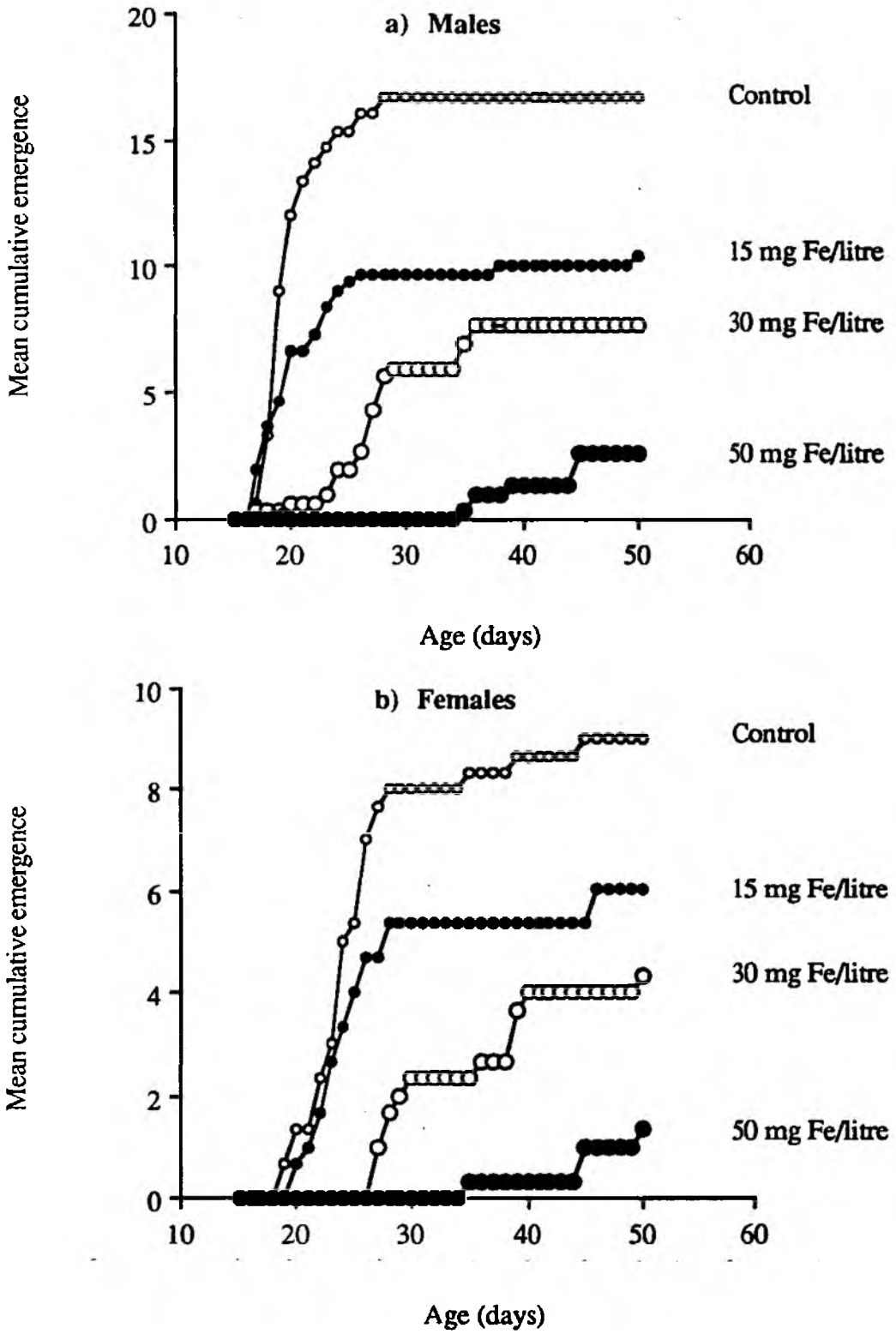

Figure 9 Effect of target iron concentration on adult emergence of a) males and b) females

4.3 Discussion

The cause of the high mortality observed in these experiments is unclear. Artificial substrate, food source and water were similar to those used in earlier experiments and so are unlikely to be factors. Aeration was maintained throughout making oxygen deficiency improbable. It is also unlikely that handling during transfer of larvae at replacement is the source. Whatever the cause it is reasonable to assume that it effected all treatments approximately equally since no significant difference in mortality between treatments was found.

Reduced emergence was seen in all iron treatments and delays occurred from 30 mg Fe litre^{-1}. Whether any of the reduction in emergence is linked to iron precipitates is uncertain since the source of the high mortality has not been identified. It is likely, however, that the delay in emergence is a reflection of the retardation of larval growth and development by iron discussed in earlier sections. A 2 day delay in emergence of *Chironomus tentans* from sediments contaminated with cadmium, zinc and chromium (1030, 17300 and 1640 ppm respectively) was recorded by Wentsel, *et al.*, (1978). Pascoe, *et al.*, (1989) noted a slight delay in *C. riparius* reared at 0.15 mg Cd litre^{-1}. The effect of cadmium on males was more pronounced than on females at this concentration since the delay between peak male emergence and peak female emergence was less than the 4 days found in the control. A similar delay between male and female emergence was found in this study in all treatments apart from 50 mg Fe litre^{-1}, where both sexes began emerging on the same day. Ferric precipitates appeared to effect males and females alike.

Time to peak emergence of larvae in the control treatment was 19 – 20 days after oviposition. Within this time frame delays in emergence of 6 and 18 days are major. Natural populations of *C. riparius* exposed to ferric precipitates might, therefore, be expected to show serious deviations from their normal life history patterns. Rasmussen (1984) described the life history of *C. riparius* in a prairie pond. In a univoltine cycle emergence and oviposition occurred by the end of May and larvae developed to small fourth instars by mid August. Most larval growth occurred from August to October and *C. riparius* over wintered as fourth instars. *C. riparius* is unable to continue growing at temperatures of 4.1°C or below (Gower and Buckland, 1978). In this system if larval growth and development were impaired larval weight at cessation of growth would be reduced. Survivorship over the winter period may be reduced and in any case delayed emergence in the next season would result. The situation might be compounded by continued ferric contamination and effects on swarming and mating success may be seen. Similar effects may occur where *C. riparius* has a multivoltine life cycle as suggested by Learner and Edwards (1966) and Gower and Buckland (1978). At best fewer generations per season might be expected.

5. UPTAKE OF IRON BY *CHIRONOMUS RIPARIUS* FROM FERRIC PRECIPITATES AND RESERVOIR SEDIMENT

5.1 Introduction

It is common to express the amount of contaminant present within the organism in terms of a total contaminant concentration or body burden per unit of dry weight. It is possible, however, to partition the total body burden of contaminant between that which is adsorbed to the surface of the organism and that which enters the organism. The latter can further be subdivided into that which is associated with the gut contents and that which is absorbed into the organism's tissues (Elwood, *et al.*, 1976; Smock, 1983b; Krantzberg, 1989; Timmermans and Walker, 1989). Partitioning of the contaminant burden can provide information concerning the method of contaminant uptake, its ultimate fate within the organism and may indicate its mode of action as a toxicant.

Observation of orange precipitates in the guts of larvae reared in iron treatments indicated that ingestion of iron precipitates is probably the major route of iron uptake. Neither the extent of internal absorption of ingested iron nor the contribution of surface-adsorbed iron was identified. The experiment described below estimates these fractions and uses them to indicate the possible mode of action of iron in the retardation of *C. riparius* larval growth and development.

5.2 Experimental procedure

Fifty 10-day old *C. riparius* larvae were randomly allocated to each of six experimental tanks. Tanks were provided with artificial substrate, organic food source and filtered reservoir water as before. All tanks were allowed 14 days for pH to equilibrate prior to the addition of larvae. Six treatments were used; a control to which no iron addition was made; four target iron treatments, 15, 30, 50 and 75 mg Fe litre^{-1} and a treatment in which artificial sediment was replaced with 1 cm depth of sediment from the North Arm of Rutland Water. No iron was added to this natural sediment treatment. Collected sediment was oven dried and kept in a desiccator prior to use.

Larvae were then reared for ten days. All the adults, pupae and a number of larvae present at the end of the test were dried to constant dry weight and digested whole for iron analysis. Digestion was performed in 0.5 ml capped polypropylene micro-centrifuge tubes at 20 °C using 50% nitric acid. Total digestion was achieved in five days. Iron content was then measured using averaged peak readings from the atomic absorption spectrophotometer. Iron content was expressed in mg Fe g^{-1} dry weight.

Estimation of gut-associated, surface-adsorbed and internally-absorbed iron fractions was made using the scheme illustrated in Figure 10. A larva may contain iron in all three compartments. When the larva pupates any unabsorbed gut contents are likely to be expelled since the pupa no longer feeds. The pupa, therefore, contains only of iron which is either surface-adsorbed or internally-absorbed. Iron associated with the gut contents may then be estimated by subtracting the pupal iron concentration from that the larvae. Similarly, the total iron concentration of the adult might be expected to contain only the internally-absorbed portion since surface-adsorbed iron will be associated with the pupal case which is abandoned on emergence. Surface adsorbed iron could then be estimated by subtracting the adult iron concentration (absorbed iron) from the pupal iron concentration. Smock (1983b) measured the body burden of adults and pupal exuviae of the mayfly *Stenacron modestum* to provide estimates of internally-absorbed and surface-adsorbed metals respectively. Obviously digestion of a specimen does not allow later

developmental stages of that individual to be examined. Mean estimates of each stage have therefore been used.

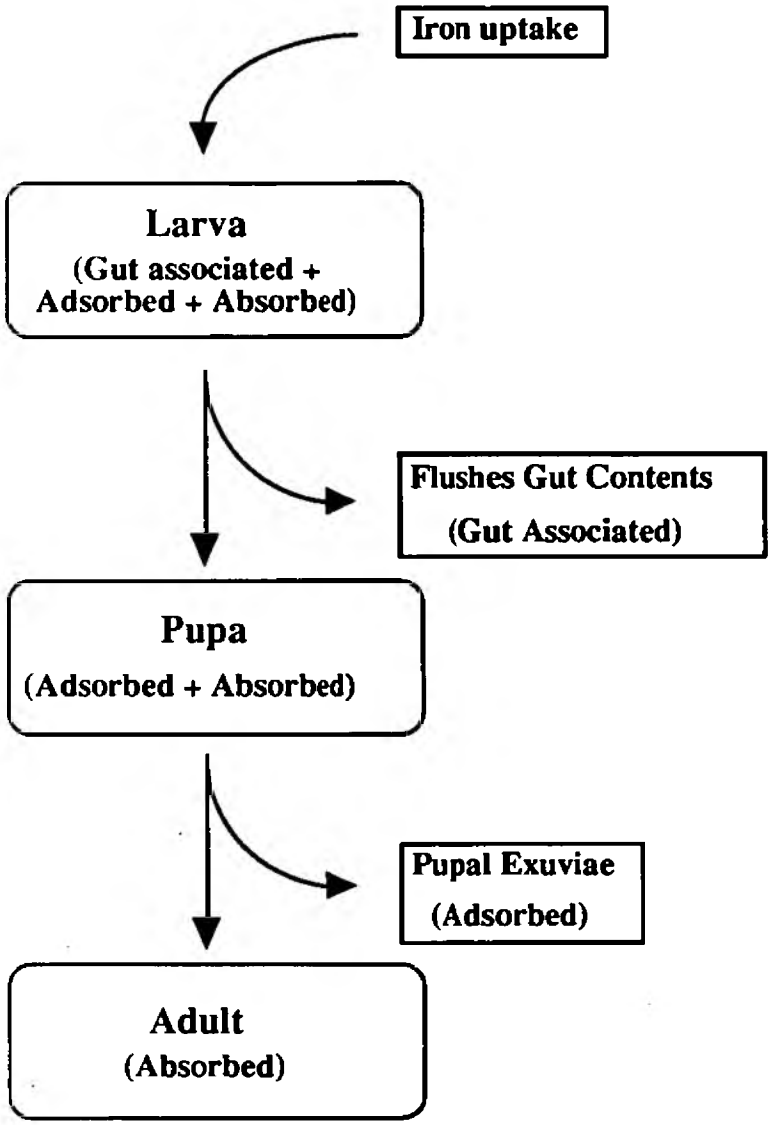

Figure 10 Partitioning of total iron content in the developmental stages of *Chironomus riparius*.

5.3 Results

Dissolved/suspended iron was below detection limits (0.056 mg Fe litre^{-1}) in all treatments. Levels of iron in particulate matter increased with target iron concentration. Background levels of total iron (iron per g dry weight of sediment) in natural sediment were greater than the highest iron-dosed treatment. Initial pH was above the tolerance limit of *C. riparius* in all treatments (Radford, 1994).

Survivorship was 94% or greater in all treatments. Larval dry weight significantly decreased (Kruskal-Wallis H statistic, 18.977, $p < 0.001$) as target iron concentration increased as in

previous experiments. Larval dry weight from natural sediments was not significantly different from the artificial sediment control (Mann-Whitney U statistic, 152, $p > 0.05$).

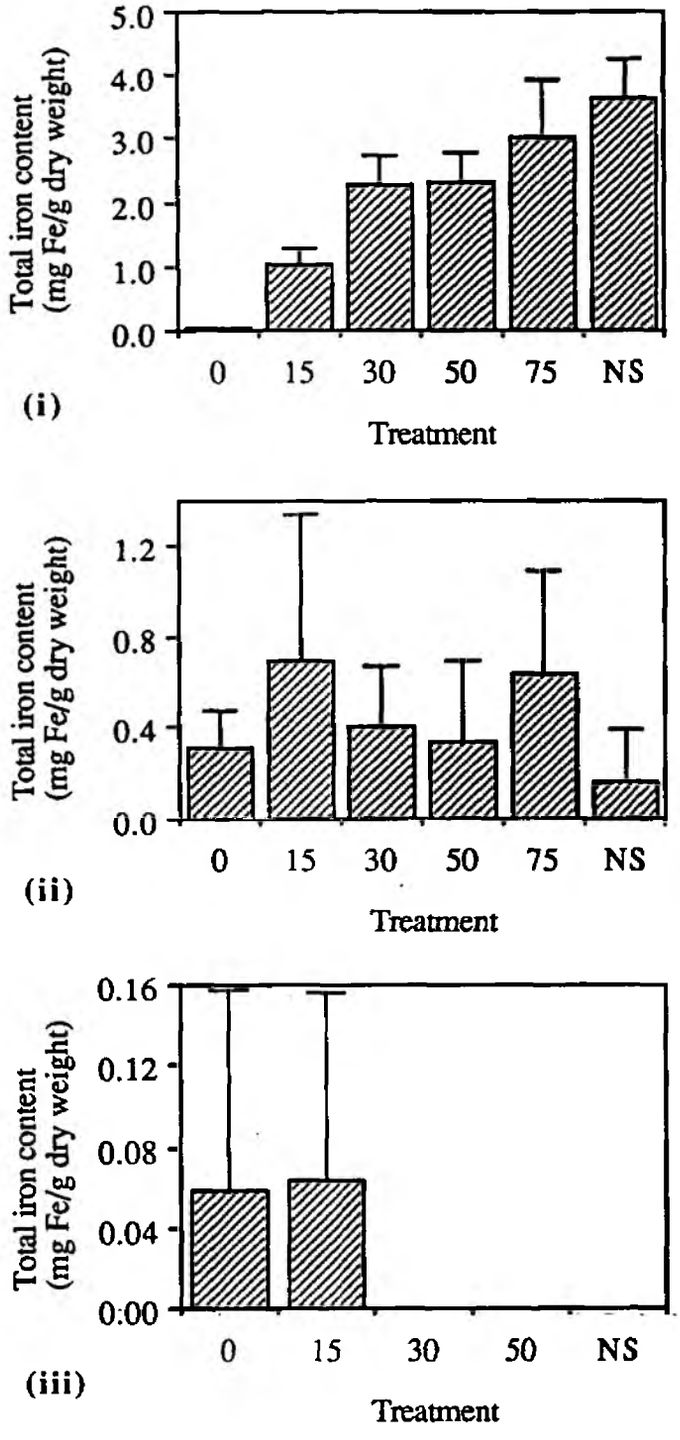

Figure 11 Total iron content from digests of (i) larvae, (ii) pupae and (iii) adult *Chironomus riparius* reared in various target iron (0 - 75 mg Fe litre^{-1}) and natural sediment (NS) treatments.

Figure 11 illustrates the total iron content from digests of larvae, pupae and adult *C. riparius* from the various treatments. The total iron content of larvae increased significantly with target iron concentration (Kruskal-Wallis H statistic, 43.014, $p < 0.001$), whilst pupal and adult iron contents did not significantly vary with target iron (Kruskal-Wallis H statistics, 4.845 and 2.503 respectively, $p > 0.05$ both cases). This indicates that only gut-associated iron varies with target iron concentration since larvae are the only stage to include this fraction. In natural sediment only iron content of larvae was significantly different from the control (Mann-Whitney U statistic, 0, $p < 0.005$).

Estimates of the partitioning of iron between gut-associated, surface-adsorbed and internally-absorbed fractions using the scheme represented in Figure 10 are given in Table 2. Estimated gut-associated iron (total larval iron concentration – total pupal iron concentration) increased with target iron concentration and was higher still in specimens reared in natural sediments. Surface adsorbed levels did not vary with target iron and internally-absorbed levels were mostly below the detection limits of the atomic absorption spectrophotometer (0.056 mg Fe litre^{-1}).

Table 2 Estimates of the partitioning of the total iron body burden of *Chironomus riparius* reared in target iron and natural sediment treatments.

Treatment	Gut associated	Surface Adsorbed	Internally Absorbed
Control	< .056	.249	.058
15 mg Fe litre-1	.344	.634	.064
30 mg Fe litre-1	1.869	.403	< .056
50 mg Fe litre-1	1.966	.334	< .056
75 mg Fe litre-1	2.375	.642	-
Natural Sediment	3.489	.161	< .056

Figure 12 represents the percentage of the total iron concentration in each of the three fractions for each treatment. The total concentration of iron in control organisms was barely detectable by atomic absorption but most (81.1%) was surface-adsorbed, none was gut-associated. In the 15 mg Fe litre^{-1} treatment 60.8% of the iron was surface-adsorbed, 33% gut-associated and 6.1% internally-absorbed. The percentage of iron that was gut-associated was very similar in the other three iron-dosed treatments (82%). The rest (18%) was surface-adsorbed. Gut associated iron provided an even larger percentage of the total from the natural sediment treatment. Over 95% of the total iron concentration was gut-associated in this treatment, leaving just 4.4% in the surface-adsorbed fraction.

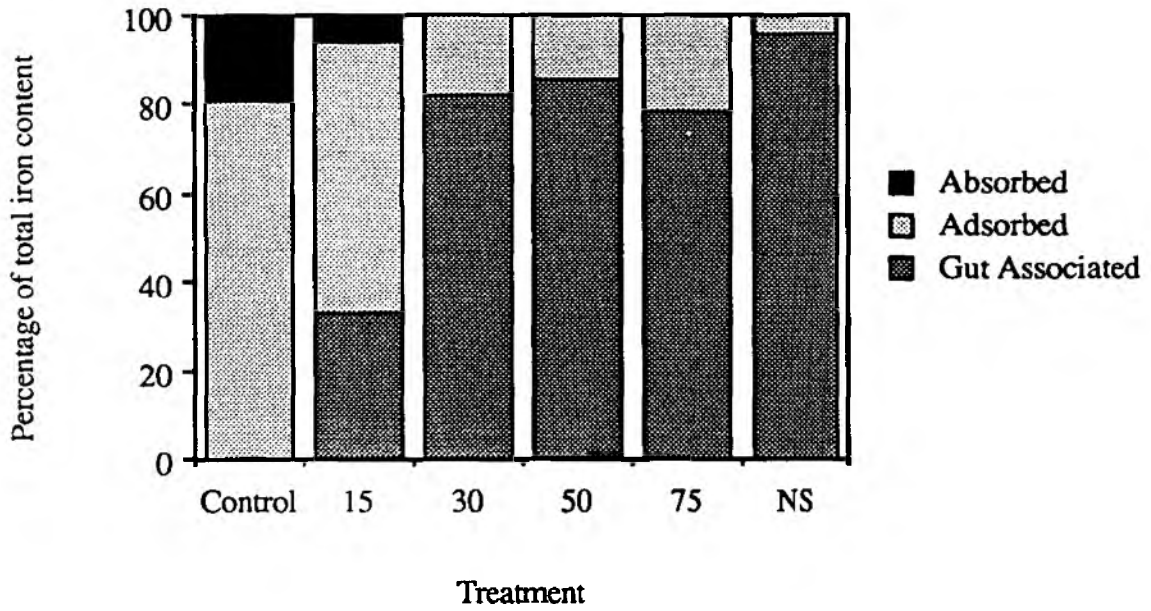

Figure 12 Percentage of total iron concentration that is gut-associated, surface-adsorbed or internally-absorbed in *Chironomus riparius* reared in various target iron (0 – 75 mg Fe litre^{-1}) and natural sediment (NS) treatments.

5.4 Discussion

No retardation of larval growth was observed from the natural sediment treatment despite exposure to higher levels of iron per unit weight of sediment than larvae reared in iron-dosed treatments. Growth restriction was observed in iron-dosed treatments. This suggests that detrimental effects of iron are more closely related to the form of iron than to the total iron concentration present in the sediments. Eighty to eighty-five percent of the total iron concentration of uncontaminated lake sediments is strongly bound in crystalline lattices (Tipping, *et al.*, 1982; Davison and Tipping, 1984; Moutin, *et al.*, 1993). Only 15 – 20% is in the form of amorphous iron hydroxides. Measurements of these fractions in sediments from the north arm of Rutland Water indicate similar proportions (Love, pers. comm.). Iron present in the iron-dosed treatments was all in the amorphous form.

Gut associated iron is the largest fraction in all treatments apart from the control and the lowest target iron concentration. This indicates that ingestion of iron was the most important uptake mechanism both from dosed artificial sediment and from natural sediment. Surface adsorption of iron appeared, however, to be of greater importance in ferric dosed treatments than in natural sediment.

Little absorption of iron was apparent and this fraction did not vary with external iron levels suggesting that the likely mode of action is physical rather than direct toxicity. It is unlikely that iron precipitates contain any useful food for chironomid larvae. Assuming that larvae presented with different food sources ingest at the same rate, a larva ingesting quantities of these precipitates will obtain less energy per unit time than a larva feeding on an undiluted food source. Greater amounts of iron precipitates will further reduce energy intake, hence the dose-dependent effect seen in this study. Natural sediments have high iron levels but also a higher proportion of organic matter than sedimented iron precipitate.

6. RELATION OF LABORATORY STUDY TO FIELD OBSERVATIONS

Smock (1983a) described *C. riparius* as sediment-dependent, which he defined as 'deposit feeders generally living within the sediment and indiscriminately ingesting this along with detritus'. *C. riparius* larvae ingest iron precipitates. Since the precipitates are inorganic this leads to a reduced energy intake per unit time. The observed growth and development retardation may be a direct consequence of this. In summary, if *C. riparius* is presented with a sedimented food source of acceptable particle size range it will feed, regardless of quality.

The response of other sediment-dependent species to deposited iron precipitates might be similar to that of *C. riparius*, provided the particle size of the precipitates is acceptable to them. The particle size of ferric iron precipitate varies greatly due to aggregation (Tipping, *et al.*, 1982). Potentially it could be included in the diet of most benthic macroinvertebrates. The layer of iron precipitate formed by ferric dosing is often flocculant in nature (Extence, *et al.*, 1992). Sediment-dwelling filter feeders are also likely therefore to be presented with ferric precipitates. Such species could also show deleterious effects through the same mechanism as sediment-dependent species, provided particle size is acceptable. Filtration of iron ore dust from sea water by the clam *Mactra lilacea* was recorded by Beckley (1981). No measurement of growth was made. Sediment-associated species ('species which to some degree selectively ingest detritus and periphyton', Smock, 1983a) may be less affected depending on their ability to selectively ingest detritus. A thin layer of iron deposited on stones or vegetation, a common occurrence in streams affected by mine drainage, may closely resemble detritus. Benthic carnivores will largely be able to avoid ingesting precipitates and since iron can be metabolised, its biomagnification is unlikely. Carnivores may, however, suffer indirectly through reduced abundance of prey species. Reduced biomass of benthic fish at sites contaminated with ferruginous mine drainage is an indication of this (Letterman and Mitsch, 1978; Scullion and Edwards, 1980a).

This study (see section 2) and those of Champion *et al.* (1991) and Extence *et al.* (1992) have observed impoverished benthic invertebrate communities coincident with high iron precipitate deposition at Rutland Water, Leicestershire. Champion *et al.* (1991) and Extence *et al.* (1992) recorded that the filter feeding bivalve *Pisidium* sp. and the sediment associated gastropod genera, *Valvata* and *Potamopyrgus*, were largely absent from high iron sites. The former study reported large numbers of dead specimens of these genera at the outlet buoy during the period of main basin dosing. Sediment dependent chironomid taxa such as *Polypedilum*, *Cryptochironomus* and the Tanytarsini tribe, filter feeding genera such as *Glyptotendipes* (Rasmussen, 1984) and the carnivorous genus *Procladius* were also absent from iron contaminated sites. Only sediment dependent oligochaetes and *Chironomus sp.*[1] remained in affected areas. These were also the dominant remaining taxa at stream sites affected by ferruginous mine drainage (Letterman and Mitsch, 1978; Scullion and Edwards, 1980b). Sediment-associated taxa such as Ephemeroptera, Trichoptera, Crustacea and Gastopoda were largely absent from these sites. So too were filter feeding molluscs and carnivorous stone fly larvae (Plecoptera).

These observations lend credence to the argument that deposited iron precipitates may adversely affect organisms of different feeding habits. It is possible that the mechanism of iron precipitate action described for *C. riparius* is applicable to these different organisms. Iron's effect on *Procladius* may be indirect through the absence of small chironomid species on which it feeds. *Chironomus* is the largest chironomid larva recorded in Rutland Water and tends to be more tolerant of contamination than other less robust chironomid genera (Anderson, *et al.*, 1980;

[1] Identification of adult flies reared from larval samples from Rutland Water (North Arm) suggested that this species was *Chironomus plumosus*.

Williams, *et al.*, 1986; Kosalwat and Knight, 1987). Oligochaetes appear to be unaffected this may be due to their deeper burrowing habits. They may largely avoid contact with the surface iron precipitate layer.

7. TOXIC IMPURITIES

Technical grade iron III sulphate was used in the laboratory study to avoid complexity from toxic impurities. Chemicals used for reservoir dosing are likely to be of poorer quality and contain more impurities. Long-term dosing introduces large quantities of dosant to the receiving system and conceivably toxic impurities may accumulate to detrimental levels. The effect of a contaminant mixture cannot be predicted by the effects of the single contaminants (Kraak, *et al.*, 1994). Assessment of the impact of dosant impurities should therefore be based on the toxicity of the mixture. Whether individual impurities remain dissolved or precipitate out with the ferric iron will also be of importance.

8. RELATIONSHIP BETWEEN DETRIMENTAL PRECIPITATE LEVELS AND DOSANT CONCENTRATION

In this study development of *C. riparius* was retarded by the presence of deposited iron precipitates at target iron concentrations $\geq$ 30 mg Fe litre^{-1}. Temporary effects were observed at 15 mg Fe litre^{-1}. Sub-lethal effects of this type can, with long-term exposure, be just as damaging as short duration lethal doses. Retarded development may seriously disrupt normal life history patterns (see section 4.3) and the lower selection pressure provided is likely to delay the acquisition of tolerance to the contaminant.

Dosing at Rutland Water averaged above 30 mg Fe litre^{-1} for the first year, 1990 – 1991 (Anglian Water Services, pers. comm.). In subsequent years dosing has been more closely related to phosphate levels in the inlet water. The average concentration has reduced to between 10 and 20 mg Fe litre^{-1} at present dosing levels.

Precipitation of iron was a rapid process both in the laboratory and at Rutland Water. The amount of precipitate formed in the laboratory was linearly related to the target iron concentration (Radford 1994). The iron content of the precipitate did not vary with target iron. Mean iron content of precipitate was 175.04 mg Fe g^{-1} dry weight (standard deviation, 17.88 mg Fe g^{-1} dry weight) across all treatments. Laboratory dosing was discrete and applied evenly over the water surface, dosing at Rutland Water is continuous and precipitation of iron is unevenly distributed. Sedimentation of precipitates was local to the inlet (NRA, pers. comm.). The accumulation of precipitate close to the inlet at Rutland Water is therefore disproportionate to the dose concentration at any one time. If a discrete dose of 30 mg Fe litre^{-1} produced enough precipitate to inhibit chironomid development then similar results might be expected from the build up of precipitate from a much lower continuous dose.

Extrapolation of a detrimental amount of precipitate to a particular dosant concentration must be approached with caution. The time-span and regime of dosing will determine how much ferric sulphate is added and whether it accumulates continuously or is periodically overlain by natural lake sediments. The position of the inlet/dosing point to the basin shape will affect the area covered by ferric precipitates. Water chemistry parameters, particularly pH and water hardness, will also affect this since they affect the extent and rate of precipitation. Water currents and wind action may affect the spread of the precipitate layer.

The only simple solution is to recommend that the deposition of iron precipitates within lakes and reservoirs be avoided. The extent of damage to a system receiving iron precipitates is likely, however, to be dependent on the proportion of the system affected. In the case of

Rutland Water approximately 10 % has sediment iron levels ≥ 90 mg g^{-1} (NRA, pers. comm.). Overall the productivity of benthic invertebrates and of fish and bird species which rely on them for food is probably little affected. With continued dosing, however, the affected area at Rutland Water is likely to increase.

This study has clearly shown that the presence of deposited iron precipitates inhibited development of *Chironomus riparius* in laboratory culture and was coincident with impoverished profundal chironomid communities in an iron-dosed reservoir. The impact of toxic impurities in the dosant on benthic invertebrates requires investigation and should be completed before in-reservoir dosing with ferric sulphate is considered for other eutrophic reservoir systems.

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