Ecotoxicological Impact of Iron III Sulphate on Chironomid Cultures and Profundal Reservoir Communities

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Dedicated to my parents and my wife Kath

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Abstract

Iron III sulphate dosing has been used as a eutrophication control measure in water supply reservoirs. The dosing results in precipitation of available phosphorus to a layer overlying the natural sediments. Stream sites contaminated with iron from mine drainage exhibit impoverished fish and benthic invertebrate communities. This study investigated the potential impact of prolonged exposure to sedimented iron precipitates on chironomids (Diptera, Chironomidae), as representatives of benthic invertebrates.

The distribution of chironomid taxa in relation to iron III dosing at Rutland Water, Leicestershire has been examined. Areas of impoverished chironomid communities coincided with areas having increased sediment iron levels. *Procladius* (Tanypodinae) and the tribe Tanytarsini, dominant elsewhere in the reservoir, were largely absent from these areas.

A laboratory investigation of the effects of sedimented iron III precipitates on *Chironomus riparius* (Meigen) has been performed. This species is easily cultured and as a non-selective benthic detritivore was a suitable test organism. Simple static-with-replacement tests of various duration were used due to the flocculant nature of the precipitates and to avoid loss of small early instar larvae.

Precipitation of iron was rapid and virtually complete after addition of iron III sulphate to filtered reservoir water. High larval mortality resulted from initial, temporary, depression of pH due to addition. Depression of pH is unlikely to be a major problem in a large well-buffered reservoir. Retardation of larval growth and development, and delayed adult emergence were related to increasing target iron concentration. Iron uptake was dominated by precipitate ingestion and little iron was internally absorbed. Reduced energy intake over time due to dilution of food by ingested precipitates is suggested to be the main mode of action. Reduced chironomid diversity at iron-contaminated field sites may be a consequence of this.

Evidence presented in this study from laboratory cultures and field observations indicates that the deposition of iron, e.g. by iron sulphate dosing, is likely to reduce abundance and diversity of benthic invertebrate communities. The potential for this to adversely affect the wider lake/reservoir communities has not been documented here but there is potential for a knock-on effect throughout the food web. The degree to which a lake/reservoir is affected may depend on the proportion of its sediment surface covered by iron deposition. The greater that proportion, the greater the potential effect. The use of iron sulphate dosing of reservoirs could therefore have serious undesirable side-effects which should be weighed against the possible phosphate-reduction benefits when seeking control of eutrophication and phytoplankton communities.

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Chapter One

General Introduction: The problem of eutrophication, iron III dosing as a means for its control and the potential hazards of iron contamination.

1.1 THE PROBLEM OF EUTROPHICATION IN LAKES AND RESERVOIRS

1.1.1 Causes of eutrophication

Eutrophication is the enrichment of aquatic ecosystems with nutrients at levels higher than those occuring naturally, causing measurable biological effects. The productivity of a lake is largely dependent on its current nutrient supply: generally, increases or decreases in limiting nutrients will be mirrored by productivity. Since the mid twentieth century man-induced artificial eutrophication (abbreviated here to eutrophication) has increased the productivity of many lakes world-wide. Particularly important is the ingress of phosphorus and nitrogen. A review of the sources of these nutrients is provided by Harper (1992), only a brief summary is given here.

Modern farming methods use fertilisers rich in these nutrients. Nitrogen compounds, particularly nitrates and ammonia are highly soluble. Leaching of nitrates substances from arable catchments into aquatic systems is the major route for nitrogen enrichment. Compounds of phosphorus have a greater tendency for low solubility, but water logging and consequent deoxygenation of wetland soils may reduce this tendency increasing concentrations in run-off. Soil erosion is the main route for phosphorus to

enter waters in particulate form. Intensive animal units e.g. fish farms and farm livestock may provide point sources of phosphorus in their run-off.

The largest source of phosphorus, however, comes from human waste. The treatment of sewage produces oxidised soluble compounds of carbon, nitrogen and phosphorus which are discharged in sewage works effluent. Approximately half of the phosphorus in effluent is from detergents, most of the rest is from human wastes. Urban and industrial effluents also contribute phosphorus through sewage treatment processes.

1.1.2 Manifestations and problems of eutrophication

The Norfolk Broads provide a well documented example of how man's influences through eutrophication have greatly changed the ecology of shallow lake ecosystems. The Norfolk Broadland consists of drained or partially drained wetlands, small slow-moving rivers, small shallow lakes created by peat-digging and a series of man-made interconnecting channels. Moss (1983) gave a full description of the area and its history. Intense use of the area for arable and livestock agriculture and the discharge of sewage effluent into rivers by an increasing human population have caused increased inputs of nitrogen and phosphorus into the ecosystem. Until the middle of this century the Broads, rivers and dykes were characterised by clear water. Plant communities were dominated by submerged plants with sparse phytoplankton populations. Intensifying eutrophication caused increased growth of weeds in the 1950s leading to fears that navigation channels would be blocked. The aquatic plants were then rapidly replaced, in the 1960s, by dense communities of phytoplankton which persist to the present day (Anon., 1993). These changes have led to a reduction in the aesthetic and conservation interest, depletion of fish populations and diversity, greater sedimentation rates and reduced protection of banks from erosion. Fish and plant-eating bird populations have also suffered during these changes.

Excessive phytoplankton production also causes problems for the management of deeper lakes and reservoirs elsewhere. A review of these problems was presented by Hayes and Greene (1984). Severe phytoplankton blooms are aesthetically unpleasant, increase the burden on filters and on degradation may produce tastes, odours and in some cases toxins (e.g. from cyanobacteria) difficult to remove during water treatment. Rapid decline of severe algal blooms can lead to oxygen depletion, especially in stratified reservoirs. In these chemically-reducing conditions, release from sediment of sulphides, ammonia and iron causes problems for water treatment and supply. Additionally, fish species that rely on cool oxygenated hypolimnia for their summer survival (particularly coregonids and salmonids) may be excluded (Moss, 1988). Eutrophication is generally perceived as a costly problem.

1.2. OPTIONS FOR EUTROPHICATION CONTROL

Poisoning of algal growths and raking out of aquatic plants are methods that have been used in the short-term to remove the symptoms of eutrophication (Moss, 1988). Hypolimnetic aeration to maintain oxygenated conditions in unmixed hypolimnia (McQueen, 1986, Moss 1990) may improve conditions for fish species of economic value, whilst artificial mixing to prevent stratification can achieve a similar role and may help to limit the production of some non-buoyant algal species (Moss, 1988).

Apart from these mainly engineering approaches most of the attempts to limit the effects of eutrophication have centred on methods to control phytoplankton production. There are two main models that have been used as a basis for the methods tried, bottom-up control and top-down control (trophic cascades). The bottom-up hypothesis proposes that changes in the availability of nutrients control each trophic level of a food web (Kerfoot, 1987). In simple terms, a reduction in the availability of potentially limiting nutrients such as nitrogen and phosphorus should be followed by a

reduction in phytoplankton production. The top-down model proposes that predation at the upper level of the food web progressively influences the structure of levels below it. This has led to the manipulation of the food web to discourage or encourage the production of particular species or groups (Kerfoot, 1987). For example, if zooplankton species that are efficient in grazing phytoplankton can be encouraged to increase in numbers then their grazing pressure may force a reduction in phytoplankton production. The rest of this section discusses the use of these theories in more detail.

1.2.1 Strategies for bottom-up control of eutrophication

Increases in phosphorus and/or nitrogen are needed to increase algal production, however, only reduction of either nutrient is required to limit that production. Arable catchments provide the major source of nitrogen enrichment (Moss, 1988). The diffuse nature of this source and the availability of atmospheric nitrogen to nitrogen fixers such as cyanobacteria, makes nitrogen input difficult to control. Phosphorus has no atmospheric reserve and generally enters the system through a number of more easily defined point sources e.g. sewage works. For this reason most attempts to reduce nutrient availability have centred around the removal of phosphate.

A variety of methods for bottom-up control have been used, either addressing the input of phosphate into a lake/reservoir or that already present within it. There are a number of methods of dealing directly with phosphate from point sources. Reduction of domestic use of phosphates within detergents is worthwhile but only has the potential to reduce the phosphate loading in raw sewage by 40 – 50%. To remove phosphate from sewage totally, further measures are required. Phosphate stripping at the sewage works is becoming more widely used and can achieve up to 95% removal of phosphorus (Moss, 1988). It is an additional step after the 'normal' (primary and secondary) sewage processes and involves the use of a suitable chemical for the precipitation of the phosphate. Iron or aluminium salts are often used (Hayes *et al.* 1984; Phillips, 1984).

An alternative to chemical precipitation can be the diversion of sewage effluent either to the sea where it is greatly diluted (Edmondson, 1970) or to a pre-reservoir basin in which prolific algal blooms reduce the load of nutrients to the main system (Hayes, *et al.,* 1984). Diversion is not always a practical option, whereas phosphate stripping can be used at any sewage treatment works.

Lake/reservoir sediments act as a sink for phosphorus and have the potential to return much of it to the water column. Thus, even if all inputs of available phosphate are removed, recovery of the system can be prevented or delayed by the internal recycling of phosphorus (Osborne and Phillips, 1978; Phillips, 1984; Phillips and Jackson, 1990). One approach is to remove phosphate-containing portions of the system. Removal of biomass, e.g. aquatic plants, is possible but compared to the sediment these contain very little of the total phosphorus load (Moss, 1988). Removal of phosphorus-rich upper layers of sediment has been used at Cockshoot Broad in the Norfolk Broads (Moss *et al.*, 1986). This shallow lake was isolated from the eutrophic River Bure by means of a dam and sediment was pumped out to increase the water level from a few centimetres to approximately one metre depth. Phytoplankton production declined soon after these measures, producing clear water and allowing recolonisation of part of the Broad by aquatic plants. Isolation alone did not produce this change; a conclusion indicated by the isolation without sediment removal of Alderfen Broad. Initial results from this lake were similar to Cockshoot. But after four years of clear water and increased submerged plant production decay of organic matter reactivated release of phosphorus from the sediments and the Broad returned to phytoplankton dominance. The success of isolation and sediment removal together may be a consequence of the nature of the lower sediment layer. At Lake Geerplas, the Netherlands, newly exposed peat sediment appeared to adopt similar phosphorus-release characteristics to the original upper layers (van der Does and Frinking, 1993). Engineering difficulties may preclude the use of sediment removal in deeper systems.

An alternative approach is to treat the system chemically with a suitable phosphate precipitant. Where this is done in a pre-reservoir (Gulati, 1990; van Liere, *et al.*, 1990) or by treatment of inflow waters through a phosphorus elimination plant (Clasen and Bernhardt, 1987) removal of phosphate, turbidity and some particulate matter from the inlet water can be achieved. Chemical treatment of the main waterbody has been used to immobilise internal phosphate. Treatment of the whole reservoir using aluminium sulphate dosing by barge has been used at Twin Lakes, Ohio, U.S.A. (Kennedy and Cooke, 1982). Use of aluminium salts is uncommon outside the U.S.A. due to reservations concerning the toxicity of aluminium (Decker and Menendez, 1974; Abram and Collins, 1981; Hayes *et al.*, 1984). A more common method has been to treat the water entering the lake/reservoir chemically (Foy, 1985; Young *et al.*, 1988; Champion, *et al.*, 1991). Iron salts, particularly iron III sulphate or chloride, are most commonly used. The intention is to remove any incoming phosphate from the water column precipitating it to the sediments. This produces a layer of iron-rich precipitate on the surface of the natural sediment, which may help to reduce internal loading. The success of such schemes is very variable and has ranged from replacement of phytoplankton dominance with aquatic plants (leading to an increase in the value for water supply (Young, *et al.*, 1988)) to negligible changes (Foy, 1985). Injection of iron salts directly into the upper layers of the sediments has also been used. The aim is specifically to combat the internal loading of phosphorus from the sediments. The increased iron content of the upper sediment layers increases the retention of phosphorus even under anoxic conditions (G. Phillips, pers. comm.). Much lower amounts of iron are needed compared to dosing inlet waters, but restrictions imposed by the injection equipment limit this procedure to use in shallow lakes.

1.2.2 Strategies for top-down control of eutrophication

The hypothesis of top-down modification of food web structure, expressed as cascading trophic interactions is harnessed as the management practice know as

biomanipulation. For example, if piscivorous fish biomass increases then biomass and possibly production of planktivorous fish will fall, in turn, herbivorous zooplankton biomass should increase, meaning greater grazing of phytoplankton whose biomass will decrease. As piscivore biomass increases it pushes phytoplankton production over its optimum. In this model the productivity of each trophic level (and energy flow through the food web) is highest at an intermediate level of predation (Carpenter *et al.*, 1985). In a real ecosystem the productivity at each trophic level is set by the nutrient supply and so actual productivity will depend on the precise interactions between species and nutrient availability.

Trophic cascades have been tested in a variety of studies. Using large (12000 litre) plastic enclosures in Lake Mitchell, a small shallow mesotrophic lake, Kerfoot (1987) altered nutrient levels, presence/absence of planktivorous fish species and presence/absence of *Daphnia*. In nutrient-enriched conditions where fish were absent, *Daphnia* numbers responded quickly to increased phytoplankton production and were able to exert control over it. It was estimated that the densities of *Daphnia* produced in this experiment (approx. 100 animals litre^{-1}) would be able to filter the whole water column of the lake twice daily and that to survive algal species would need a doubling rate of around 8.5 hours. This exceeds the reproduction capabilities of most algal species. Where fish were present in these enclosures *Daphnia* numbers were suppressed, producing a shift in size structure towards smaller zooplankton and leading to elevated phytoplankton standing crops. Drenner *et al.* (1990) suggested that the effects of nutrients and fish are not independent from each other. Using 5500 litre tanks to examine the combined effects of three nutrient levels and the presence/absence of bluegill fish (*Lepomis macrochirus*), they reported a greater effect on chlorophyll a levels from the ratio of N:P than from fish presence. *Daphnia* was not present in their studies and they conclude that the trophic cascade uncouples at the zooplankton/phytoplankton link in eutrophic systems lacking this large zooplankton. *Daphnia* appears to be a key organism if biomanipulation is to be used for control of

phytoplankton production. As the size of the zooplankton grazer increases so does its absolute grazing rate (cells animal^{-1} unit time^{-1}) but its mass-specific excretion rate decreases. In this way large herbivorous zooplankton alter the species composition and size structure of phytoplankton communities directly by selective grazing and indirectly by recycling of nutrients by excretion (Carpenter *et al.*, 1985). The complex nature of these trophic relationships was highlighted by Threlkeld (1987) who found that three different fish species all resulted in significant changes in zooplankton community structure but that none produced significant phytoplankton enhancement. Clearly the relationship between zooplankton suppression and phytoplankton enhancement is not a simple one. Indirect bottom-up factors such as nutrient recycling are likely to be important as well as more direct top-down factors. Exact relationships will depend on many factors, not least the precise species composition of the lake community. Vanni and Findlay (1990) investigated the effect of two different zooplankton predators on the zooplankton composition and phytoplankton production. Both the vertebrate predator (yellow perch) and the invertebrate predator (*Chaoborus*) gave similar effects, decreasing zooplankton size structure and biomass and therefore decreasing grazing on phytoplankton, though, only enclosures containing yellow perch showed enhanced phytoplankton. The authors suggest that fish predators excrete and egest more nutrients than *Chaoborus* and that decomposition of dead fish will also release nutrients directly to phytoplankton.

Results from field-based studies in shallow lakes have given some promising support to the notion of using biomanipulation for suppression of phytoplankton. Phillips and Kerrison (1991) used large enclosures at Barton Broad in Norfolk to exclude fish. Phytoplankton dominance was suppressed and good growth of submerged plants returned to the enclosure. This result was even more pronounced in enclosures where netting had been used additionally to exclude coot from eating submerged vegetation. Similar results were achieved after biomanipulation of the whole of Lake Zwemlust, the Netherlands (van Donk *et al.*, 1990). The lake was drained and fished to

eliminate populations of zooplanktivorous and benthivorous fish. Northern pike were used to restock the lake along with adult rudd whose offspring were intended to provide food for the pike. Zooplankton were provided with shelter and pike with spawning sites by the addition of *Chara* plants, *Salix* twigs and *Nuphar* roots. One year after biomanipulation very low phytoplankton abundance was recorded, accompanied by increased transparency and a shift from rotifer to daphnid dominance of the zooplankton community. The authors noted that all this was despite an increase in nitrogen and phosphorus attributed to bioturbation of the sediments by increasing chironomid numbers.

It seems likely that top-down and bottom-up strategies may be needed in combination to ensure greater chance of eutrophication control. Benndorf (1987) reviewed the long-term data for a number of full-scale biomanipulation experiments and concluded that the success of food-web manipulation was closely related to the prevailing nutrient situation. A combination of top-down control and decreases in the phosphorus load of the system was recommended. Moss (1990) also proposed that combinations of eutrophication-control methods will often be needed for efficient control. The combination of measures employed will depend on the precise nature of the system; e.g. the precise phosphate load and its sources, the size and depth relating to stratification, the community structure, the geographical position and history of the site; and on the required end product of the scheme. Moss (1990) stated that a change in dominance from phytoplankton to aquatic plants is often a requirement of restoration of shallow eutrophic lakes e.g. the Norfolk Broadland. He suggested that in shallow lakes the role of internal phosphorus release from sediments is more critical than in larger waterbodies and so biomanipulation may be essential in these systems to push the change to plant dominance. In deeper systems, external loading of nutrients is more critical and aquatic plants tend to be of less importance. The aim in these systems is more to lower phytoplankton productivity and biomass (in the case of reservoirs, to lower the costs of treatment for water supply) rather than to alter community structure.

Methods of reducing the external phosphorus load and engineering methods such as hypolimnetic aeration may be the most fruitful methods (Moss, 1990).

The current study examines the detrimental effects of one of the eutrophication-control measures discussed above i.e. direct chemical precipitation of phosphate within water supply reservoirs. This method introduces large amounts of iron sulphate to the ecosystem via the inlet waters. The natural role of iron in aquatic ecosystems and its potential toxicity are discussed in the rest of this chapter.

1.3 BIOLOGICAL ASPECTS OF IRON CHEMISTRY

1.3.1 Natural role of iron in biota

Iron is an important plant nutrient, essential for the synthesis of chlorophyll, though only very small amounts are required. Deficiency of iron can cause chlorosis, the symptoms of which are pale, unhealthy leaves. Some electron carriers (e.g. cytochromes) employ iron as a binding site and thus it has an important role in the energy-conversion reactions of photosynthesis and respiration (Weier, *et al.,* 1982). Iron is also a vital constituent of the respiratory pigment haemoglobin found in mammals and some invertebrates (Ewer, 1942).

1.3.2 Chemistry of iron in freshwater lakes and their sediments

Iron exists in several oxidation states but only iron III and iron II are stable and common. The interchange between these two states, particularly at the sediment/water interface, is of great consequence in aquatic ecosystems. Iron becomes available to aquatic systems *via* the weathering of iron silicates and carbonates (Mance and Campbell, 1988). In oxygenated waters dissolved iron is present as oxidised iron III. Sediments underlying oxygenated water generally have an oxic red/brown upper

layer overlying an anoxic black layer. Iron is present in the iron III form as hydrous oxides ($Fe_2O_3.nH_2O$) in the former layer and as iron II associated with sulphide ions ($Fe^{2+}S^-$) in the latter layer. Iron II is more soluble than iron III and will tend to exist in dissolved form in the interstitial water. Diffusion of iron II to the oxic layer results in oxidisation to iron III and precipitation at the sediment/water interface. Thus, no net release of iron to the overlying water results (Mortimer, 1941; Mortimer, 1942; Davison and Tipping, 1984).

Where thermal stratification of a water body occurs in the summer months oxygen depletion of the hypolimnion and the surface sediments can result. In these conditions reductive dissolution of hydrous iron III oxide takes place resulting in the release of iron II to the overlying water. Release of compounds bound to hydrous iron III oxides accompany this reduction. Of particular importance is the release of phosphates. As discussed previously internal loading of this nutrient from the sediments in this way is of significance and can hinder attempts to alter the trophic status of the system. Oxidation of iron II to iron III and its precipitation occurs at the thermocline. The concentration of dissolved iron II is limited by the solubility of iron II sulphide (FeS), which eventually precipitates from the water. When the water body becomes mixed again in the autumn any iron II is rapidly oxidised and most returns to the sediment surface (Mortimer, 1941; Mortimer, 1942; Davison and Tipping, 1984). The half life of iron II at pH 7 in oxygenated waters is just a few minutes (Mance and Campbell, 1988).

The solubility of iron III is strongly affected by pH. For almost all the iron III to be in solution (99%) the pH must be approximately zero. Cotton and Wilkinson (1962) reported that as the pH rises to about 2 – 3 colloidal hydrous iron III oxides are formed. Ultimately these are precipitated as a red/brown gelatinous mass. Sholkovitz and Copland (1981) found, however, that the maximum extent of precipitation in river water occurred between pH 1.2 – 2.0 and that some solubilisation of iron resulted at

pH's from 3 – 11. The colloidal particles are roughly spherical/ellipsoidal with average dimensions of 0.05 – 0.5 μm (Tipping, *et al.*, 1982; Davison and Tipping, 1984). These are finer than the standard method of measuring dissolved metals which is filtration through a 0.45 μm membrane filter, so use of this method for iron may overestimate the 'dissolved' fraction (Kennedy, *et al.*, 1974; Mance and Campbell, 1988). Sholkovitz and Copland (1981) suggested that at least 20 – 30% of measured 'dissolved' iron existed in fine colloidal particles. Sedimentation of these colloidal particles relies on aggregation, either by self-association or by association to other particulate matter (Tipping, *et al.*, 1982; Davison and Tipping, 1984). Humic and tannic acids and inorganic anions such as phosphates and silicates may help to stabilise the colloidal iron in the 'dissolved' state, i.e. able to pass through 0.45 μm filters, by preventing aggregation. The presence of cations, particularly divalent ones (Ca^{2+} and Mg^{2+}), reduces the efficiency of phosphate to stabilise the colloidal particles resulting in greater precipitation of iron (Cameron and Liss, 1984). Flocculation of the colloid co-transports phosphates and humic acids to the sediments (Davison and Tipping, 1984).

Only about 15 – 20% of the iron in lake sediments is in the amorphous hydrous iron III oxide form. Approximately eighty percent is within the clay mineral chlorite and the rest is organic iron (Tipping, *et al.*, 1982; Davison and Tipping, 1984; Moutin, *et al.*, 1993). The chlorite fraction is a crystalline lattice form that sediments faster than the amorphous form and is unavailable for redox reactions. Only the slow-sedimenting amorphous fraction is involved both with phosphate uptake and release, depending on the prevailing redox conditions (Davison and Tipping, 1984).

1.4 IRON AS A CONTAMINANT

Information concerning the potential toxicity of iron pollution in the aquatic environment is limited. Other metals such as mercury, cadmium, copper, zinc, lead, aluminium, tin and nickel have received much more attention than iron both in field and

laboratory studies. Iron is more abundant than these metals (apart from aluminium) and is considered to be of low toxicity. Iron is, however, included as a List II substance under the European Community's Dangerous Substances Directive, which requires member states to reduce pollution of fresh and salt waters resulting from discharge of listed substances. Iron-rich discharge from abandoned mine workings and colliery spoil is the most common form of iron pollution in freshwaters (Mance and Campbell, 1988). The drainage from these sources is often acidic, resulting from the oxidising action of air, water and bacteria on sulphur exposed within coal seams or the surrounding strata (Decker and Menendez, 1974; Dills and Rogers, 1974; Letterman and Mitsch, 1978). In the reaction iron II sulphide is oxidised to iron II sulphate and sulphuric acid:

$$2FeS_2 + 7O_2 + 2H_2O \rightarrow 2FeSO_4 + 2H_2SO_4$$

Oxidation of iron II to iron III occurs when discharge enters the receiving stream. The speed of this process will depend on the resultant pH conditions of the stream. If neutralisation of the acid drainage occurs, oxidation will be rapid and precipitation of iron III oxide is likely. If the drainage remains acid, oxidation will be slow. Areas affected by mine drainage are often characterised by the presence of a layer of orange/yellow iron precipitate on the stream bottom (Letterman and Mitsch, 1978). The aesthetic appeal of the stream for recreation can be impaired. Where the drainage remains acidic direct toxicity problems from the low pH and the presence of dissolved iron may override the effects of iron precipitates (Dills and Rogers, 1974; Scullion and Edwards, 1980). Iron is not the only metal contaminant of systems affected by mine drainage. Copper and zinc were found in the River Hayle, Cornwall as a result of mine drainage but iron was found to be the most abundant metal in river water and sediments (Brown, 1977).

The majority of the laboratory and field studies concerning the effects of iron on fish and aquatic invertebrates are related to mine drainage contamination. Generally, iron II is considered to be more toxic than iron III. The former is mostly encountered in

dissolved form whereas iron III iron is often particulate. The size of iron III particles and the extent to which they sediment may affect the stress to aquatic organisms. Sykora, *et al.* (1972a) and Smith, *et al.* (1973) reported that at 2 mg litre^{-1} of particulate iron III hydroxide, hatching of fathead-minnow (*Pimephales promelas*) eggs was reduced to 23%, but with increasing iron concentration percentage hatch improved. At 50 mg Fe litre^{-1} no adverse effect on hatching was observed. The proposed explanation was that the particle size increased with increasing iron concentration. At the lowest concentrations the particles were small enough to clog the pores of the egg membranes and thus have a direct physical effect. Growth and survival of this species was impaired at the high iron concentrations used in these studies. Direct effects on fish species appear to be greater if iron is in dissolved or suspended form rather than being allowed to sediment. Decker and Menendez (1974) exposed 14 month old brook trout (*Salvelinus fontinalis*) to dissolved iron in the form of iron II sulphate. Toxicity of dissolved iron through 96 hour LC50s increased with decreasing pH (1.75 mg Fe litre^{-1} at pH 7.0 to 0.41 mg Fe litre^{-1} at pH 5.5). Ninety-six hour LC50s for dissolved iron III sulphate on juvenile brown trout (*Salmo trutta*) and juvenile rainbow trout (*Salmo gairdneri*) in flow through tests were 8.5 and 2.9 mg Fe litre^{-1} respectively (Abram and Collins, 1981). Furmanska (1979) observed loss of activity, paralysis and ataxia in the fish *Lebistes*, and the 48 hour LC50 was 73.17 mg iron III chloride litre^{-1}. Similar effects were observed on *Asellus* and the mollusc *Planibarius* (48 hour LC50s of 81.08 and 117.18 mg iron III chloride litre^{-1} respectively). These results give credence to the idea that iron II is more toxic than iron III, though different species obviously have different tolerances.

Working with juvenile (90 day old) brook trout, Sykora, *et. al.* (1972b) and Smith and Sykora (1976) observed a significant growth reduction through exposure to 12 mg litre^{-1} of suspended iron III hydroxide. Vigorous aeration of the test aquaria and maintenance of pH above neutral minimised sedimentation and levels of dissolved iron. Juvenile (90 day old) coho salmon (*Oncorhynchus kisutch*) appeared more sensitive to

iron hydroxide suspensions exhibiting reduced growth at 1.27 mg Fe litre^{-1} (Smith and Sykora, 1976). Impairment of visibility affecting food consumption was suggested as the reason for the decline in growth for these species.

If iron is allowed to settle then little direct effect on adult fish is likely. No reduction in survival of yearling rainbow trout (*Salmo gairdneri*) was found at target concentrations up to 10 mg Fe litre^{-1} in tanks where iron III sulphate added in one dose to the water surface was allowed to precipitate and settle (Abram and Collins, 1981). It is clear that the exact nature of the iron contamination is important. This will be determined in part by the pH, oxygen content and mixing regime of the receiving water.

Brown trout populations in a tributary of the River Taff, Wales affected by neutral ferruginous drainage were examined by Scullion and Edwards (1980a). Average population densities upstream and downstream of the drainage were 0.18 and 0.03 m^{-2} respectively. The viability of eggs and larvae were also reduced at the contaminated site. Iron levels were 2.39 mg Fe litre^{-1} downstream of the discharge compared with 0.71 mg Fe litre^{-1} upstream. Much of the iron will have been in suspended fine particulate form. Similarly, Letterman and Mitsch (1978) found that fish biomass downstream of neutral mine drainage discharge was reduced to 11 kg ha^{-1} from 228 kg ha^{-1} above the discharge. Reductions in abundance and diversity of macro-invertebrates were given as the reason for poor fish populations in both studies. An 80 – 90% reduction in invertebrate abundance was reported by Scullion and Edwards (1980b) and Letterman and Mitsch (1978) found 0.1 – 1.5 g wet weight m^{-2} in the deposition area compared to 14 g wet weight m^{-2} upstream of the discharge. This reduction of food resource was coupled with low diversity. Oligochaetes and chironomids tended to dominate high iron sites. These species may be largely inaccessible to fish species both through their burrowing habits and through reduced visibility in the presence of iron precipitates.

Few species of invertebrates have been exposed to iron in laboratory studies. *Daphnia middendorrffiana* was exposed to 30 mg litre^{-1} solutions of iron II chloride at controlled pH (4.5) in 48 hour tests (Havas and Hutchinson, 1982). Substantial mortality resulted but the authors were reluctant to attribute this firmly to iron as a drop in pH to 4.2 and some precipitation of iron occurred. Biesinger and Christensen (1972) found 48 hour and 3 week LC50 values of 9.6 and 5.9 mg Fe litre^{-1} respectively for *Daphnia magna* exposed to solutions of iron III chloride. Reproductive impairment of 16% at 4.38 mg Fe litre^{-1} and 50% at 5.2 mg Fe litre^{-1} was found in the same study during 3 weeks exposure. Larvae of three insect species displayed varying tolerance to iron II sulphate solution. *Acroneuria lycorias* (a stonefly) and *Hydropsyche betteni* (a caddis fly) appeared to be relatively tolerant with 50% survival at 16 mg Fe litre^{-1} for 9 and 7 days respectively. For *Ephemerella subvaria* (a mayfly), on the other hand, a 96 hour LC50 of 0.32 mg total iron litre^{-1} was reported (Warnick and Bell, 1969).

Few studies of the impact of particulate iron on invertebrates have been made. One study using *Gammarus minus* (Sykora, *et al.*, 1972a) found that the age of the precipitate may affect the toxicity. Fresh suspended iron precipitates were more detrimental in 7 and 21 day tests than 6.5 hour-old precipitate. The majority of the above studies have concentrated on mortality as their measure of toxicity. Gerhardt (1992) examined the effect of iron on the motility, gill ventilation and feeding, as well as survival, of the mayfly *Leptophlebia marginata*. Iron was added to stream water as iron II sulphate solution at two pHs, 4.5 and 7. At pH 7 the dominant dissolved iron species was iron III, whilst at pH 4.5 the dominant dissolved species was iron II with precipitation of iron, presumably iron III, after 5 days. At pH 7 no significant differences in motility or feeding were observed between control animals and those in all iron treatments upto 50 mg Fe_{tot} litre^{-1}. Feeding and motility were significantly reduced in all iron treatments (range 10 - 50 mg Fe_{tot} litre^{-1}) at pH 4.5. In the highest concentration at this pH animals stopped feeding for two weeks and some mortality (20%) resulted. The larvae were found to be constipated, having bloated midguts

packed with food which allowed no transport in either direction. At both pH levels iron compounds were found in the gut and additionally associated with the body surface and gills at pH 4.5 only. No iron was found in the other tissues. Uptake of iron II may have directly destroyed the gut membrane by ulceration or by the formation of precipitates onto the membrane and may have affected the muscular system preventing peristalsis. In a similar study by the same author (Gerhardt, 1994) escape behaviour of the mayfly was lost due to the formation of iron crusts on the body surface. This may have prevented uptake of oxygen and other ions. Iron did not appear to be taken up by the mayfly, other than by surface adsorption. Gerhardt concluded that iron was regulated by *Leptophlebia* and that cytotoxic effects were not responsible for changes in the mayfly's survival or behaviour.

Some trends are clear from the review presented above. The exact speciation and solubility of the iron is important: dissolved iron II is probably more toxic than dissolved iron III but this latter species is more likely to occur in particulate form. If the particulate iron is suspended it may interfere with the ability of visual feeders to find prey and if it sediments out it may eliminate natural niches for invertebrates and for fish spawning. The Environmental Quality Standard (EQS) document for iron (Mance and Campbell, 1988) reports that water hardness appears to have little effect on the toxicity of iron but that at pHs below neutral an increase in toxicity may occur. The EQS value suggested by this report for the protection of freshwater life is 2 mg Fe_{tot} $litre^{-1}$. It is mainly based on direct effects from field observations of fisheries and biotic scores relating to total iron levels. Indirect effects of iron contamination such as habitat smothering are not included in the EQS but are deemed by the authors to be as important as direct toxicity of iron, and avoidance of iron deposition is recommended.

1.5 IRON III DOSING: POTENTIAL FOR CONTAMINATION

Iron-toxicity studies have been largely restricted to field observations and LC50 tests, but these clearly suggest that the discharge of iron to freshwater ecosystems can be detrimental and should be avoided. It is clear, therefore, that the introduction of an iron III dosing scheme for phosphate inactivation in a reservoir may incur a deleterious ecological effect. The success or failure of iron III dosing as a eutrophication control measure is not the concern of this study, though obviously it is of major concern for those who have the task of long-term management of such sites. The concern of this project was the potential of the dosing scheme to have a damaging impact on the fauna of the ecosystem, and in particular on benthic invertebrates.

As discussed earlier (section 1.2.1) the principal aim of iron III dosing is to precipitate available phosphate to the sediments. The greatest accumulation of iron is liable, therefore, to be at the sediment surface. The most direct effect of this is likely to be on benthic invertebrates either directly from iron toxicity or indirectly through the smothering of their habitat by iron-rich precipitates. Beckley (1981) reported that the clam, *Mactra lilacea* filtered iron ore dust from sea water but eliminated it within faeces. Lamb and Bailey (1981) observed high mortality and impeded movement in the midge *Tanytarsus dissimilis* when exposed to concentrations of aluminium sulphate (alum) that had precipitated to form a whitish sediment of aluminium hydroxide. Decline of invertebrate communities may have a deleterious effect on fish populations that rely on them for food (Letterman and Mitsch, 1978; Scullion and Edwards, 1980a). Since the solubility of iron varies with factors such as pH and oxygen content, increases in dissolved and suspended iron are possible. A particular risk may come from the potential reduction of iron III precipitate to dissolved iron II during periods of anoxia. Relatively, however, the impact of increased levels of dissolved iron II iron during variable periods of anoxia may be of little consequence compared to the long-term exposure of the system to iron III precipitates. Information on the latter is confined to

field observations of streams receiving mine drainage. This project has attempted to redress this with particular reference to the use of iron III compounds for eutrophication control.

The study has concentrated on the effects of iron III dosing on chironomids as representatives of benthic invertebrates. Chironomids form a dominant part of profundal invertebrate communities and through their burrowing and detritivorous habits are likely to come into direct contact with the iron III floc. Additionally, certain species are particularly practical for use in the laboratory (see Chapter Three) and much work on their sensitivity to a number of metals is available in the literature. Extensive long-term benthic surveys have been performed by the National Rivers Authority and Anglian Water Services at a number of iron III dosed reservoirs in the Anglian region of the U.K. This study has, therefore, been largely laboratory based to avoid too much repetition of these surveys. Chironomid samples provided by the National Rivers Authority from one of the dosed reservoirs have, however, been examined as part of this study with the aim of elucidating changes in chironomid community.

Laboratory cultures of a chironomid species, *Chironomus riparius* (Meigen), have been used to examine the effect of iron III addition and the sedimentation of resultant precipitates. Exposure of test organisms to sedimented precipitates is uncommon (but see Lamb and Bailey, 1981) and the absence of standard procedures has required the development of applicable methodology. Addition to reservoir water has been used to ascertain the extent of precipitate formation and sedimentation and to assess the impact of any pH depression caused by the addition. That iron III addition can cause pH depression has been noted in several studies (Abram and Collins, 1981; Havas and Hutchinson, 1982). The principal aim of the study has been to appraise the effects of long-term exposure to iron precipitates both in terms of mortality and of sub-lethal parameters. Is the generally held view that iron III iron is not harmful, correct? If not, are iron III precipitates directly toxic or does the floc physically impair the test

organisms? The final aim is to apply the findings of the laboratory studies where possible to the field situation with reference to iron III dosing as a management tool.

SUMMARY

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).

Methods to control eutrophication are based on either top-down or bottom-up control. The former often involves the introduction of piscivorous fish species or the removal of zooplanktivorous species. This allows larger zooplankton to dominate and graze more efficiently on phytoplankton. Bottom-up control involves the reduction of nutrient loading to the system and so limits phytoplankton production. Phosphorus, rather than nitrogen, is deemed easier to limit and so most nutrient loading schemes 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. 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. The measures needed to control eutrophication vary from system to system but often some combination of approaches is necessary to achieve the desired response (Moss, 1990).

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.

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 iron III 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 including the suggestion that deposition of iron should be avoided.

The concern of this study is that the use of large quantities of iron III 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 iron III floc which results from iron III dosing. Appraisal of the effects of prolonged exposure to iron III 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. Comment on the results in relation to iron III dosing as a eutrophication control measure will be made.

Chapter Two

Field study of the impact of iron III dosing at Rutland Water, Leicestershire on the distribution of chironomids.

2.1 INTRODUCTION

Rutland Water is a lowland pump storage reservoir situated to the east of Leicester. The reservoir was impounded in 1976, and receives water from the Rivers Welland and Nene through a submerged inlet in the South Arm (Figure 2.1). It is the largest pumped storage reservoir in Britain with a surface area of approximately 1260 ha (3112 acres) and maximum and mean depths of 34m and 10.6m respectively (Knights 1981; Hayes and Greene 1984; Champion, *et al.,* 1991). Rutland Water has high amenity value, including an artificially stocked trout fishery, in addition to use for water supply. The reservoir has been designated as a Site of Special Scientific Interest (SSSI) and as a Ramsar site principally for internationally important waterfowl populations of Shoveler (*Anas clypeata*), Gadwall (*Anas strepera*), and Teal (*Anas crecca*) (Appleton 1981).

Rutland Water is typical of many reservoirs in the Anglian region in that it is eutrophic due to high nutrient loadings from its source rivers. Awareness of the potential for this problem at Rutland Water was present at the planning stage and a system of 'Helixor' tubes were placed in the main basin during the reservoir's construction (Figure 2.1). The aim of this system was to prevent stratification and thus limit the hypolimnetic oxygen depletion associated with eutrophic lakes (Harper and Bullock, 1982). Whether destratification is achieved in the long North and South Arms

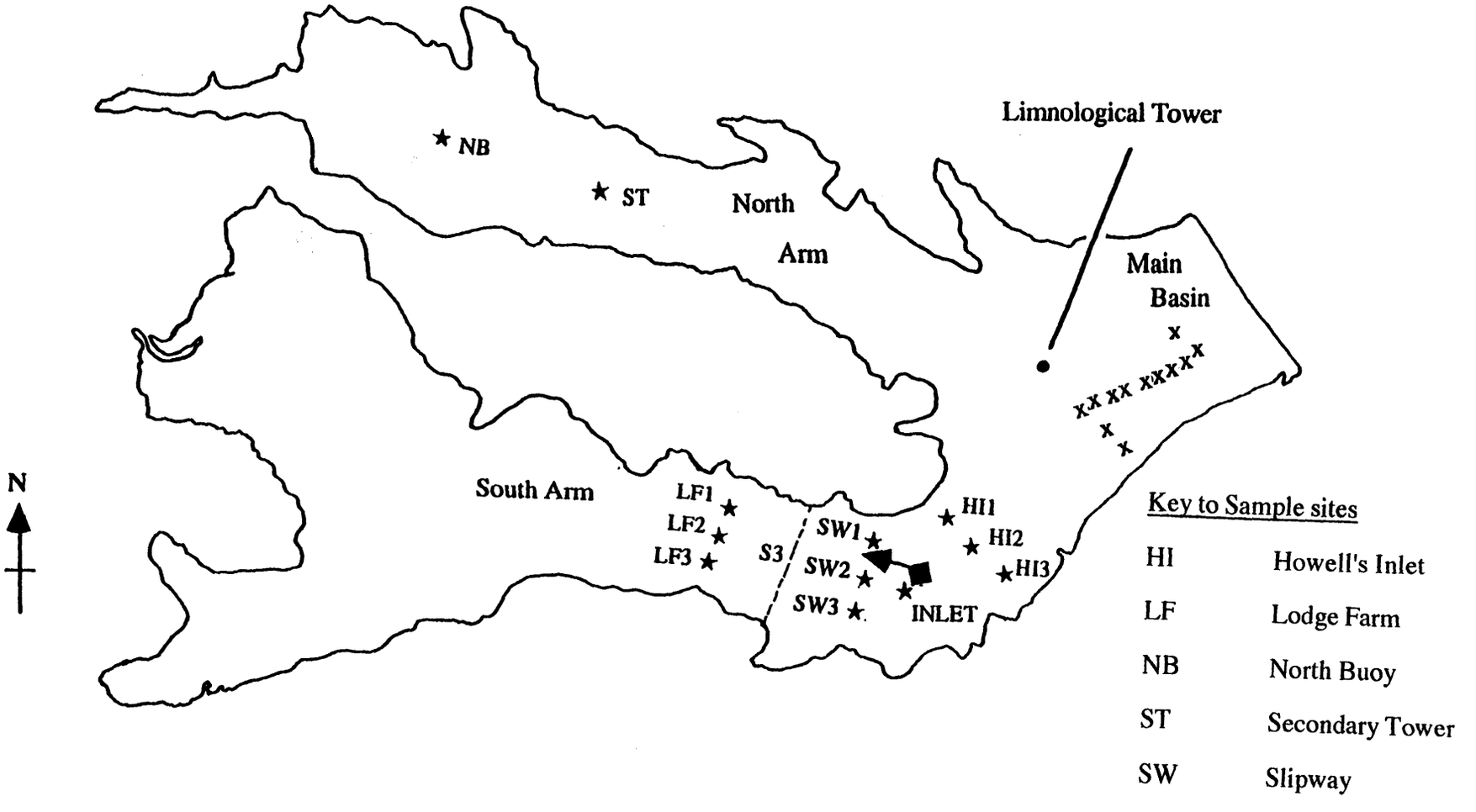

Figure 2.1 *Outline map of Rutland Water, Leicestershire showing the positions of the inlet jet, limnological (draw-off) tower, 'helixor' destratification cylinders (x) (Harper & Bullock, 1982), National Rivers Authority grid sampling sites (★), and the S3 transect. Modified from Extence,* et al., *(1992).*

of Rutland Water is questionable and the requirement of additional eutrophication control measures has become clear in the light of persistent nuisance algal blooms.

In 1989 a combination of favourable weather conditions and high phosphate availability lead to extensive blooms and scums of the cyanobacteria *Microcystis aeruginosa* (Champion *et al.* 1991). Besides the potential problems for water treatment that such blooms imply (Hayes and Greene 1984), the death of a number of sheep and dogs that had drunk from the reservoir was associated with toxins produced by the 1989 bloom. Public concern was voiced and considerable disruption to recreational activities occurred at Rutland Water and at many reservoirs across the UK. Anglian Water Services (AWS) requested and received consent to dose Rutland Water and other badly affected reservoirs with iron III sulphate for phosphate inactivation in order to combat algal blooms and allow continued use of the reservoir as an amenity. This followed a similar, apparently successful dosing scheme adopted by Anglian Water Services at Foxcote Reservoir, Buckinghamshire in 1981 (Hayes and Greene 1984; Young, *et al.*, 1988).

The initial period of iron III dosing at Rutland Water commenced in June 1990 and ran until October of the same year. Originally iron III sulphate was added via the submerged inlet, but due to cessation of pumping from the Rivers Welland and Nene because of low water levels, later additions were made from a barge. Barge dosing was concentrated in the main basin. A second period of dosing began in December 1990 and has continued to the present with only a few short breaks usually concurrent with periods of pumping from the less eutrophic River Welland. Only dosing via the inlet waters has been used during this second period (Champion, *et al.*, 1991).

The impact of the dosing scheme both in positive terms i.e. control of phosphate/algal levels and in negative terms i.e. the possibility of detrimental effects on the ecology of the reservoir has been the subject of a National Rivers Authority

monitoring programme (Champion, *et al.*, 1991; Extence, *et al.*, 1992). Part of the programme has focused on benthic macroinvertebrate communities. Routinely, the National Rivers Authority takes most macroinvertebrate identification to species level; however, the chironomid portions of profundal (>12 metres depth) benthic samples were only identified to family level and were provided for further examination by the author. Chironomids form a dominant part of most benthic communities occurring in high abundances and often with wide diversity. Members of the family exhibit a range of feeding habits e.g. deposit and filter feeders, and exist within different trophic levels e.g. detritivores and predators (Pinder, 1986). Potentially, therefore, much information can be gained by identification of chironomids beyond family. This chapter discusses trends in chironomid community structure in relation to iron III dosing.

2.2 PROCEDURE

Duplicate Ekman grab samples from twelve deep water sites (>12 m) have been taken on a bi-monthly basis since December 1991. The positions of these grid survey sites are shown in Figure 2.1. Modification of the Ekman grab for use with a messenger was required to sample sites with high iron precipitate loadings due to their flocculent nature (Brierley, pers comm.). Samples were returned to the laboratory for sorting and preservation.

Identification of chironomid larvae from the December 1991 samples was made to genus using the following procedure:- Internal tissues of larvae were cleared by placing in Histoclear solution for approximately one week. Dissected head-capsules were mounted on slides using Euparal. Slides were placed on a 60 ^{o}C hotplate for about two weeks for the Euparal to dry. Histoclear is soluble in Euparal and so no rinsing between these steps was necessary. Head-capsules from large larvae were removed prior to placing in Histoclear solution, whilst small larvae were placed directly into Histoclear and head-capsules were removed prior to mounting.

For the majority of larvae it is necessary to view the head-capsule from its ventral surface. Where orientation of the head-capsule was wrong some key features were obscured and full identification could not be achieved. For the December 1991 samples a number of larvae could only be identified as *Tanytarsus/Micropsectra*, the two genera of the Tanytarsini tribe found in Rutland Water (Brown 1981; Brown and Oldham 1981), because of this problem. It is worth noting, however, that only *Tanytarsus* larvae were identified from correctly oriented Tanytarsini head-capsules. Identification was based on Cranston and Reiss (1983); Fittkau and Roback (1983) and Pinder and Reiss (1983).

For speed, chironomid larvae from the other bi-monthly grid surveys were placed into one of four easily identifiable groups; *Chironomus*, *Procladius*, Tanytarsini tribe and others. *Chironomus*, which tend to be large larvae, may be distinguished by having two pairs of ventral tubules and two pairs of well developed anal tubules (Pinder and Reiss 1983). *Procladius* is the only tanypod genus identified from these samples, it has an oval head-capsule with retractile antennae and a well developed ligula (Cranston and Reiss 1983; Fittkau and Roback 1983). The Tanytarsini tribe tend to be small larvae and can be recognised from other members of the sub-family Chironominae, at dissection microscope level, by having long antennae (in relation to head capsule size) mounted on a well developed pedestal (Pinder and Reiss 1983). Figure 2.2 illustrates the features described above. This scheme avoids the need for time-consuming dissection, clearing and mounting of specimens, whilst allowing identification of the dominant taxa encountered.

The similarity between sites on each sampling date was examined using the Bray-Curtis Coefficient (Bray and Curtis, 1957). This is a similarity coefficient calculated between every pair of samples from taxa abundances (numbers per metre square). Since most taxa were present in the majority of samples the use of a similarity coefficient based on taxa abundances rather than presence/absence of taxa was deemed

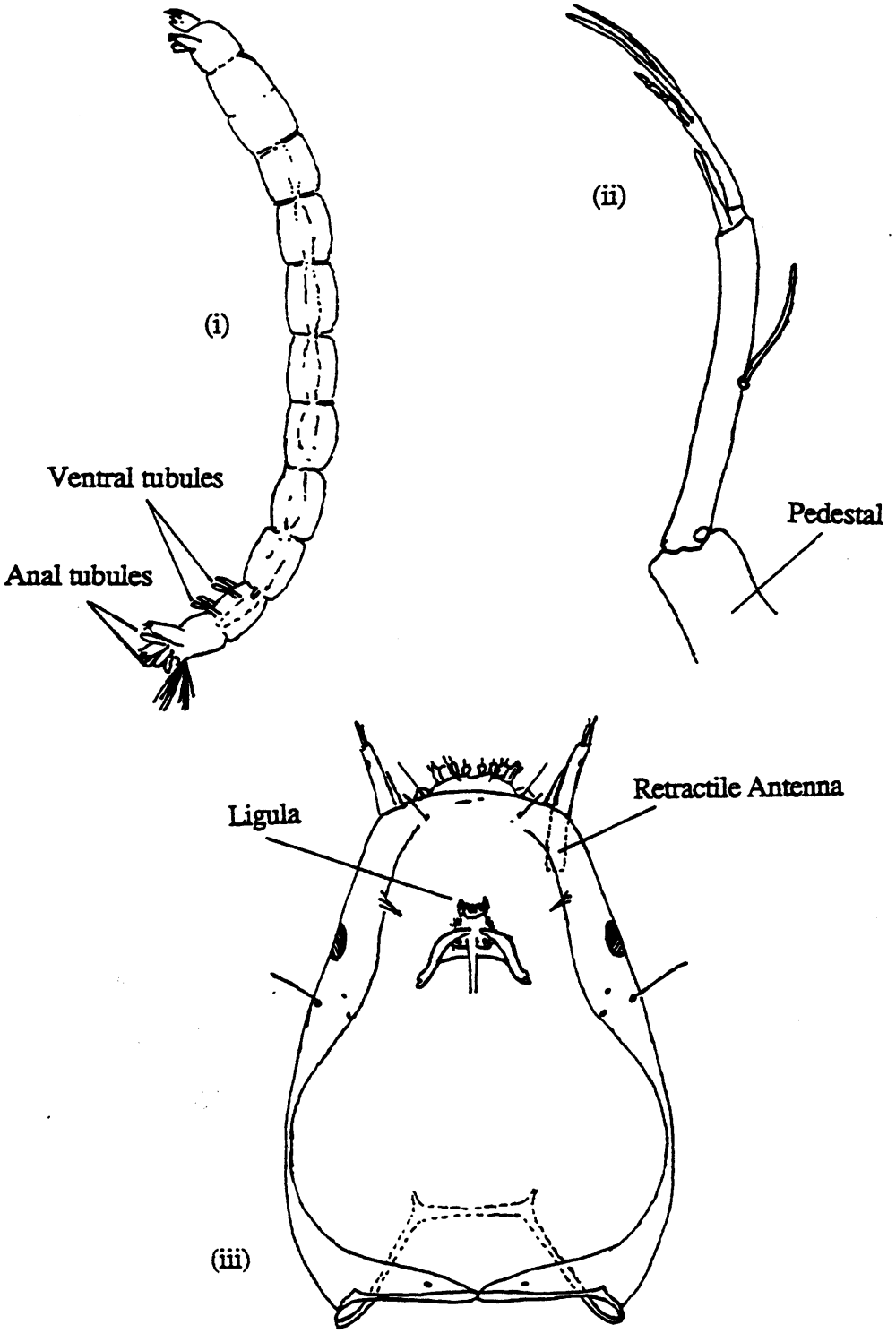

Figure 2.2 *(i)* Chironomus *larva showing the position of ventral and anal tubules, (ii) antenna of* Tanytarsus *(tribe Tanytarsini) showing well developed pedastal and (iii) head capsule of* Procladius, *oval in shape with retractile antennae and well developed ligula (Cranston and Reiss, 1983; Pinder and Reiss, 1983).*

appropriate. The Bray-Curtis coefficient is dominated by the abundant species and is at its best when species diversity is low and sample size is small (Krebs, 1989). The chironomid communities at Rutland Water are dominated by a few abundant species and sample sizes are often small, thus Bray-Curtis was felt to be a suitable choice of similarity coefficient. Cluster analysis of the resulting matrices was by hierarchical agglomerative clustering.

2.3 RESULTS

2.3.1 December 1991 grid survey

All the samples were taken from sites deeper than 12m where invertebrate communities generally tend to be profundal in nature, low diversity but very high densities (Jonasson 1978). This is mirrored by the Chironomidae. In total 26 chironomid genera have been recorded at Rutland Water (Brown 1981; Brown and Oldham 1981) but only 8 of these were found in the NRA's profundal samples, these are listed in Table 2.1.

Table 2.1 *Chironomidae from Rutland Water December 1991 grid survey.*

Subfamily	Tribe	Genera
Tanypodinae		*Procladius*
Chironomidae	Chironomini	*Chironomus*
		Polypedilum
		Glycotendipes
		Cryptochironomus
		Microtendipes
		Chironomus/Polypedilum
	Tanytarsini	*Tanytarsus*
		Tanytarsus/Micropsectra

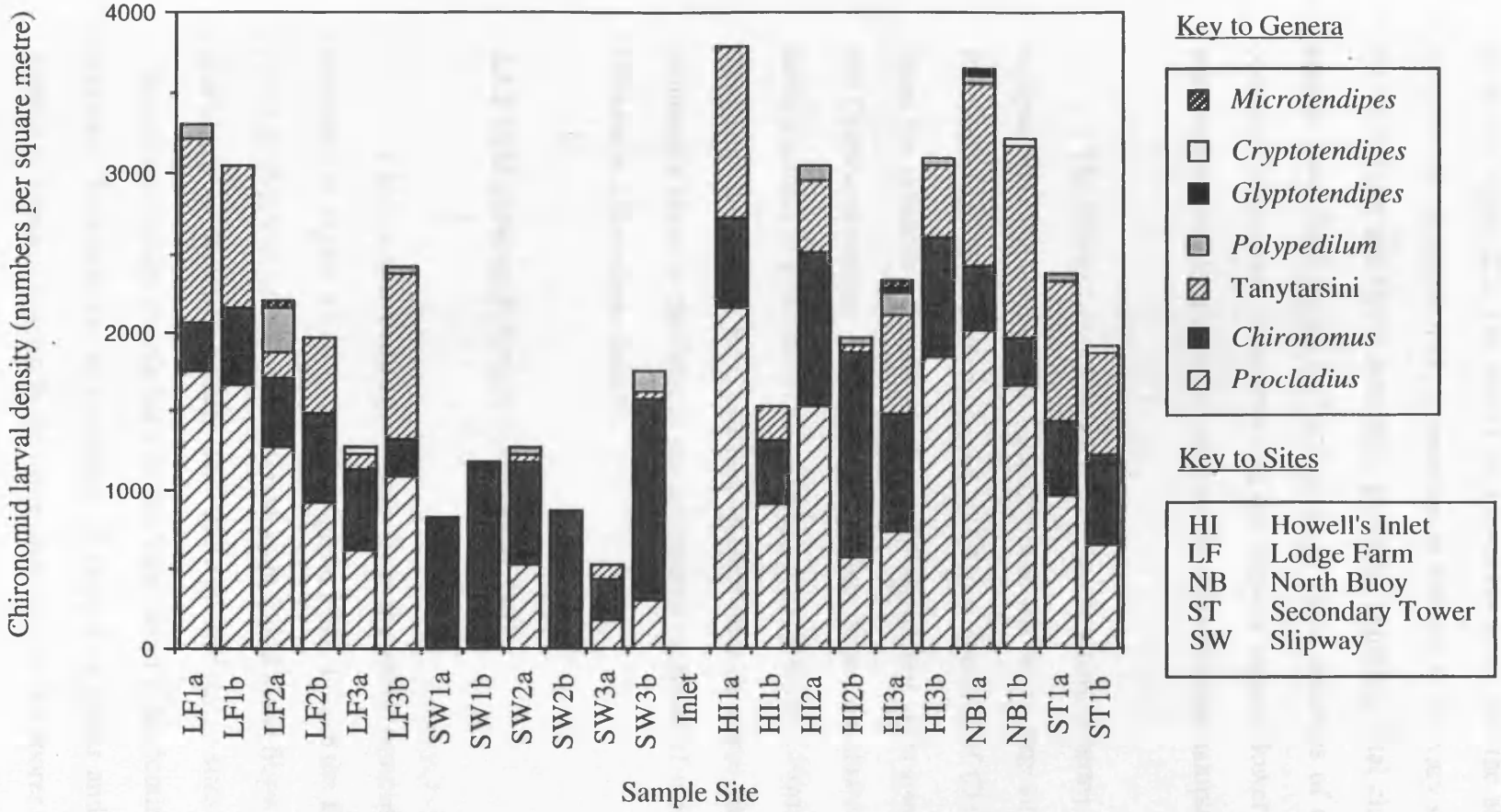

Figure 2.3 *Abundance (numbers per square metre) of chironomid taxa at Rutland Water, results of December 1991 grid survey of grab samples relating to the sites marked in Figure 2.1. Note: Tanytarsini refers to specimens identified as* Tanytarsus *and/or* Tanytarsus/Micropsectra.

The densities of these genera found in the December 1991 grid survey are shown in Figure 2.3. The results of this survey show that the abundance and species composition of chironomid communities in Rutland Water vary spatially. Lodge Farm, Howell's Inlet and North Arm sites generally exhibited total chironomid densities of greater than 2000 larvae m^{-2} and in some cases densities of over 3000 larvae m^{-2} occurred. In contrast, densities along the Slipway transect lower than 1000 larvae m^{-2} were typical. No chironomid larvae were found in the Inlet sample.

The Slipway sites also vary from the other sites in terms of chironomid species composition. The Lodge Farm, Howell's Inlet and North Arm sites were dominated by *Procladius* and the Tanytarsini tribe with lower numbers of *Chironomus* and in some cases the presence of small numbers of *Polypedilum*, *Microtendipes*, *Glyptotendipes* and *Cryptochironomus*. The Slipway sites were largely dominated by *Chironomus* with lower numbers of *Procladius*, Tanytarsini and *Polypedilum*. Numbers of *Chironomus* at these sites differ little from numbers at other sites. It seems likely that *Chironomus* dominance close to the Inlet is due to reduced presence of other taxa rather than an increase in *Chironomus* density.

2.3.2 Grid surveys; February – November 1992

Chironomid community data from the other bimonthly grid surveys are illustrated in Figure 2.4 (i) – (vi). Throughout 1992, as with the December 1991 survey, lower abundances of chironomid larvae were found at the Slipway and Inlet sites than elsewhere in Rutland Water. The Slipway and Inlet sites were dominated by *Chironomus*, whilst *Procladius* and the Tanytarsini tribe dominated elsewhere in the reservoir. Furthermore, this absence of large *Procladius* and Tanytarsini densities appears to explain completely the lower abundance at the poorer sites.

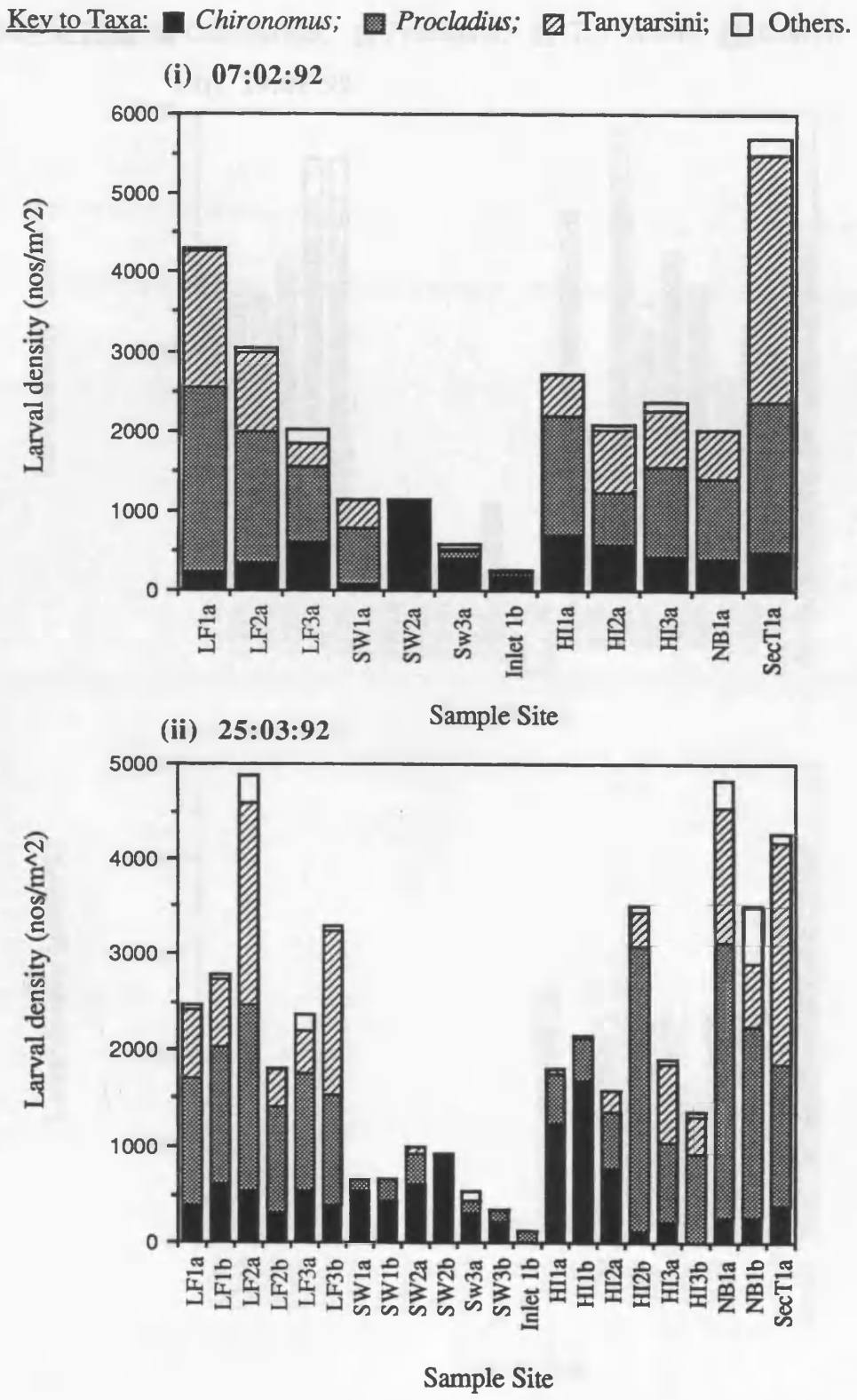

Figure 2.4 *Larval density of four chironomid taxa from benthic grid surveys at Rutland Water, (i) February 1992, (ii) March 1992.*

Key to sites: *HI = Howell's Inlet, LF = Lodge farm, NB = North Buoy, SecT = Secondary Tower and SW = Slipway.*

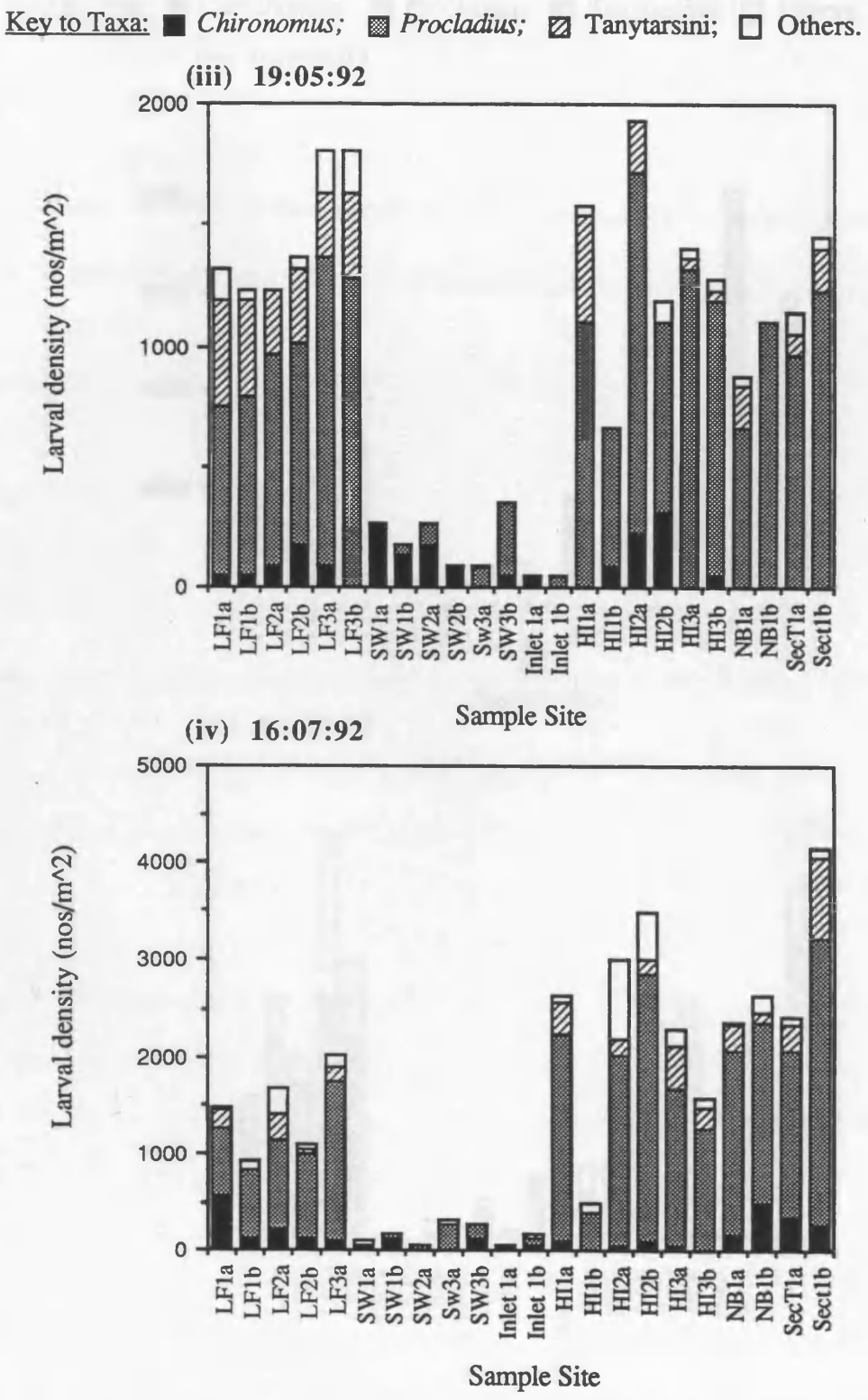

Figure 2.4 *Larval density of four chironomid taxa from benthic grid surveys at Rutland Water, (iii) May 1992, (iv) July1992.*

Key to sites: *HI = Howell's Inlet, LF = Lodge farm, NB = North Buoy, SecT = Secondary Tower and SW = Slipway.*

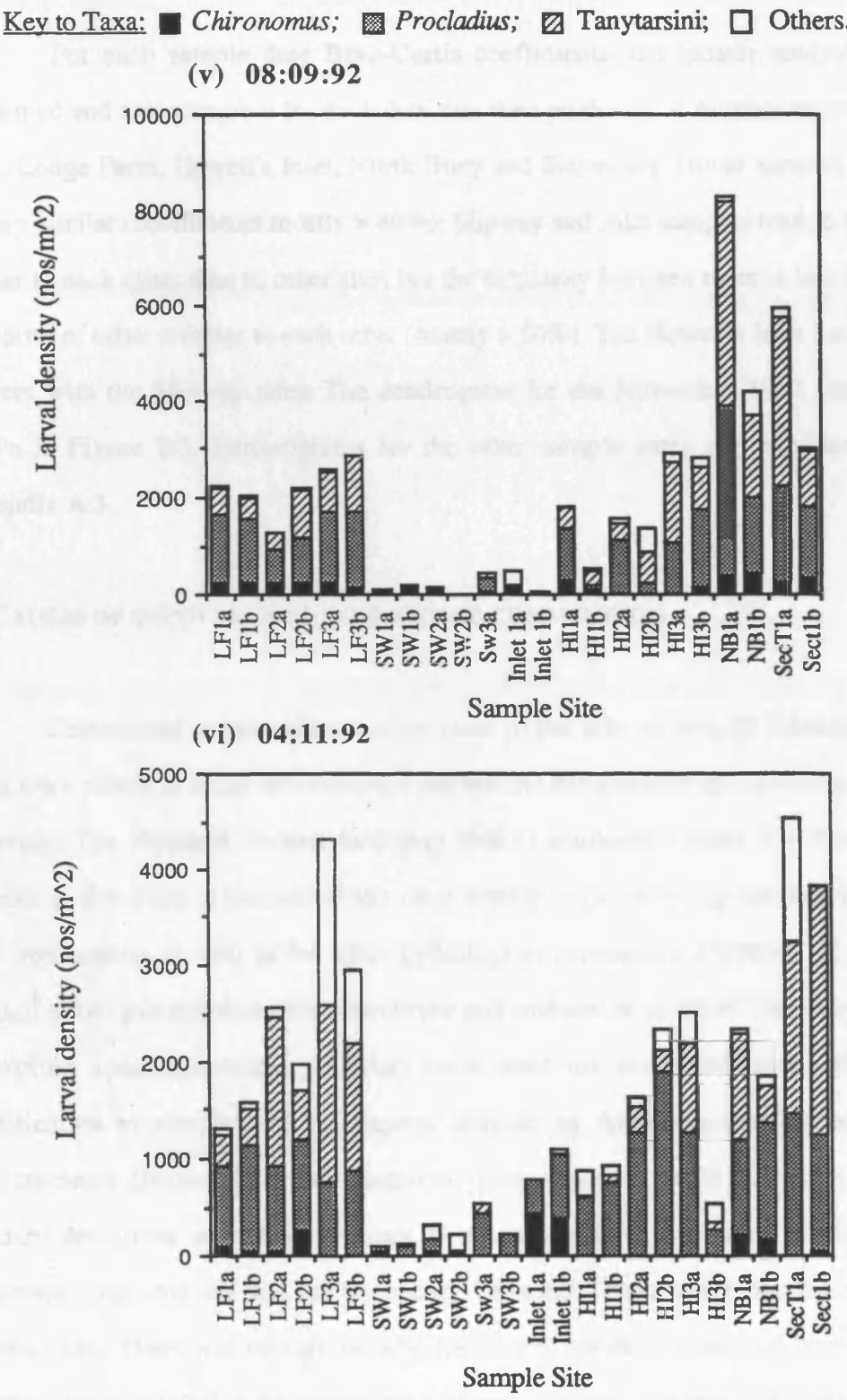

Figure 2.4 *Larval density of four chironomid taxa from benthic grid surveys at Rutland Water, (v) September 1992, (vi) November 1992.*

Key to sites: *HI = Howell's Inlet, LF = Lodge farm, NB = North Buoy, SecT = Secondary Tower and SW = Slipway.*

For each sample date Bray-Curtis coefficients and cluster analysis were performed and a dendrogram for each date was then produced. A number of trends are clear. Lodge Farm, Howell's Inlet, North Buoy and Secondary Tower samples tend to be very similar (coefficients mostly > 80%); Slipway and Inlet samples tend to be more similar to each other than to other sites but the similarity between them is less than the similarity of other samples to each other (mostly > 50%). The Howell's Inlet 1 site often clusters with the Slipway sites. The dendrogram for the November 1992 samples is shown in Figure 2.5. Dendrograms for the other sample dates are provided in the Appendix A.3.

2.4 CAUSES OF IMPOVERISHED CHIRONOMID COMMUNITIES

Chironomid communities at sites close to the inlet of iron III sulphate-dosed water were poorer in terms of abundance and species composition than elsewhere in the reservoir. The National Rivers Authority (NRA) routinely collect 0 – 5 m water samples at five sites at Rutland Water on a weekly basis analysing for dissolved and total iron content as well as for other hydrological parameters. Filtration of samples through a 0.45 μm cellulose filter membrane and analysis of acidified filtrate by atomic absorption spectrophotometry (AAS) were used for dissolved iron estimation. Acidification of samples and subsequent analysis by AAS was used for total iron measurements (National Rivers Authority, pers. comm.). Table 2.3 gives means, standard deviations and Mann-Whitney U-test results from two sites; the Inlet as a potentially high iron site and the Secondary Draw-off Tower in the North Arm as an iron-free site. There was no significant difference in levels of dissolved iron between the two sites ($p > 0.05$), but a significant difference in total iron levels ($p < 0.001$) was present. Elevated total iron levels at the Inlet site are likely to be due to the presence of suspended iron precipitate particles. That there is no increase in dissolved iron at the

Inlet site suggests that all the dosed iron is precipitated and that this must happen rapidly.

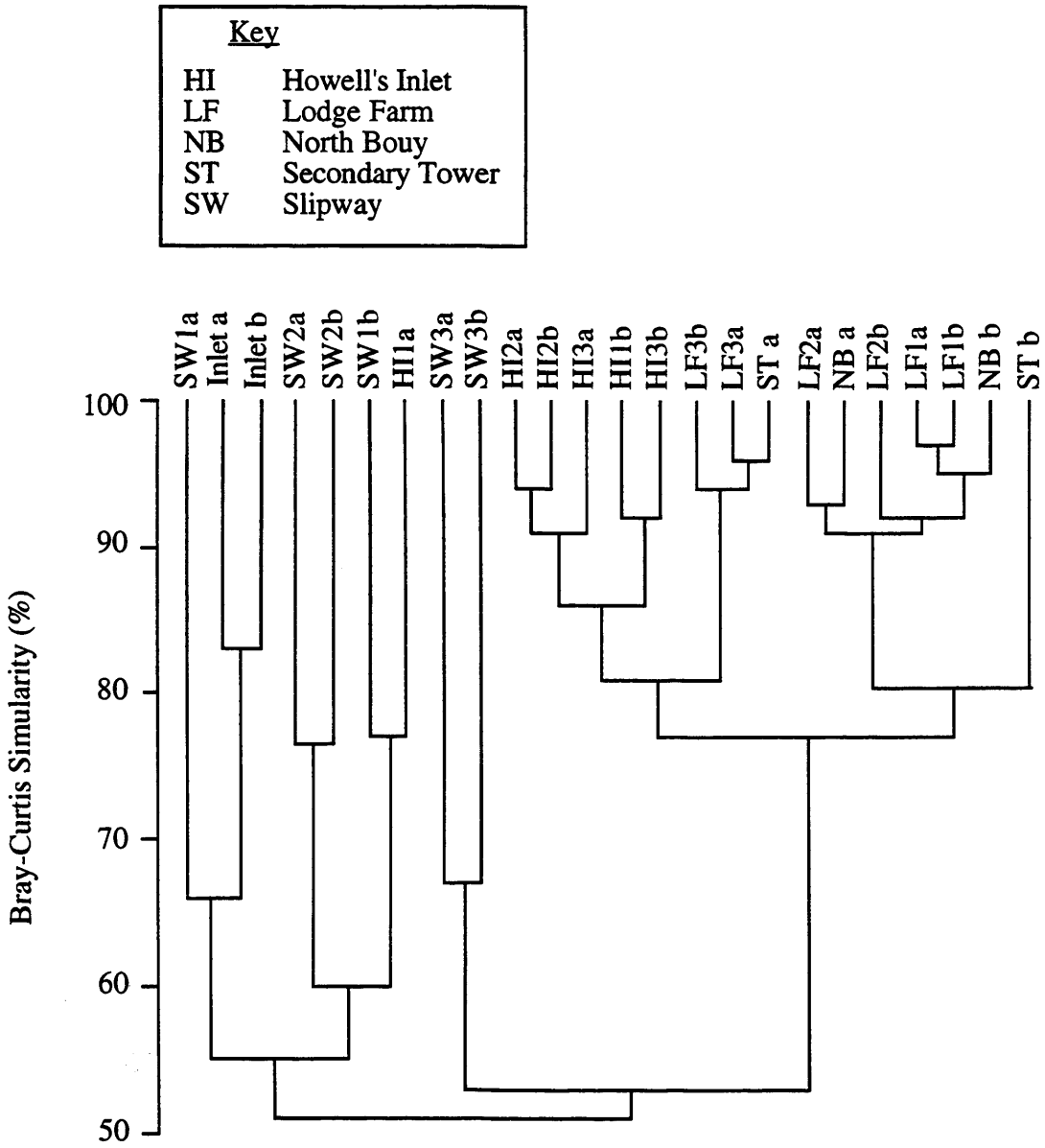

Figure 2.5 *Dendrogram of Bray-Curtis similarity coefficients from the NRA November 1992 grid survey of grab samples taken from the sites marked in Figure 2.1. Sorting of the Bray-Curtis matrix was achieved using hierarchical agglomerative cluster analysis.*

Table 2.3 also shows that pH levels at the Inlet were significantly lower than at the Secondary Tower ($p < 0.001$). The pH of iron III sulphate solution is very low

and has been shown in laboratory tests to depress the pH when added to solutions (see later chapters). It is possible that the addition of iron III sulphate at Rutland Water has caused a local depression of pH around the inlet; however, the lowest recorded pH at the inlet during the period examined was pH 7.75 and thus serious effects from pH depression in the water column are unlikely.

***Table 2.2**: Description parameters and Mann-Whitney U-test values for comparison between 0-5m water samples taken and chemically analysed by the National Rivers Authority between 5th May 1992 and 7th September 1992 from a high iron site (Inlet) and an iron free site (Secondary Tower). All figures have been calculated from raw data supplied by the National Rivers Authority.*

		Secondary Tower	Inlet	Mann - Whitney U Test Calculated U	Tabulated U	p
Dissolved iron	Mean	.012	.016	123	83	> 0.05
(mg Fe litre^{-1})	SD	.005	.015			
	n	16	16			
Total iron	Mean	.021	1.182	7	51	< 0.001
(mg Fe litre^{-1})	SD	.014	1.79			
	n	18	15			
pH	Mean	8.23	8.05	35	60	< 0.001
	SD	.072	.171			
	n	19	16			

Total iron levels in sediments for the first grid survey (December 1991) are presented in Figure 2.6 (supplied by the National Rivers Authority). 5 cm cores were taken through the top of the grab samples. These were digested and analysed using atomic absorption spectrophotometry. Similar levels, approximately 60 mg Fe g^{-1} dry weight of sediment, were found at all the Howell's Inlet, Lodge Farm and North Arm (North Buoy and Secondary Tower) sites. The Inlet site and sites 1 and 2 on the

Slipway transect show highly elevated iron levels upto 558.8 mg Fe g^{-1} dry weight (Inlet site).

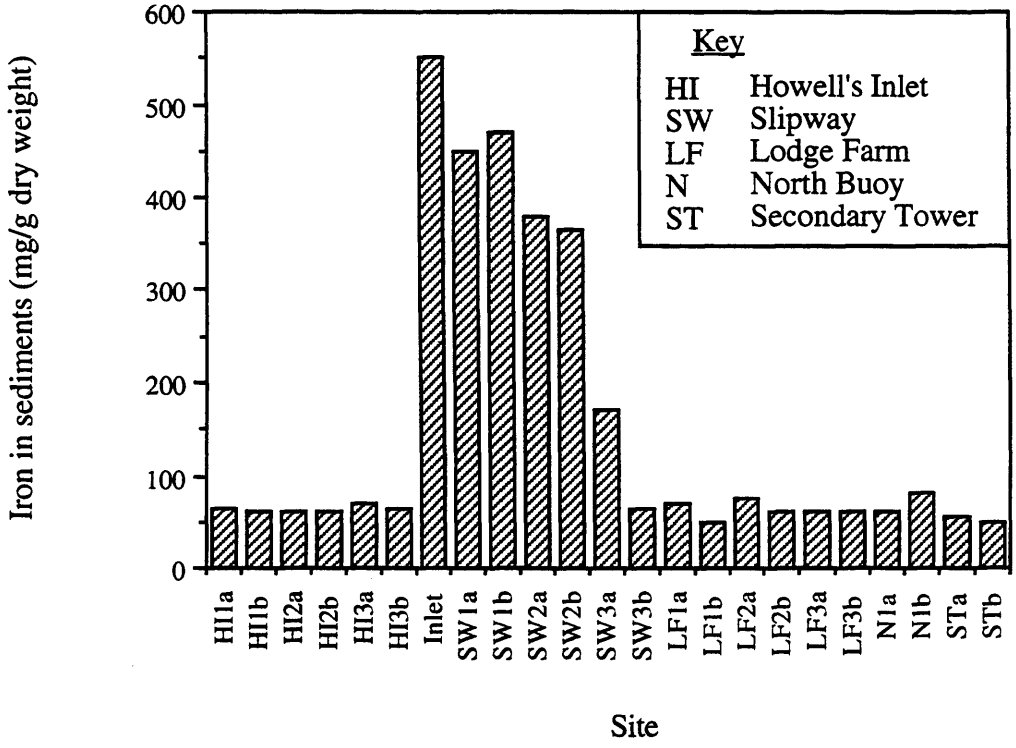

Figure 2.6 *Total iron in sediment levels recorded from modified Ekman grab samples taken by the National Rivers Authority, 4th December 1991.*

The distribution of iron precipitates appears to correspond well with the distribution of impoverished chironomid communities. This is illustrated by the scatter plot of chironomid density against sediment iron concentration (mg Fe g^{-1} dry weight) modified from data supplied by the NRA (Figure 2.7). Above approximately 100 mg Fe g^{-1} dry weight of sediment only low chironomid densities were found.

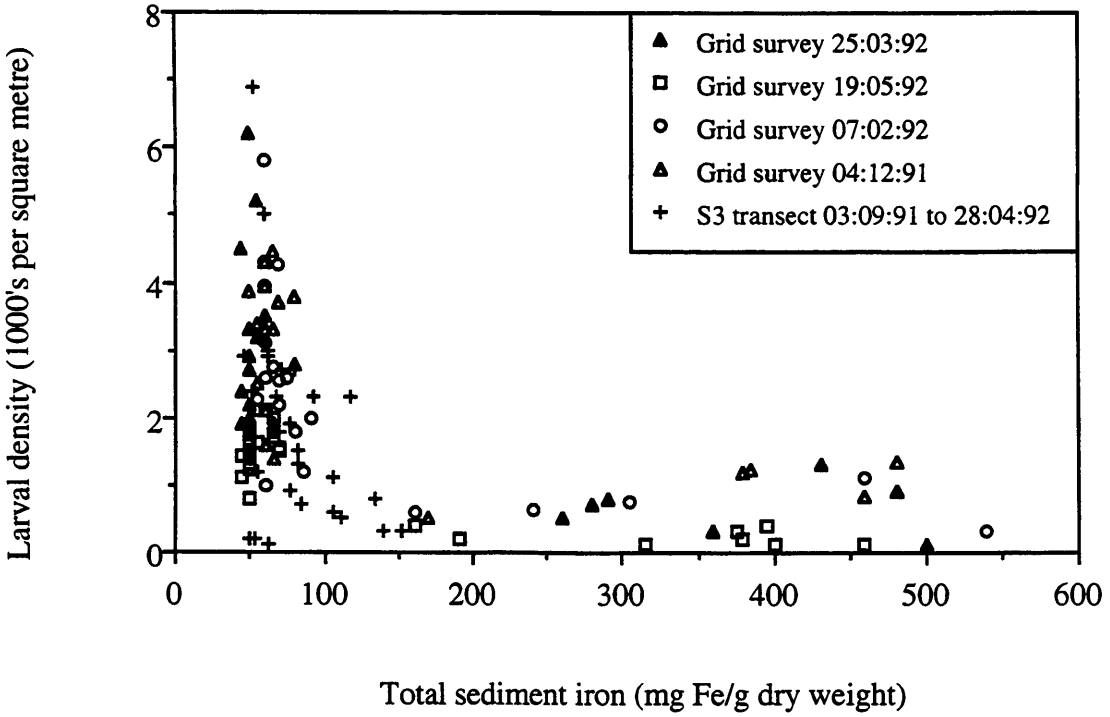

***Figure 2.7** Density of chironomid larvae against levels of total iron in sediments at Rutland Water, modified from Extence and Brierley pers comm.. Samples are from four of the NRA grid surveys and from NRA samples from the S3 transect (South Arm between Slipway and Lodge Farm transects).*

2.5 DISCUSSION

Sites close to the inlet of iron III dosed water at Rutland Water had poorer chironomid communities in terms of abundance and species composition than the rest of the reservoir. Similar results have been found by the National Rivers Authority monitoring programme for other benthic taxa, though individually chironomids provide the most marked differences (Extence, *et al.*, 1992). During the initial period of barge dosing (June – October 1990) the monitoring scheme covered three transects; North Arm, South Arm and Main Basin. Cluster analysis showed that most sites were relatively uniform in taxa present; chironomids, oligochaetes, *Pisidium* sp. and ostracods predominated. Shallow margin sites and sites in which clusters of the zebra mussel

(*Dreissena polymorpha*) occurred tended to harbour more diverse communities. Sites on the south end of the Main Basin transect and at the Outlet Buoy (where loading of iron III sulphate onto the barge took place) averaged fewer taxa than deep, non-*Dreissena* sites. Iron staining was seen at all of these poor community sites. Following the shift from barge dosing to inlet dosing in December 1990 a similar pattern of invertebrate distribution was found except that the poor community sites were now situated along the South Arm transect close to the inlet. Recovery of sites in the Main Basin seems to have occurred (Champion, *et al.*, 1991). Grid survey samples instigated by the National Rivers Authority in December 1991 have consolidated the early results. Abundance and diversity of invertebrates at sites close to the inlet appear to be reduced (Extence, *et al.*, 1992).

The movement of areas of poor invertebrate communities from the Main Basin to the South Arm with the switch of dosing from the barge to the inlet, is in itself an indication that iron III dosing leads to qualitative and quantitative loss of benthic invertebrates. The absence of pre-dosing data for the distribution of invertebrates at Rutland Water, however, hinders these conclusions. An alternative explanation might be that invertebrate communities are damaged by scouring by high flow from the inlet. In the case of chironomids this seems unlikely since species closely related to those in the reservoir survive well in the high scouring conditions of river systems. Also large amounts of flocculent iron precipitates are maintained very close to the inlet, which suggests that flow from the input causes little disturbance in that region (National Rivers Authority, pers. comm.)

It seems unlikely that the differences in invertebrate communities observed at Rutland Water are due to an increase of dissolved iron since there appeared to be no difference in levels of dissolved iron between the Inlet site and the Secondary Tower in the undosed North Arm. A significant difference in pH was observed between these two sites but since the lowest pH recorded from 0 – 5 m samples was 7.75 it is unlikely that

pH reduction has seriously affected invertebrate communities. The first National Rivers Authority grid survey (December 1991) indicated elevated total iron in sediment levels at sites close to the inlet, up to approximately 10 times greater than the background levels elsewhere in the reservoir. Grab samples from these sites are likely only to contain iron precipitates. The levels recorded at these sites are therefore artificially high when compared to sites where some 'natural' sediment is included in the sample. However, they serve to illustrate that sedimentation of iron precipitates close to the inlet is very high and occurs rapidly.

It is possible to partition sediment iron into three classes, amorphous, organic and crystalline. Generally, the latter form predominates (approximately 80% of total sediment iron) within the clay mineral, chlorite and is not available for participation in redox reactions (Davison and Tipping 1984). It is the amorphous iron (hydr)oxide fraction that is involved both with phosphate uptake and its release, depending on the prevailing redox conditions, and generally makes up 15 – 20% of the total sediment iron (Tipping *et al.,* 1982; Davison and Tipping 1984; Moutin *et al.,* 1993). 71% of the total iron in sediments from a North Arm site at Rutland Water was found to belong to the bound crystalline fraction, whereas 94% of total iron in cores taken from a site close to the inlet was in the amorphous fraction (Love, pers. comm.). Experiments examining the stability of this amorphous fraction from cores taken from Rutland Water under low oxygen conditions have been undertaken by Andrew Love at the University of Birmingham. Little release of phosphate occurred from South Arm cores down to oxygen saturation of 25%; only at 10% oxygen did release rates increase dramatically (A. Love, pers. comm.).

Bray-Curtis similarity measures and cluster analysis indicated that sites close to the inlet of iron III sulphate dosed water are dissimilar from the rest of the reservoir in terms of their chironomid communities. Chironomid diversity and abundance are both low at these sites. The presence of high iron levels in the sediments corresponds

strongly with the restricted communities. Whether the response is to toxicity of iron or to a physical effect of its presence is unclear from these data. The laboratory-based project explained in the following chapters has aimed to clarify this situation and to quantify the response of a chironomid species, *Chironomus riparius*, to the addition of iron III sulphate. The genus *Chironomus* has been recommended as a benthic test organism for its ease of rearing and testing and because it exhibits high control survival and its larvae remain in intimate contact with the sediment (Nebecker *et al.*, 1984). *C. riparius* was favoured over *C. plumosus,* the *Chironomus* species probably present in the Rutland Water samples, because the latter requires too large an area for mating swarms for it to be viable in laboratory culture (Powlesland, 1984). As is described in the following chapter *C. riparius* is very easy to culture and egg masses were readily available from already successful cultures in other research institutions. Attempts to culture members of the Tanytarsini tribe from Rutland Water failed and no identification of Tanytarsini to species was made.

SUMMARY

Rutland Water is a lowland pump storage reservoir of high amenity value. The occurrence of large nuisance blooms of algae, particularly toxic cyanobacteria, is a consequence of the eutrophic status of the reservoir. In order to combat nuisance algal blooms Anglian Water Services (since June 1990) have employed the use of iron III sulphate as a coagulant for phosphate precipitation to the sediments. Dosing of iron III 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 (NRA) since the summer of 1990. Preserved samples of chironomid larvae from profundal benthic grid surveys were sent to the author for identification beyond family level. Initial samples were identified to genus; later samples were separated into four easily distinguished groups; *Chironomus*, *Procladius*,

Tanytarsini tribe and others. This scheme avoids time-consuming preparation of specimens, whilst allowing identification of the dominant chironomid taxa.

Chironomid communities were dominated by *Procladius* sp. and the Tanytarsini tribe at the majority of sample sites, but these groups appeared to be largely absent from sites close to the inlet of iron III dosed water. Bray-Curtis similarity coefficients indicated that the Inlet site and sites along the Slipway transect close to the inlet were dissimilar from the other sample sites at Rutland Water. At these sites 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.

Chapter Three

Chironomus riparius culture and toxicity testing methods.

3.1 INTRODUCTION

The main experimental procedures employed in this study are outlined in this chapter. Any variations or extensions of these basic procedures will be discussed within the chapters relating to particular experiments. The first half of the chapter deals with the test organism, *Chironomus riparius*, its ecology, life cycle and the use of culture techniques to allow its use as a test organism. The second half of the chapter explains how tests using iron III sulphate precipitates were carried out.

3.2 *CHIRONOMUS RIPARIUS*: TAXONOMY, ECOLOGY AND LIFE CYCLE.

Chironomus riparius (Meigen) is a member of the dipteran family Chironomidae, the non-biting midges (sub-family Chironominae). The genus *Chironomus* contains several hundred species and has a world-wide distribution. Larvae of the genus are predominantly found in soft sediments of standing waters and less frequently in flowing systems (Pinder and Reiss, 1983). Commonly, red pigmented larvae (including *C. riparius*) are known as blood worms. All life stages of *Chironomus*, particularly larvae and pupae are poorly described taxonomically (Lindeberg and Wiederholm 1979). General diagnostic characteristics of larvae of the genus are two pairs of ventral tubules on the 11th segment and the shape of the mentum which has a trifid median tooth (Pinder and Reiss, 1983). *Chironomus riparius* larvae have been described

as being of the *thummi*-type[1] of the genus in that they have two ventral tubules at least as long as the 11th segment, but no lateral tubules on the 10th segment (Lindeberg and Wiederholm, 1979).

Natural populations of *Chironomus riparius* are common both in lotic ecosystems and in ponds and shallow margins of lakes. The highest densities of *C. riparius* in Stephenson Pond, Alberta occurred between 0.5 and 1.0 metres depth, though larvae were found at 2.1 metres, the maximum depth of the pond (Rasmussen, 1984). In particular, its presence in association with organic pollution in lotic systems has been documented (Learner and Edwards, 1966; Gower and Buckland, 1978; Pinder, 1986). Sewage effluent discharges into Moat Brook, Staffordshire resulted in the replacement of *Polypedilum laetum* with *Chironomus riparius* as the dominant member of the Chironomidae (Gower and Buckland, 1978). In these eutrophic situations the presence of large mating swarms of this midge has become a source of complaint, despite the fact that the adults do not bite and as far as is known they are not associated with the transmission of pathogens (Learner and Edwards, 1966). *C. riparius* can also dominate in temporary or new water bodies or in water subject to pollution other than organic in origin (Pinder, 1986).

Like many chironomid species *C. riparius* larvae are tubiculous. Rasmussen (1984) found that the tubes were more or less vertically aligned and extended 2 – 3 cm into the mud with the open end a few millimetres above the substrate. Tubes were of lighter construction and lacked the heavy mucous lining of tubes of the other chironomid studied, *Glyptotendipes paripes*. The tube type of *C. riparius* is characteristic of deposit feeding chironomid larvae (Rasmussen, 1984). Detritus is the most frequently recorded material in chironomid guts (Pinder, 1986). It has been suggested that much of this material is indigestible and that the rapid passage of material through the guts of deposit and filter feeders allows little time for extensive digestion (Pinder, 1986). This suggests

[1] No distinction can be made between *Chironomus riparius* and *Chironomus thummi*. The former is the correct name for the species because it was first described (Credland 1973a).

that such larvae do not select what they eat. Indeed, Smock (1983a) describes deposit feeders as sediment dependent in that they indiscriminately ingest sediment along with detritus. There is some evidence, however, that *C. riparius* may be able to select against ingesting large quantities of bacteria in favour of some other component of detritus (Pinder, 1986).

The life cycle of *C. riparius* , summarised in Figure 3.1, is generally described as multivoltine from observations of lotic populations (Gower and Buckland, 1978; Learner and Edwards, 1966). The generation time was said to be related to temperature (Learner and Edwards, 1966). Rasmussen (1984), however, found that the species exhibited a univoltine life cycle in Stephenson Pond, Alberta. He put this apparent anomaly down to the availability of food rather than temperature at the sediment-water interface. Pupation, emergence and oviposition occur in the spring. Under certain conditions determined by light intensity, temperature and wind velocity male flies can collect in large mating swarms, generally close to their aquatic habitats. Females are attracted to the swarm, and coupling takes place. After mating, eggs are laid in a gelatinous mass just below the water surface attached to aquatic plants and other objects (Learner and Edwards, 1966). It is likely that *C. riparius* like most chironomid species, only lay one eggmass per adult female (Pinder, 1986) Eggmasses from natural populations were found to contain between 650–680 eggs each (Gower and Buckland, 1978). Observations from laboratory cultures indicate that once hatched first instar larvae utilise the gelatinous covering of the eggmass as an immediate food source. The first instars are planktonic but they descend to the sediments as second instars. Once in the sediments the larvae develop through two further stages to fourth instars. The species over-winters within this final instar stage. Pupation appears to be inhibited by low temperatures (Learner and Edwards, 1966). In Rasmussen's study (1984) pupation, emergence and oviposition were mostly complete by the end of May. The first instar stage was short-lived, by early June the majority of larvae were second instars. By mid July most larvae had attained the third instar stage and by mid August most were small

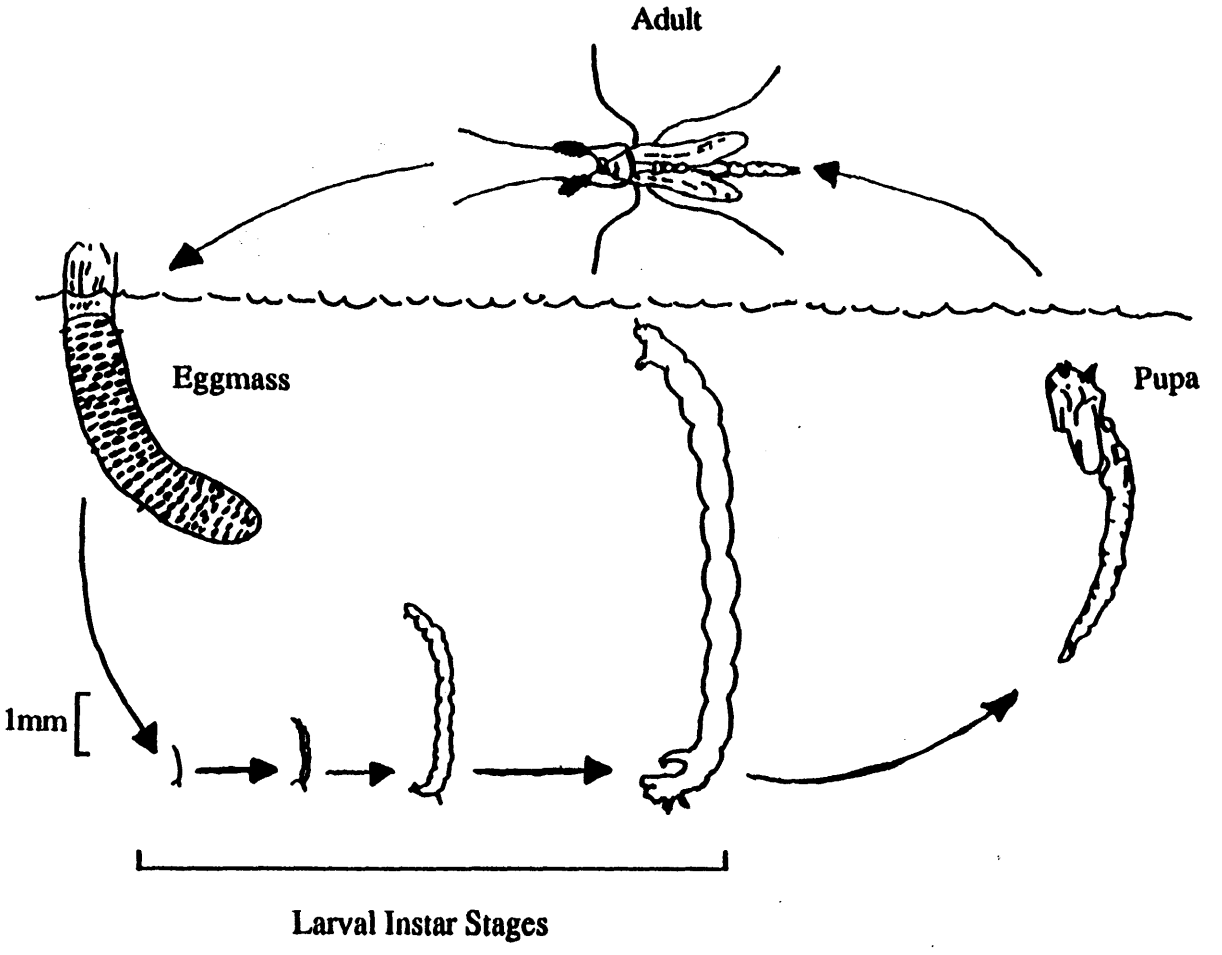

Figure 3.1 *Life cycle of the midge* Chironomus riparius *(Meigen), modified from Timmermans (1991)*

fourth instars. The majority of larval growth occurred in this stage and appeared to coincide with peaks of phytoplankton production.

3.3 ADVANTAGES OF *CHIRONOMUS RIPARIUS* AS A TEST ORGANISM

Insect species are often a major component of aquatic ecosystems and can dominate macroinvertebrate communities in terms of both numbers and biomass. Their use along with other macroinvertebrates for environmental classification and as bioindicators of pollution has been widespread (Williams, *et al.,* 1984). For example, chironomid communities have been used as indicators of trophic status in Lake Michigan. Chironomid taxa were placed into three groups; 0, 1 or 2 as indicators of increasing nutrient load. An average of the taxa score for a site will be close to 2 if the site is eutrophic and close to 0 if oligotrophic. Littoral areas of the lake were found to be mesotrophic (average index 1.26) by this system and profundal areas were oligotrophic (average index 0.41) (Winnell and White, 1985). It is only relatively recently, however, that the need to include macroinvertebrate toxicity tests for the preparation of water-quality standards has been recognised.

It is desirable to look at the effects of contaminants in the field, however laboratory tests are often required to provide control of the many variables involved in toxicity studies. Ideally, organisms used in these tests will be easy to maintain in the laboratory, preferably as continuous cultures so that studies involving the whole life cycle may be made. For most aquatic insects this is not possible often because the aerial adult phases they possess have environmental requirements for mating and/or oviposition not easy to accommodate within the laboratory (Pascoe and Edwards, 1989). For example, *Chironomus plumosus* a large profundal species exposed to iron III dosing at Rutland Water (see Chapter Two) is unable to mate except when in flight and requires large volumes in which to swarm (Powlesland, 1984). This clearly makes *C. plumosus*

impractical for laboratory use. *Chironomus riparius* is an example of an aquatic insect for which continuous cultures are possible.

The simple culture technique described below enhances *C. riparius'* usefulness for toxicity testing. All life stages of the species are constantly available and the relatively short generation time at 20 °C means that more than one life stage or the whole life cycle can be handled within single short duration tests. Life stages of the species are easily distinguished from each other, which can be important where more than one is involved in a single test. That the species exhibits high fecundity (section 3.4.2) and is physically robust can also be important for toxicity testing (Pascoe, *et al.*, 1989). *Chironomus* exhibit high control survival (Nebeker *et al.*, 1984). Large numbers of larvae (300 – 400) of the same age and of similar size can be provided by one eggmass. The relative robustness of the species allows easy handling, measurement of larval weight and easy distinction between larval stages (section 3.5). Besides mortality a number of sub-lethal effects of toxicants on *C. riparius* can be examined. Through the use of whole or partial life cycle tests parameters such as percentage hatch, larval growth, larval development and the timing and magnitude of adult emergence may be measured.

Chironomus riparius is a burrowing detritivore which indiscriminately ingests sediment with detritus. This makes it ideal for testing of sediment bound contaminants (McCahon and Pascoe, 1988) or, as in this study, testing of inorganic precipitates present as a layer on top of the sediments. *Chironomus* is recommended as a benthic test organism for solid phase toxicity testing by Nebeker *et al.* (1984) due to its ease of rearing, handling and because they feed on and remain in intimate contact with the sediment.

A summary of toxicity tests in which *C. riparius* and other chironomid species have been used is given in Table 3.1.

***Table 3.1** Summary of toxicity tests in which* C. riparius *and other chironomid species have been used.*

3.4 LABORATORY CULTURES OF *CHIRONOMUS RIPARIUS*

3.4.1 Culture technique

The culture technique used in this study is relatively simple and maintains large numbers of the species continuously. The technique was based on those of Credland (1973), and McCahon and Pascoe (1988) for *C. riparius* and on Batac-Catalan and White (1982) for *C. tentans*. Some alternative food and substrate sources were used. Cultures were started from eggmasses supplied Dr. David Pascoe from existing cultures at the University of Wales College of Cardiff.

Cultures were set up in plastic aquaria approximately 46x26x25 cm. A wooden framed muslin cage was fitted to the top of each aquarium, allowing the adults some room in which to fly without escaping (see Figure 3.2). Light is provided on a 16-hour on, 8-hour off basis. Credland (1973b) found that different light intensities and photo-periods had little effect on culture characteristics, but McCahon and Pascoe (1988) found that the use of 'True Lite' fluorescent tubes, to simulate daylight, improved adult emergence. In this study the light intensity provided was probably a lot lower than in the other studies mentioned but adult emergence and oviposition were maintained at levels high enough to easily support experiments.

That the generation time of fly cultures is dependent on temperature is well documented. Development of the midge *Tanytarsus dissimilis* from egg to adult was shown to take 12 days at 28 °C, 13 days at 25 °C and 16 days at 21 °C. At temperatures above 28 °C no decrease in developmental time was seen and at temperatures above 33 °C detrimental effects occurred (Nebeker 1973). Credland (1973b) found that of *C. riparius* cultures established at temperatures between 15 and 25 °C ones in the higher part of the range were more satisfactory. At 24 °C the generation time was 29 – 31 days.

Powlesland (1984) reported similar findings, a 28 day life-cycle at 22 °C and in the present study cultures kept at 20 ± 0.5 °C exhibited generation times of 25 – 30 days.

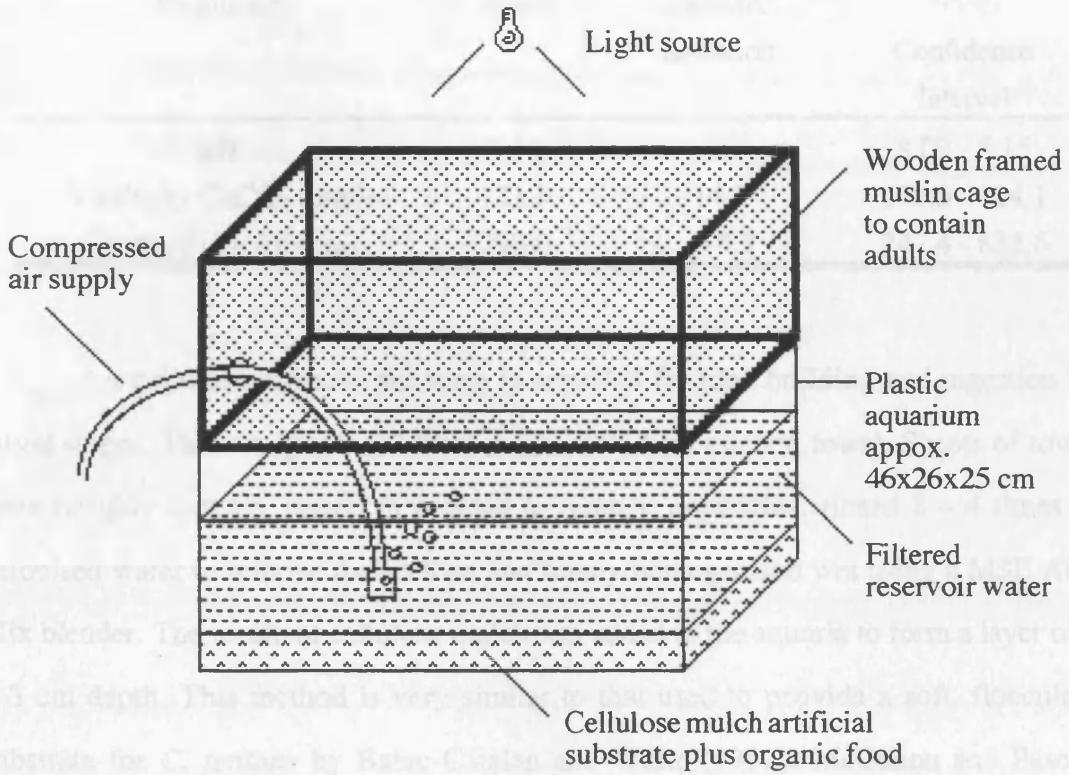

Figure 3.2 *Oblique view of culture aquarium for rearing continuous cultures of* Chironomus riparius. *Aeration of the filtered reservoir water was constant.*

The cultures were provided with reservoir water collected regularly from Whitwell in the North Arm of Rutland Water and filtered through a 53 μm mesh phytoplankton net. Important chemical parameters of the surface water of the North Arm are given in Table 3.2. Cultures were continuously aerated to maintain a high oxygen saturation. This is less critical for C. *riparius* since, like all Chironomidae containing haemoglobin in their haemolymph are very tolerant of low oxygen concentrations. A related species, *C. anthracinus*, only showed inhibited growth at an oxygen saturation of below 4% in Lake Esrom, Sweden (Pinder, 1986).

Table 3.2 Major water chemistry parameters for surface water samples from the North Arm of Rutland Water 29th May 1990 to 7th September 1992.

Parameter	Mean	Standard deviation	95% Confidence Interval
pH	8.12	.16	8.09 - 8.15
Alkalinity $CaCO_3$ (mg/l)	121.3	14.8	118.5 - 124.1
Conductivity (µS/cm)	790.0	118.3	747.4 - 832.6

A cellulose artificial substrate is provided for tube building and ingestion by larval stages. This was produced from recycled undyed kitchen towel. Sheets of towel were roughly torn up, rinsed in acetone to remove impurities, rinsed 3 – 4 times in deionised water to remove the acetone and finally homogenised wet using a MSE Ato-Mix blender. The resultant cellulose mulch was added to the aquaria to form a layer of 2 – 3 cm depth. This method is very similar to that used to provide a soft, flocculent substrate for *C. tentans* by Batac-Catalan and White (1982). McCahon and Pascoe (1988) used Whatman No.1® filter paper as their source of cellulose. Filter paper does not require acetone washing, but is relatively expensive and the mulch provided is coarser and harder to handle.

On a twice weekly basis 5 ml of a 50 g litre^{-1} suspension of *Xenopus* Diet No.1 was added to each culture to supplement the larval diet. Dry pellets of the *Xenopus* Diet No.1 (supplied by Blades Biological - Educational Supplies) were ground with a pestle and mortar prior to suspension in deionised water. Food suspensions were kept refrigerated when not in use. Food sources recommended in other studies have included Dog Kisses®, powdered liver (Bievier, 1971) and various commercial fish foods (Credland, 1973), e.g. Tetramin® (McCahon and Pascoe, 1988), Tetra conditioning food for tropical fish (Batac-Catalan and White, 1982). The composition of these foods probably varies very little and all seem to have been successful.

3.4.2 Egg counts

Counts of the number of eggs in eggmasses removed from cultures for experiments were made using a Watson dissecting microscope. The mean number of eggs per eggmass from 72 counts was 336.3, standard deviation, 87.6 and range 193 to 575. This mean value is much lower than that quoted by Gower and Buckland (1978) of 650 – 680 eggs per eggmass for a wild lotic population. Powlesland (1984) reported similar findings for his cultures of *Chironomus riparius*. From 20 counts he found a mean of 428.5 eggs per eggmass (range 247 – 710). It is possible that the reduced development time experienced by laboratory cultures maintained at room temperature (20 °C) results in decreased weight at emergence and therefore in lower numbers of eggs per female. Differences in food availability between cultures and wild populations may also have some bearing.

3.5 IDENTIFICATION OF LARVAL INSTAR STAGE USING HEAD CAPSULE WIDTH.

The head capsules of chironomid larvae are chitinous and therefore during each larval instar only growth of the soft body of the larva can occur. Moulting of the head capsule takes place between instars. The size of the head capsule can, therefore, be used to identify the instar stage of a larva.

Artificial substrate collected from cultures was sorted for larvae which were placed in 70% alcohol for preservation. Very small larvae are difficult to find by this method and so, in addition, some larvae from recently hatched eggmasses were also examined. The head capsule width and body length of each larva was measured using a Nikon compound microscope fitted with an eyepiece graticule. The results are summarised by the scatter plot presented in Figure 3.3. Simple regression analysis for head capsule width against body length gave the equation;

Body length (mm) = -2.2982 + 2.3299*10^{-2}. Head capsule width (μm)

$R^2 = 0.82$

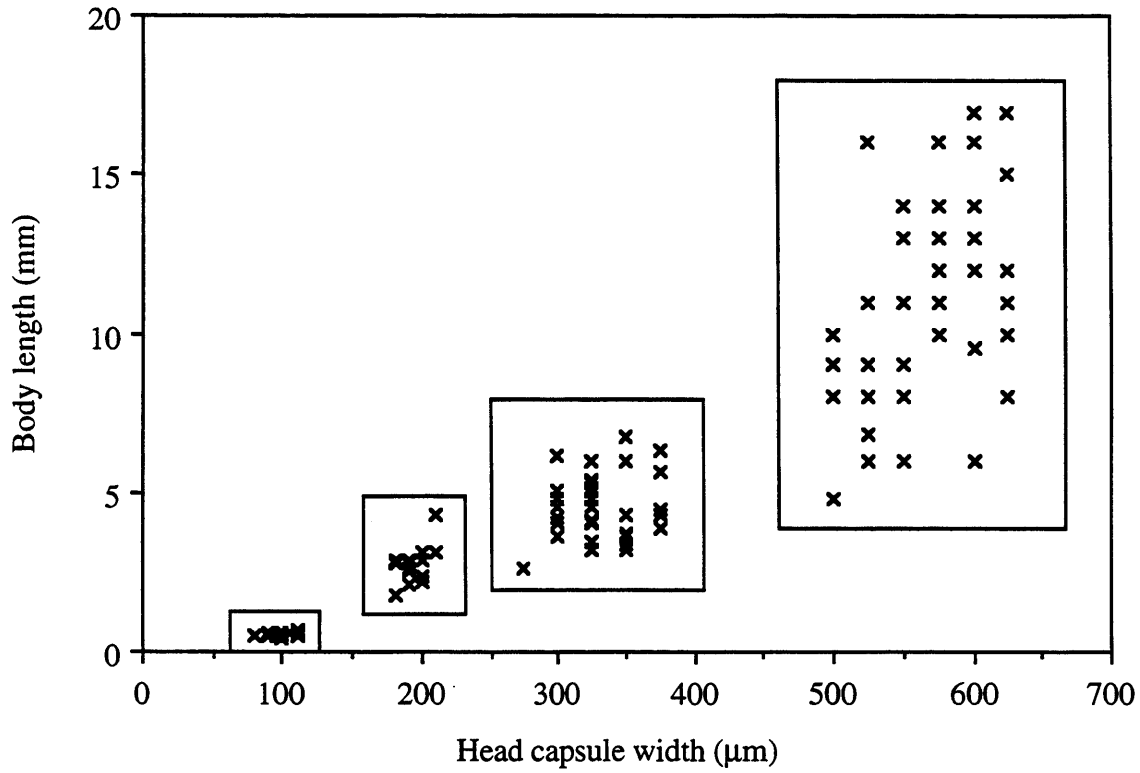

Figure 3.3 *Head capsule width versus body length for* Chironomus riparius *larvae from laboratory culture.*

The data clearly show four distinct groups which represent the four instar stages. Some overlap in body length is present between the instars. Growth of the body occurs during each larval stage so larvae about to moult from one instar stage may have a similar body length to newly moulted larvae in the subsequent instar. The greatest variation in body length occurs in the final instar stage reflecting that most of the larval growth occurs within this stage. No overlap in head capsule width is observed between groups due to the need to moult the head capsule before any increase in its size. Measurement of the head capsule width can therefore be used to identify the instar stage

of the larva using the scheme given in Table 3.3. These figures are in close agreement with mean values provided by Powlesland (1984), also shown in Table 3.3.

***Table 3.3** Identification of larval instar stage of* Chironomus riparius *using measurements of head capsule width, all measurements are given in μm except for the number of larvae measured in each instar. (*) represents mean values cited from Powlesland (1984).*

Instar Stage	Range	Mean (*)	Standard Deviation	Number measured
First	80 - 110	99.6 (87.9)	8.4	26
Second	180 - 210	193.2 (191.3)	10.6	19
Third	275 - 375	331.4 (329.0)	26.4	51
Fourth	500 - 625	570.1 (525.0)	37.5	66

3.6 PREPARATION OF IRON III STOCK SOLUTIONS

Two iron salts, iron III sulphate and iron III chloride, have been used for phosphate inactivation in lakes and reservoirs. Sulphate was chosen for use in this study principally because it is the salt that has been used to dose the water supply reservoirs in the Anglian region. Also, it is possible that the use of chloride may have given rise to increased salinity within the tests. Hydrated technical grade iron III sulphate ($Fe_2(SO_4)_3.22H_2O$) obtained from Fisons Scientific Equipment was used for all experiments. It is fairly certain that Anglian Water Services use a rather impure source of iron III sulphate for their dosing schemes but the precise nature and amounts of these impurities are not available to me. The use of technical grade chemical in this study means that some small sacrifice of comparison between laboratory tests and the field

situation may have been made but that any effects seen were due to the presence of iron III precipitates rather than the toxicity of various impurities in the dosant.

Stock solutions of the iron III sulphate were prepared by weighing out 3.57 g of the hydrated salt using an Oertling NA114 microbalance and dissolving it in one litre of deionised water at room temperature. The hydrated salt is a fine yellow/green powder and forms a clear orange/brown solution when dissolved. Deionised water is used as the dilutent rather than a standard reservoir water to minimise iron precipitation of solutes. The low pH (approximately pH 2) of the stock solution also aids the retention of iron in solution. This procedure produces a 500 mg Fe litre^{-1} stock solution (mean 497.7 mg Fe litre^{-1}, standard deviation 7.595 mg Fe litre^{-1}).

3.7 PRECIPITATE PRODUCTION

It is difficult to transfer accurately a known concentration of precipitate from a stock to a test chamber and so production of precipitate must occur *in vitro*. Appropriate amounts of 53 µm filtered reservoir water were placed in each test chamber and different quantities of the 500 mg Fe litre^{-1} stock solution were then added to bring the medium up to the required total volume (100 ml for 10-day tests, 2 litres for 25-day tests, see later sections) and provide the required range of target total iron concentrations. The iron stock solution was applied evenly over the surface of the water using a pipette and aeration of the medium (25-day tests) helped to ensure mixing.

Production and sedimentation of the orange precipitate occurred rapidly, in all tests, forming a layer on top of the artificial substrate. Constant aeration of the medium (25-day tests) and the high pH (pH 8 – 8.5) of the filtered reservoir water ensured that precipitation of iron was almost complete and that the iron stayed in the oxidised (Fe^{3+}) form. Amounts of iron remaining in the test medium as dissolved or suspended iron were generally low, for all target iron concentrations. Details of these levels will be set

out in individual results chapters to follow. Depression of pH often accompanied addition of the stock solution and depended on the amount added. The extent to which depression of pH affected individual experiments will be discussed in their relevant chapters.

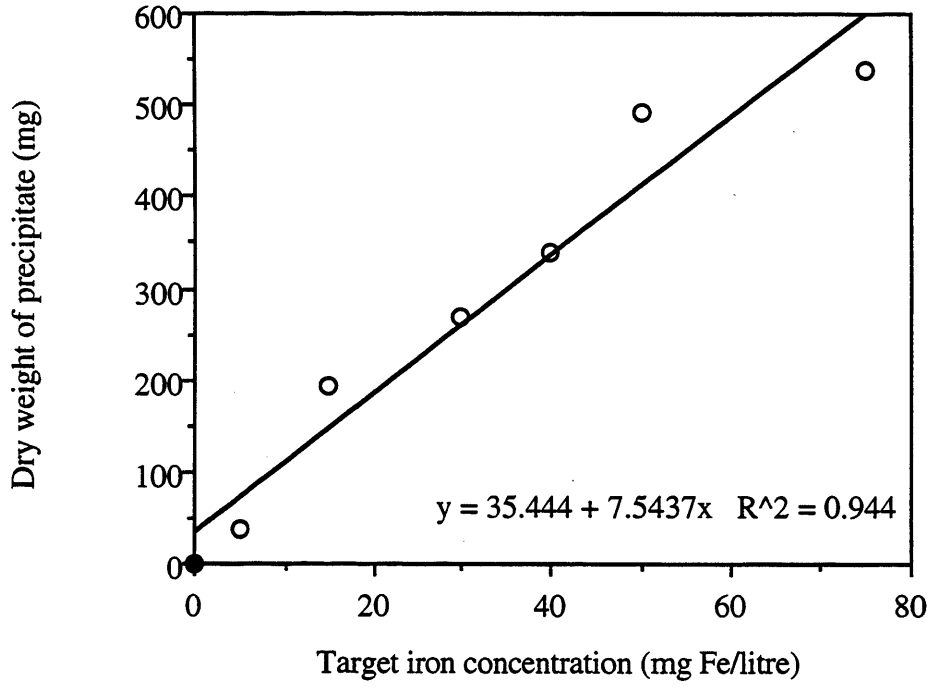

Figure 3.4 *Dry weight of iron precipitate formed in different target iron concentrations. Note, the point* (●) *is inferred, a target iron concentration of zero should produce no precipitate.*

The amount of precipitate produced increased linearly with the target iron concentration (Figure 3.4). These results were not replicated and so it is uncertain how reliable these results are. The iron content of the precipitate (mg Fe g^{-1} dry weight) was independent of the target concentration. The iron content of precipitate formed at target iron concentrations from 5 to 75 mg Fe $litre^{-1}$ fell within the range 150.24 to 196.72 mg Fe g^{-1} dry weight of precipitate with no relationship to target concentration (R = 0.071). Mean concentration was 175.04 mg Fe g^{-1}, standard deviation was 17.88 mg Fe g^{-1}.

Thus, the production of a range of target concentrations results in a range of quantities of iron precipitate of similar composition in terms of iron.

3.8 EXPERIMENTAL CONDITIONS

All experiments were carried out in a constant temperature room running at 20 ± 0.5 °C. Light was provided on a 16-hour on, 8-hour off basis. Fresh artificial substrate (cellulose mulch, section 3.4.1) and an organic food source (suspensions of ground *Xenopus* food pellets) were provided in each test. Reservoir water filtered through a 53 µm phytoplankton mesh was used as the base for the test medium. All glassware and plastics were rinsed before and after use, first in 1% nitric acid and then in deionised water and then allowed to air dry covered by laboratory paper.

3.9 PARTIAL LIFE CYCLE TESTS OF TEN-DAY DURATION

These preliminary tests follow a 10-day test procedure based on that of Taylor, Maude and Pascoe (1991) for exposing second to fourth instar *Chironomus riparius* larvae to solutions of toxicants. For each test at least five eggmasses were obtained from laboratory culture within 18 hours of oviposition. Eggmasses are laid just below the water level in the culture tanks and remain adhered to the tank wall by their gelatinous coating until the eggs hatch. Eggmasses already present in the cultures were either detached from the tank wall and allowed to sink to the bottom (hatching success appears unaffected by this) or the water level in the tanks was raised clear of these existing eggmasses. Either case allowed freshly laid eggmasses to be identified and collected the following day.

Isolated eggmasses were incubated as a single culture at 20 °C for 10 days. This culture was provided with cellulose mulch substrate, organic food suspension (5 ml of 50 g ground *Xenopus* No.1 diet pellets litre^{-1} deionised water) and filtered reservoir

water. At the end of the incubation period the larvae had hatched and developed to second instar stage.

Square petri dishes (10 x 10 x 1.5 cm) were used as experimental chambers. To each chamber, containing an appropriate amount of filtered reservoir water, cellulose mulch was added, dispersed within the medium and allowed to settle to give an approximate depth of 3 mm. 5 ml of the organic food suspension (25 g *Xenopus* No.1 diet litre^{-1} deionised water) was added to each dish. Preliminary observations indicated that the addition of an adequate food supply at the start of an exposure period yielded better larval growth than provision of a smaller amount with subsequent additions during the exposure period. Addition to dishes of an initial amount of food larger than 5 ml at the start did not yield greater larval growth.

Appropriate amounts of iron stock solution were then added to bring the total volume of test medium to 100 ml and produce the required range of target iron concentrations. Aeration of the medium was deemed unnecessary due to the large surface area (exposed to the atmosphere) to volume ratio of the medium. Partial removal of the petri dish lids for a few hours each day reduced the potential for oxygen depletion within the medium and limited evaporative loss from the treatments.

Ten individual second instar larvae from the incubated culture were allocated to each chamber and left for an exposure period of ten days. As larvae develop their increasing size allows greater ease of handling, but they also become more tolerant of contaminants (Gauss *et al.*, 1985). Nebeker *et al.*, (1984) suggested that second instar *Chironomus* larvae offered a good compromise between handling ease and the use of sensitive life stages. Control larvae (i.e. in chambers containing only filtered reservoir water, cellulose mulch and food source) had developed to fourth instar stage at the end of the test period. Surviving larvae were then removed from the dishes (numbers noted) to labelled tubes of tap water for rinsing. This helped to separate off small quantities of

cellulose mulch/particulate matter often removed with the larvae. The remaining contents of the dishes were then vacuum filtered using Nalgene® filter holders and receivers fitted with Whatman® 47 mm diameter, 0.45 μm pore cellulose nitrate membrane filters to separate solution and particulate matter. This method may slightly over-estimate the amount of iron in solution as some fine particles containing iron may pass through the 0.45 μm filter (Kennedy, *et al.*, 1974). Particles of iron III oxide from the water column of Esthwaite Water were found to have mean diameters of between 0.05 and 0.5 μm (Tipping, *et al.*, 1982; Davison and Tipping, 1984) and thus only the largest of these would be stopped by a 0.45 μm filter. I have no information on the particle size of iron III precipitates formed in this study, but I suspect that it is very variable due to aggregation of particles and observation suggests that the majority of precipitate particles are removed by the filter used. Preparation of samples for iron analysis using atomic absorption spectrophotometry is detailed in section 3.11.

Larval growth was used to assess chronic toxicity of iron and was measured using larval wet weight. Rinsed larvae were blotted 'surface' dry on absorbent paper. Some measurements of individual larvae were made but in the majority of experiments surviving larvae from each dish were weighed together and an average wet weight per organism was calculated. All measurements were made using an Oertling NA114 microbalance accurate to 0.1 mg.

As a preliminary investigation into the effects of iron III precipitates these 10-day duration tests were able to provide some useful information concerning the range of target concentrations that warranted further study. The limited size of the test units did not allow very large numbers of larvae to be used and so variation was likely to be high, even when several replicates were performed. Also, the small size of these units did not allow even simple chemical parameters such as pH or oxygen saturation to be measured. Without knowledge of these parameters firm conclusions as to the cause of variation

between units is difficult. All these experiments could give is some indication of possible effects of iron III precipitates.

3.10 WHOLE LIFE CYCLE TESTS OF 25-DAY DURATION

The procedure described below provides the basis of the majority of experiments performed in this study. The 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. First instars of *Chironomus tentans* were found to be between 12 and 27 times more sensitive to dissolved copper than fourth instars in 96 h EC_{50} tests examining immobilisation of larvae (Gauss, *et al.*, 1985). Eggs were said to be more resistant than either larval stage. Similarly, larvae of *C. riparius* were found to have increased tolerance to cadmium and nickel solutions with increasing instar stage. The 24 h LC_{50} of cadmium to fourth instars was approximately 950 times greater than the corresponding value for first instars (Williams, *et al.*, 1986) and 48 h LC_{50} values for nickel were significantly higher for second instars of this species than for first instars (Powlesland and George, 1986).

The following procedure is a simple static-with-replacement system and was chosen as normally preferred flow-through systems seemed inappropriate for use with a flocculent 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. It has been stated that static tests should be avoided (Seelye and Mac cited in Nebeker *et al.*, 1984) because flow-through tests provide greater control over the medium i.e. over dose concentration, oxygen saturation, pH and water hardness. Flow-through systems have often been used where the toxicant is in dissolved form. In such systems the medium is constantly replaced so allowing exposure to a constant toxicant

dose. In this study the toxicant was in precipitate form and required fairly static conditions in which to form and sediment. Use of a flow-through system would not provide any greater certainty of toxicant dose than a static system. The problem of oxygen depletion in static tests can be overcome by constant aeration of the medium (Nebeker *et al.*, 1984). The reservoir water used in this study had fairly constant pH and alkalinity levels (Table 3.1) and pH was monitored in most of the tests performed.

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. Tanks were left overnight for iron precipitates to form and sediment on to the substrate. 5 ml of 50 g litre^{-1} food suspension was added to each tank. The tanks remained covered and aerated throughout. An illustration of the experimental set up is given in Figure 3.5.

Eggmasses collected within 18 hours of oviposition were placed in a drop of reservoir water on a microscope slide and covered with a coverslip. This spreads the eggmass out allowing the number of eggs to be counted by examination under a Watson dissection microscope at 8.75 times magnification. A single eggmass was allocated to each tank and was carefully placed onto a strip of filter paper that had been allowed to settle onto the substrate settled precipitate layer. This provides easy location of the eggmass. Eggs were left for four days to hatch, after which the remains of the eggmasses were removed from the tanks and the numbers of unhatched eggs counted. Powlesland and George (1986) found that eggs of *Chironomus riparius* that remained

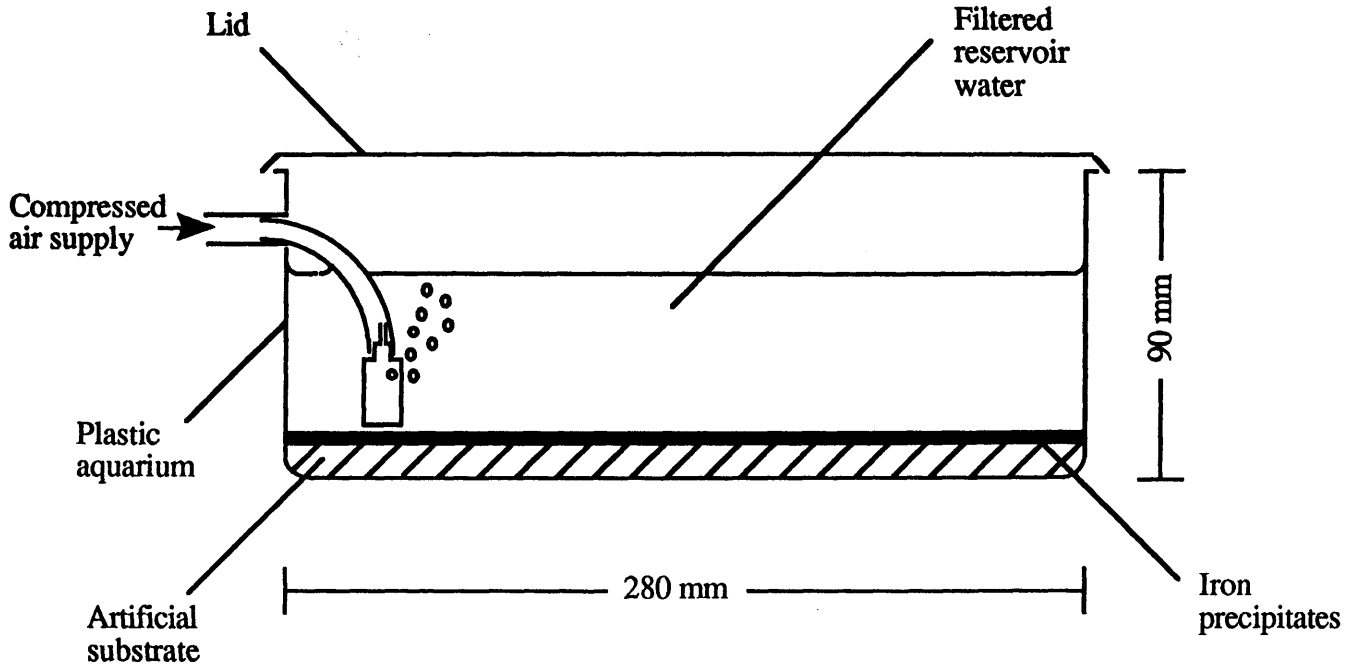

Figure 3.5 *Illustration of experimental chamber showing iron precipitate layer. Tank width was 160mm.*

unhatched after three days were unlikely to develop further. Counts of initial egg numbers and of unhatched eggs allowed the determination of hatching success.

Once hatched the progress of larval development was monitored via two measurements; larval weight and larval instar stage. Initially, measurements of larval wet weight were made using an Oertling NA114 microbalance capable of measuring to 0.1 mg but later the availability of a Ci Electronics Microforce MkIIc balance allowed measurements to 0.01 mg to be made allowing use of larval dry weight. Dry weight measurements are preferable to wet weight measurements because they imply a constant value and so less variation appears in the sample. Larval weight is most useful once the larvae have reached the final (fourth) instar stage as this is when the majority of larval size increase occurs. Identification of larval instar stage was made using measurement of head capsule width (section 3.5) and is most informative prior to larvae reaching the final instar stage.

Larvae were sampled from tanks by using a 10 ml Oxford macropipette fitted with a modified 10 ml pipette tip. This had the tip removed so as to produce a slowly tapering tube of length 120 mm and diameter 13 mm reducing to 9 mm. This allowed both substrate and larvae to be sampled simultaneously, by effectively removing a small core of larvae and substrate from the tank. Positioning of each sample was made relative to a conceptual grid. Grid squares were selected using random number tables. Squares which had previously been sampled were not subsequently re-sampled. Two such samples were made on each sample day and combined. Samples of this nature were made on days 6, 14, 20 and 25 of the experimental period. In hindsight, modification of this procedure to ensure greater numbers of larvae in each sample may have been useful. In low density treatments (perhaps due to high mortality) larval numbers recovered in samples hindered their inclusion in statistical analyses. Larvae were removed from the sample and placed in labelled tubes containing tap water for rinsing. The instar stage of each larva was identified from head capsule widths using a Nikon compound microscope

fitted with an eyepiece graticule. Live larvae were placed on a microscope slide with a small amount of tap water. A coverslip placed over them helps to keep them still. Where measurement of wet weight was used, larvae were blotted 'surface' dry on absorbent paper and weighed individually. For dry weight measurements, larvae were placed in numbered repli dish cells and desiccated for a week for a constant dry weight to be achieved. Again larvae were weighed individually. After weighing, larvae were placed in a conical flask containing 50 ml of 10% nitric acid in preparation for digestion (see section 3.11). A single conical flask was used for each sample.

After removal of larvae the remainder of the sample was vacuum filtered through a 0.45 μm pore cellulose nitrate filter membrane using Nalgene® filter apparatus. The membrane and filtered particulate matter were placed in a covered petri dish and allowed to dry fully to constant dry weight prior to digestion for iron analysis. Samples of particulate matter were also taken in tanks prior to the addition of animals i.e. day 0 for initial tanks and day 14 for replacement tanks (see below). The particulate matter was made up of the artificial cellulose substrate, sedimented iron III precipitates and larval faecal pellets.

The medium was sampled for total iron using the same apparatus as for larvae and particulate matter. Two 10 ml samples were taken from each tank to give a 20 ml combined sample. The medium of each tank was sampled on days 0, 6, 14, 20 and 25 of each test. Samples were placed in 30 ml Sterilin® tubes prior to preparation for iron analysis.

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, the day before replacement (day 13). Each tank contained cellulose mulch substrate, 2 litres of the appropriate medium and 5 ml of 50 g litre^{-1} food suspension. Larvae from each initial tank were carefully sorted from the medium and particulate

matter and counted on transfer to the appropriate replacement tank. The initial tank was then discarded. This provided an accurate census of the number of surviving larvae and allowed the conditions in the tanks to be renewed. Larvae ingest large amounts of material including the cellulose mulch, organic food source and iron precipitates during the tests. Over time supplies of these get low and may have caused additional stress to the larvae i.e. competition for food and for space for burrowing activities. Replacement of tanks ensured that supplies of mulch and food source were renewed and that larvae were kept exposed to the intended level of iron precipitates. Faecal pellets that tended to build up during the tests were also removed by this process.

Towards the end of the test, *circa* day 20, the numbers and sex of adults that emerged from the tanks each day were recorded. Careful lifting of the lid and use of a pooter enabled adults to be removed from the tanks. Adult males are generally smaller than female flies and have plumose rather than simple antennae. At the end of the test (day 25) after any adults were removed and the final medium, substrate and larval samples had been taken the remaining larvae were sorted and counted to give a final census.

3.11 PREPARATION OF MEDIUM, PARTICULATE MATTER AND LARVAE SAMPLES FOR ATOMIC ABSORPTION SPECTROPHOTOMETRY (AAS).

3.11.1 Test medium

The 20 ml samples of test medium were acidified using 2 ml of 'PrimaR' grade fuming nitric acid giving a 10% nitric acid solution. This dissolves any suspended iron particles and prevents any precipitation of iron. Acidified samples were stored in 30 ml Sterilin® tubes prior to atomic absorption spectrophotometry.

3.11.2 Particulate matter (substrate/precipitate)

Digestion of particulate matter samples was based on that used by the National Rivers Authority (1991). For each sample the combined dry weight of filtered particulate matter and filter membrane were measured using an Oertling NA114 microbalance. Each sample was then transferred to a separate conical flask containing 50 ml of 10 % nitric acid (prepared by adding 5 ml of 'PrimaR' grade nitric acid to 45 ml of deionised water) and a few anti-bumping granules. Samples were digested on a hotplate at approximately 170 °C for 45 minutes after which they were removed from the hotplate and allowed to cool to room temperature. 2.5 ml of 'AAS' grade hydrogen peroxide were added and the samples digested on the hotplate for a further 30 minutes. Samples were again allowed to cool to room temperature prior to filtration through Whatman® No. 541 hardened ashless filter papers into 50 ml volumetric flasks. This removes any artificial substrate, anti-bumping granules and the, usually, intact filter membrane from the digested sample. The volume of the sample was then made up to 50 ml using deionised water. Clean filter membranes were put through the digestion procedure to provide 'blanks'.

3.11.3 Chironomid larvae

Weighed larvae were placed in 50 ml of 10% nitric acid and put through the same digestion procedure as particulate matter. Cooled samples were filtered and made up to 50 ml using deionised water. This method appeared to work well but, some chitinous structures (head capsule) remained undigested at the end of the procedure.

3.12 ATOMIC ABSORPTION SPECTROPHOTOMETRY (AAS)

Stock solutions for AAS were prepared using 'AAS' or equivalent grade reagents. Blank, 0.05, 0.2 and 1.0 mM stocks were produced from a stock of known iron concentration (100 mM) in 100 ml of 10% nitric acid.

A Varian Techtron AA-6 atomic absorption spectrophotometer was used for all iron analyses. The lower detection limit was 0.001 mM Fe. Multiplication of these readings by the atomic weight of iron (approximately 56 g) gives a value in mg Fe litre^{-1}. Test medium concentrations of iron were expressed in this form. A correction for weight was made for particulate matter and larval digestions. The number of mg of iron produced by digesting x grams of particulate or larval matter in 50 ml of 10% nitric acid can be found from:

$$y * 50 / 1000$$
$$= y / 20$$

Where y equals the concentration of iron in the digested sample found from AAS expressed in mg Fe litre^{-1}. This figure can then be expressed in terms of mg Fe g^{-1} wet or dry weight by dividing through by x grams, i.e.

$$y / 20 * x$$

For particulate matter digestion x equals the measured dry weight of particulate matter and filter membrane minus the mean weight of the filter membrane in grams. The mean filter membrane weight was 8.353×10^{-2} g, (S.D. = 4.4×10^{-4} g, n = 10). For larvae x equals the combined weight of larval material (g) into the digestion.

Chapter Four

Effects of iron III sulphate addition on cultures of *Chironomus riparius* (Meigen)

4.1 INTRODUCTION

As discussed earlier, evidence from the limited literature on iron toxicity and from the field study presented in chapter two suggests that iron III dosing in reservoirs may exert a detrimental impact on benthic invertebrate communities. The aim of dosing schemes is to precipitate phosphate to the sediments. It is this build up of an iron-rich layer that presents the greatest threat to benthos. Mance and Campbell (1988) advised that the deposition of iron precipitates should be avoided from evidence of reduced invertebrate communities supplied by field observation. The majority of laboratory studies investigating the toxicity of iron to invertebrates have been concerned primarily with dissolved iron species (Warnick & Bell 1969; Biesinger & Christensen 1972; Furmanska 1979; Havas & Hutchinson 1982; Gerhardt 1992), though some note the potential for detrimental effects from the presence of iron precipitates. Only one study (Sykora, *et al.*, 1972) has examined the effects of suspended iron III sulphate on the freshwater shrimp *Gammarus minus* and a caddis fly larva *Cheumatopsyche*. The effects of aluminium sulphate, which has been used to coagulate phosphate in a similar way to iron II sulphate, on the midge *Tanytarsus dissimilis* were examined by Lamb and Bailey (1981). Aluminium sulphate precipitated from solution and was associated with increased mortality of *Tanytarsus* larvae at concentrations of 80 – 480 mg litre^{-1}. Clearly, further study is needed to examine the possible deleterious effect of large scale iron III dosing.

The experiments described in this chapter were designed to explore the consequences of iron III addition and in particular of the presence of iron precipitates on cultures of *Chironomus riparius*, a riverine/littoral midge species. This species is convenient for laboratory toxicity studies and as a burrowing detritivore was thought to be particularly useful for the study of contamination by sedimented iron precipitates (see section 3.3).

The common approach to a problem of this nature is to perform short duration (24 – 96 hour) acute tests using mortality as an indicator of toxicity. Such tests supply quick results and may provide a useful range of concentrations for further study, but mortality is a crude measure and allows little understanding of the mode of action of the contaminant. Since long-term exposure to a sub-lethal contaminant concentration may have equally severe consequences compared to brief exposure to higher levels, the information provided by acute tests is limited. Where iron III dosing is used as the principal eutrophication control method near continuous dosing is common and therefore contamination of sediments with iron precipitates can span several years. Examination of significant sub-lethal effects is therefore important along with study of more obvious mortality effects. Two different duration tests have been carried out in this study. The shorter of the two (10-day duration) uses mortality and larval growth as parameters to provide a range of target iron concentrations likely to be of interest in longer tests (25-day duration).

4.2 EXPERIMENTAL PROCEDURE

4.2.1 Partial life cycle tests, ten-day duration

The procedure followed has been previously described in section 3.9. Table 4.1 summarises the six 10-day tests discussed in this chapter giving the test medium diluent used, the number of replicates per treatment and the target iron concentrations produced

Table 4.1 *Summary of the six 10-day experiments performed, indicating the test medium diluent the number of replicates per treatment and the range of target iron concentrations used in each experiment (marked with x).*

Experiment	Test medium diluent	No. of replicates per treatment	Target iron concentrations (mg Fe/litre)										
			0.0	0.5	5.0	25.0	32.5	42.5	45	47.5	50.0	55.0	125.0
A	Deionised water	5	x	x	x	x					x		x
B	Deionised water	3	x			x	x	x			x		
C	Filtered reservoir water	5	x	x	x	x					x		x
D	Filtered reservoir water	3	x			x	x	x			x		
E	Filtered reservoir water	3	x			x	x	x	x	x	x	x	

in each experiment. In experiments A and B deionised water is used as the test medium diluent instead of filtered reservoir water. It was hoped that this action would allow the iron III to remain in solution. These experiments (A and B) have been analysed separately from the experiments using filtered reservoir water as the test medium diluent.

Initial experiments used a wide range of target iron concentrations, 0.5 to 125 mg Fe litre^{-1}, with the intention of including concentrations which would give both little or no response and high or total mortality of chironomid larvae. Later a narrower range, 25 to 50 mg Fe litre^{-1} was used, within which lethal and sub-lethal responses to the dosing had been observed. The final experiment included digestion of chironomid larvae for iron analysis (see section 3.11).

4.2.2 Whole life cycle tests, 25-day duration

The procedure for these experiments is explained in section 3.10. Four replicate experiments were used. Target iron concentrations of 5, 15, 30 and 50 mg Fe litre^{-1} were used. Each experiment also included an undosed control treatment (0 mg Fe litre^{-1}), whose tanks were provided with 2-litres of filtered reservoir water and with amounts of artificial substrate and food source equal to other treatments. Measurement of water pH was made using a Jenway 3100 microprocessor pH meter with a Gerplas combination electrode. Readings were made prior to addition of animals in both the initial and replacement tanks and on each sampling day. Measurements of larval wet weight and instar stage were made as described in section 3.10.

The complete censuses of eggs and larvae during the exposure period provided information on the timing of mortality within each treatment. The percentage of non-viable eggs was calculated from egg counts at the start of the experiment and on day 4. Occasionally, first instar larvae died prior to leaving the eggmass (initially first instars feed on the eggmass coating). These were counted on day 4 together with the non-viable

eggs. Mortalities of larvae between leaving the eggmass and the first larval census (day 14) and between the first and second (day 25) larval censuses were also recorded.

4.3 RESULTS

4.3.1 Partial life cycle tests, ten-day duration

These shorter term tests utilised either deionised water or filtered reservoir water as the test medium. The former was intended as a measure to keep the majority of the iron in the dissolved form. Levels of dissolved iron and precipitated iron (indicated by particulate iron levels) are given in Table 4.2. Dissolved iron tended to increase with increasing target iron concentration. This trend was clearest where deionised water was used to dilute the iron stock solution. Similarly, the amounts of precipitated iron (indicated by the measured levels of iron in solutions produced by digesting the dried residue of sediment from each test) increase with increasing target iron concentration. This indicates that in both types of medium the majority of iron precipitated from solution. Use of deionised water to retain iron in solution effectively failed.

Table 4.2 *Levels of dissolved iron and iron in particulate matter for 10-day duration tests. All values are given as mM iron.*

	Deionised water diluent		Filtered reservoir water diluent	
Target iron concentration	Dissolved iron	Particulate iron	Dissolved iron	Particulate iron
0 mg Fe/litre	0 – 1	3 – 18	0 – 2	4 –18
25 mg Fe/litre	1 – 12	305 – 557	6 – 32	220 – 457
50 mg Fe/litre	60 – 113	993 – 3670	0 – 6	178 – 3361

Particulate iron readings indicate the amount of iron precipitate in each treatment. The precipitate has an empirical formula and so its iron content is relatively constant regardless of the target iron concentration. However, the particulate iron readings will vary not only with the amount of precipitate but also with the amount of artificial substrate in the sample. For this reason all analysis has been compared to the target iron concentrations for each treatment.

Percentage mortality figures and means, standard deviations and 95% confidence intervals of larval wet weight are given in Tables 4.3 and 4.4 for deionised water and filtered reservoir water experiments respectively. Total mortality occurred at 32.5 mg Fe litre^{-1} and above in deionised water and at 125 mg Fe litre^{-1} in filtered reservoir water.

Table 4.3 *Percentage mortality and statistical descriptions of larval wet weight measurements for 10-day (240h) experiments exposing second to fourth instar* C. riparius *larvae to iron precipitates using deionised water as the medium. 95% Confidence intervals were calculated using the Student's t-distribution.*

Target concentration (mg Fe/litre)	No. replicates	Larval wet weight (mg)			% Mortality
		Mean	S.D.	95% C.I.'s	
0.0	8	8.170	.821	7.484 - 8.856	0.00
0.5	5	8.584	.240	8.286 - 8.882	1.85
5.0	5	8.730	.673	7.894 - 9.566	4.00
25.0	8	7.284	.635	6.697 - 7.871	25.00
32.5	3	-	-	-	100.00
42.5	3	-	-	-	100.00
50.0	8	-	-	-	100.00
125	5	-	-	-	100.00

Table 4.4 *Percentage mortality and statistical descriptions of larval wet weight measurements for 10-day (240h) experiments exposing second to fourth instar* C. riparius *larvae to iron precipitates using filtered reservoir water as the medium. 95% Confidence intervals were calculated using the Student's t-distribution.(*100% mortality in two of three replicates).*

Target concentration (mg Fe/litre)	No. replicates	Larval wet weight (mg)			% mortality
		Mean	S.D.	95% C.I.'s	
0.0	11	7.145	.981	6.486 - 7.804	6.25
0.5	5	7.498	.286	7.143 - 7.853	5.56
5.0	5	7.762	.955	6.576 - 8.948	6.00
25.0	11	7.602	.574	7.216 - 7.988	2.50
32.5	6	7.655	.547	7.655 - 8.299	6.67
42.5	6	7.618	.943	6.628 - 8.608	0.00
45.0	3	8.073	.791	5.666 - 10.480	3.33
47.5	3	6.137	.006	6.119 - 6.155	3.33
50.0	11	5.563	1.368	4.644 - 6.482	40.91
55.0	3*	5.357	-	-	76.67
125.0	5	-	-	-	100.00

Mortality response curves for both media are presented in Figure 4.1. It is standard to use a probit transformation for percentage mortality and/or a $\log_{10}$ transformation of the dose to linearise the sigmoid reponse curve for estimation of LC_{50} values (Finney 1971; Gauss, *et al.*, 1985). For these data, however, the increase in mortality is so steep that neither probit nor log transformations nor combinations of them, allowed any greater resolution of the LC_{50} value. For this reason only the untransformed data are shown here and were used in the LC_{50} calculation.

Values for 240 hour LC_{50}'s were 27 and 51 mg target iron $litre^{-1}$ for iron in deionised water and filtered reservoir water respectively. Toxicity of iron in deionised water was, therefore, approximately twice that in filtered reservoir water.

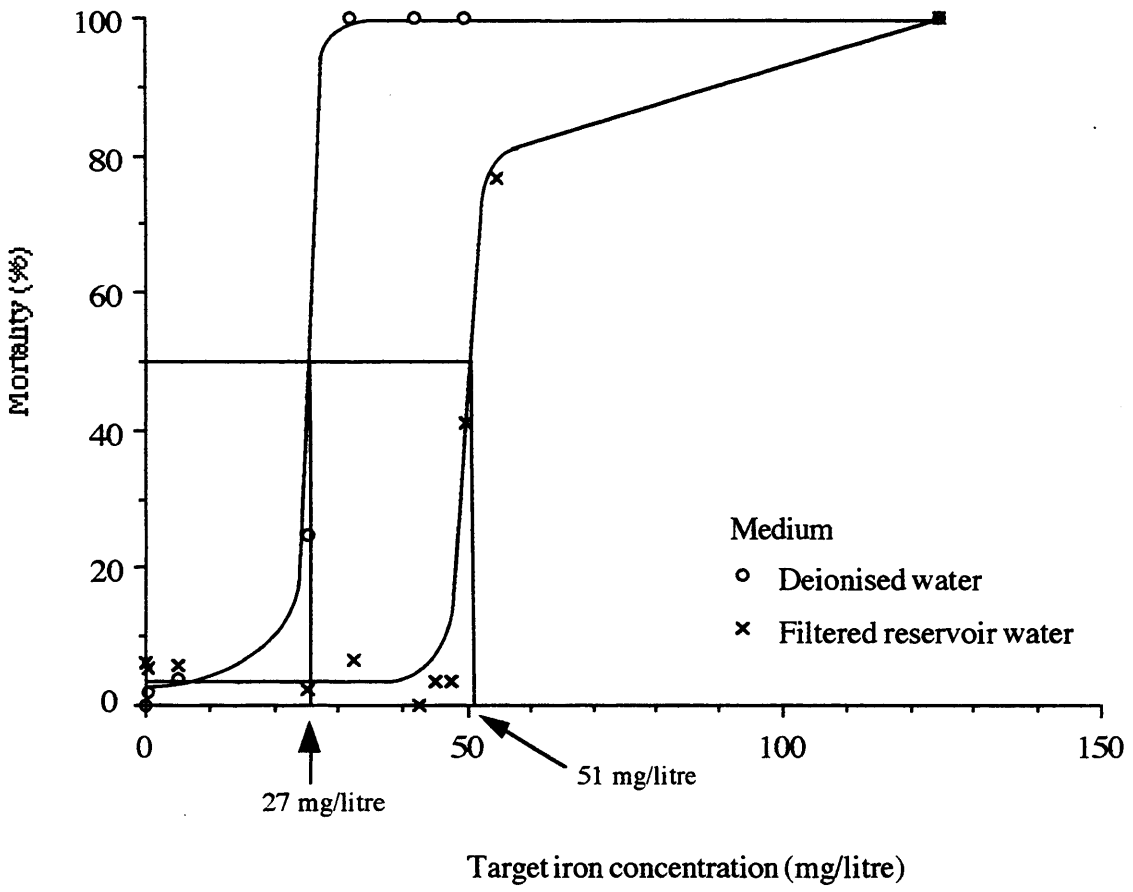

Figure 4.1 *Percentage mortality response curves for the two test mediums. LC_{50} values are 27 and 51 mg target Fe litre^{-1} for deionised water and filtered reservoir water respectively.*

Analysis of the larval weight data in these experiments has been done using an ANOVA technique called General Linear Modelling (GLM). GLM is a block analysis technique in which the effect of two or more determining factors and their interactions can be tested. The main factor to be tested here is whether target iron concentrations affect larval growth. Due to practical restraints, however, repeats of experiments were carried out on different dates and so different runs may also be a factor affecting the analysis. Where filtered reservoir water was used as diluent three experimental runs were performed and a total of 11 different target concentrations used. However, GLM requires that all blocks in the analysis contain data. Since different target iron concentrations were used on different runs some blocks would not contain data. Only three target iron

concentrations (0, 25 and 50 mg Fe litre^{-1}) were used in every experimental run. The resulting GLM was a 3x3 block design for treatments x runs. The analysis requires that the variable be approximately normally distributed and that the variances of the blocks are homogeneous. Block variances were tested for homogeneity using the F_{max} ratio (Sokal and Rohlf, 1969). Where deionised water was used as diluent a 2x2 design was used because only two runs were performed and no larval weight data were gathered from the 50 mg Fe litre^{-1} treatment due to total mortality of larvae. Results of these statistical analyses are shown in Table 4.5. A significant difference in larval wet weight between target iron treatments has been shown for both test media ($p < 0.01$), but a significant difference due to experimental run is also present in both cases ($p < 0.02$). There is no significant interaction of treatment and run ($p > 0.05$). Larval wet weights resulting from the filtered reservoir water tests are illustrated in Figure 4.2.

Table 4.5 *Two-way general linear model (GLM) statistics for differences in larval wet weight due to target iron treatments and experimental run from 10-day duration tests.*

	Deionised water			
	Degrees of freedom	Mean squares	F-statistic	Probability
Target iron concentration	1	3.672	15.85	< 0.005
Experimental Run	1	3.993	17.24	< 0.005
Interaction	1	0.458	1.98	> 0.05
Error	11	0.232		
	Filtered reservoir water			
	Degrees of freedom	Mean squares	F-statistic	Probability
Target iron concentration	2	10.114	13.84	< 0.0005
Experimental Run	2	3.474	4.75	< 0.02
Interaction	4	1.785	2.44	> 0.05
Error	24	0.731		

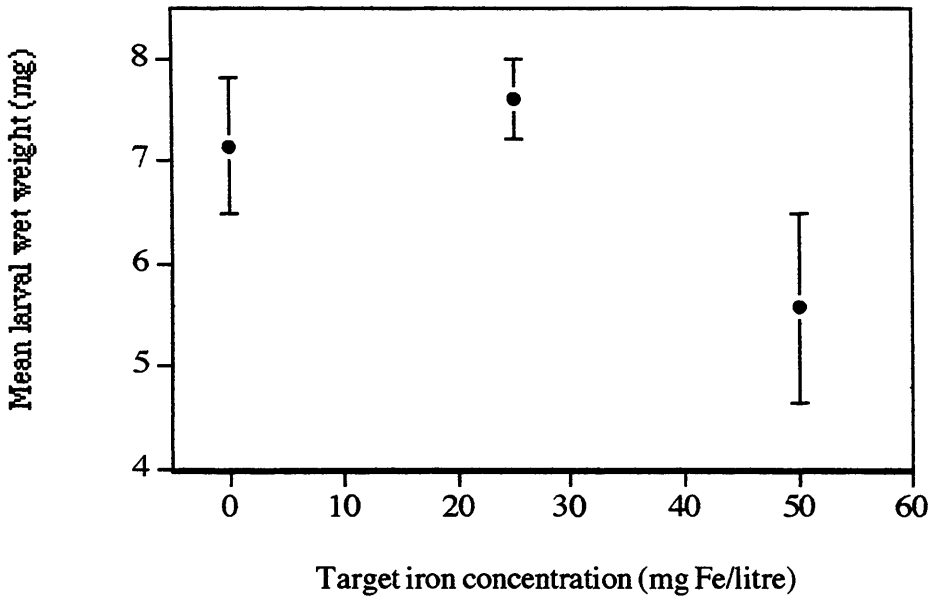

Figure 4.2 *Mean larval wet weight at the end of 10 day exposure to varying target iron concentrations in filtered reservoir water. Error bars represent 95% confidence intervals form the Student's t-distribution.*

Iron content of digested chironomid larvae (experiment E only) shows an exponential increase with target iron concentration. A scatter plot of log iron content against target iron concentration is shown in Figure 4.3.

4.3.2 Whole life cycle tests, 25-day duration

Precipitation of iron was rapid and virtually complete as with shorter duration experiments. To explore the variance in levels of dissolved/suspended iron and precipitated iron the experimental design suggests a three-factor block analysis; five target iron treatments, four experimental runs and six different sampling days within the procedure. Unfortunately, this gives only one sample value per block allowing no calculation of variance and therefore no three-way general linear model could be performed. Instead, two two-way block designs were used, one combining treatment and

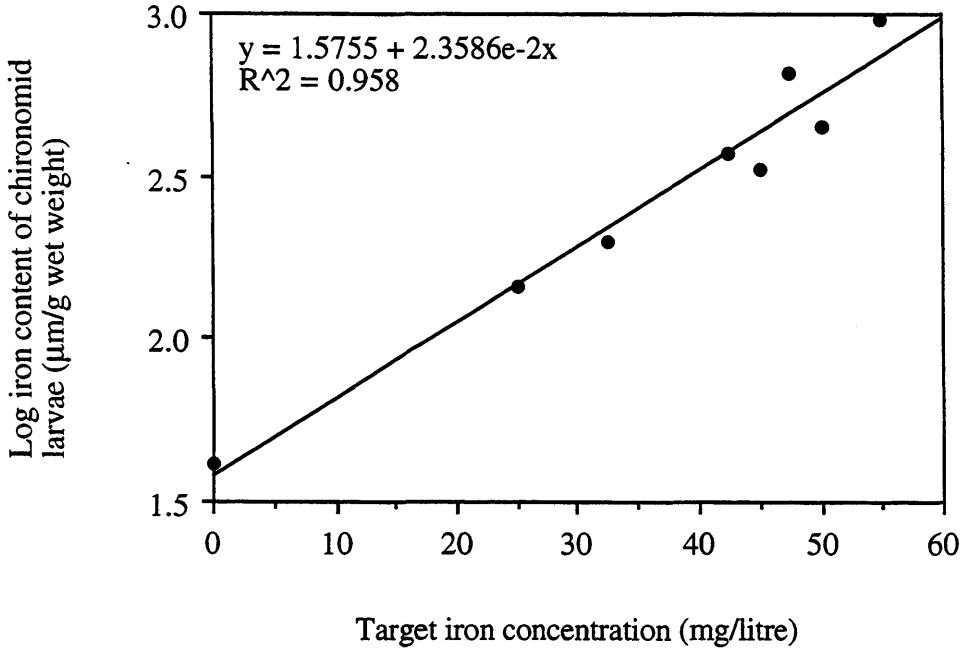

Figure 4.3 *Iron content of chironomid larvae (log scale) recovered from target iron treatments in experiment E of the 10-day duration tests.*

experimental run and the other combining treatment with sample day. For both dissolved/suspended and precipitated iron $\log_{10}$ transformation of the raw data was required to achieve homogeneity of variances (checked using F_{max} ratios).

Results of these analyses are presented in Tables 4.6 and 4.7. For both dissolved/suspended and precipitated iron significant differences between target iron treatments were present. Figure 4.4 shows that dissolved/suspended iron levels were only significantly different from the control treatment in the 50 mg Fe litre^{-1} treatment. Levels of iron precipitate, indicated by measurement of mg iron per g dry weight of particulate matter sampled from tank sediment cores, increased significantly with increasing target iron concentration (see Figure 4.5). This illustrates the greater importance of precipitated iron within the treatments. Experimental run made no

Table 4.6 *Results of two two-way GLM"s examining the effects of target iron treatment, experimental run and sample day on dissolved/suspended iron concentrations in 25-day duration tests.*

	Degrees of freedom	Mean squares	F-statistic	Probability
Target iron concentration	4	3.435	32.41	< 0.0005
Experimental Run	3	0.143	1.35	> 0.05
Treatment/Run Interaction	12	0.111	1.05	> 0.05
Error	72	0.106		
Target iron concentration	4	3.283	52.96	< 0.0005
Sample Day	5	0.492	7.94	< 0.0005
Treatment/Day Interaction	20	0.115	1.86	< 0.05
Error	63	0.062		

Table 4.7 *Results of two two-way GLM"s examining the effects of target iron treatment, experimental run and sample day on precipitated iron levels in 25-day duration tests.*

	Degrees of freedom	Mean squares	F-statistic	Probability
Target iron concentration	4	17.091	370.42	< 0.0005
Experimental Run	3	0.074	1.61	> 0.05
Treatment/Run Interaction	12	0.070	1.51	> 0.05
Error	97	0.046		
Target iron concentration	4	16.671	360.82	< 0.0005
Sample Day	5	0.208	4.50	< 0.001
Treatment/Day Interaction	20	0.025	0.53	> 0.05
Error	87	0.046		

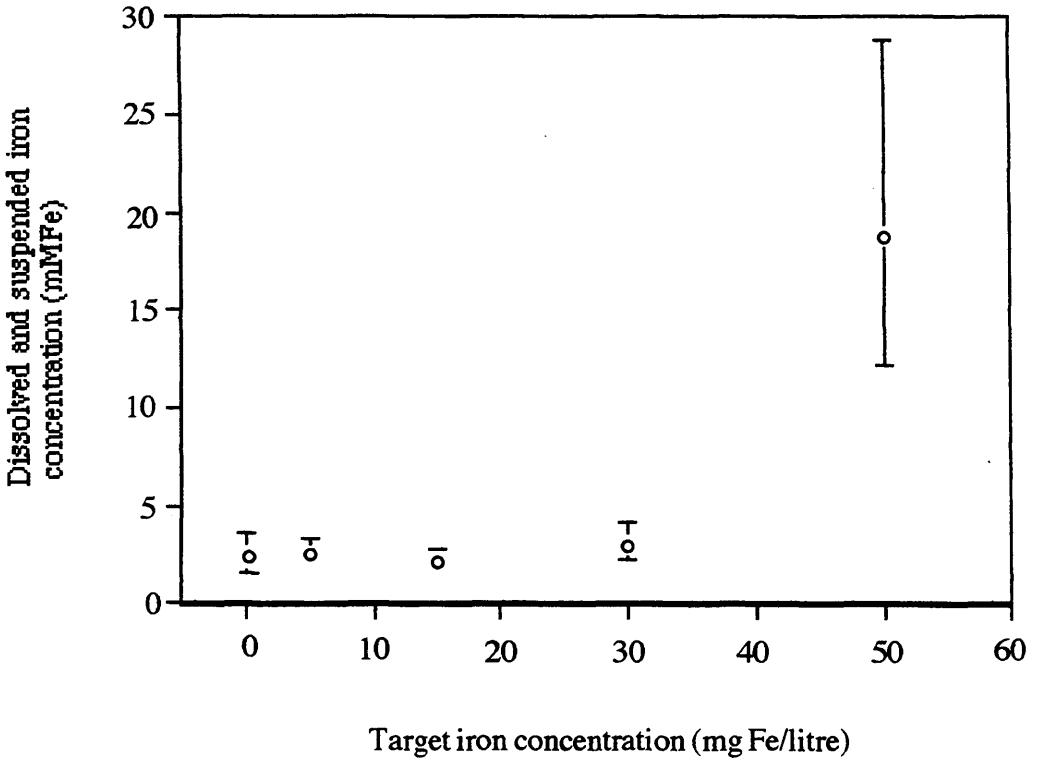

Figure 4.4 *Mean levels of dissolved/suspended iron in target iron treatments. Error bars represent 95% confidence intervals form the Student's t-distribution.*

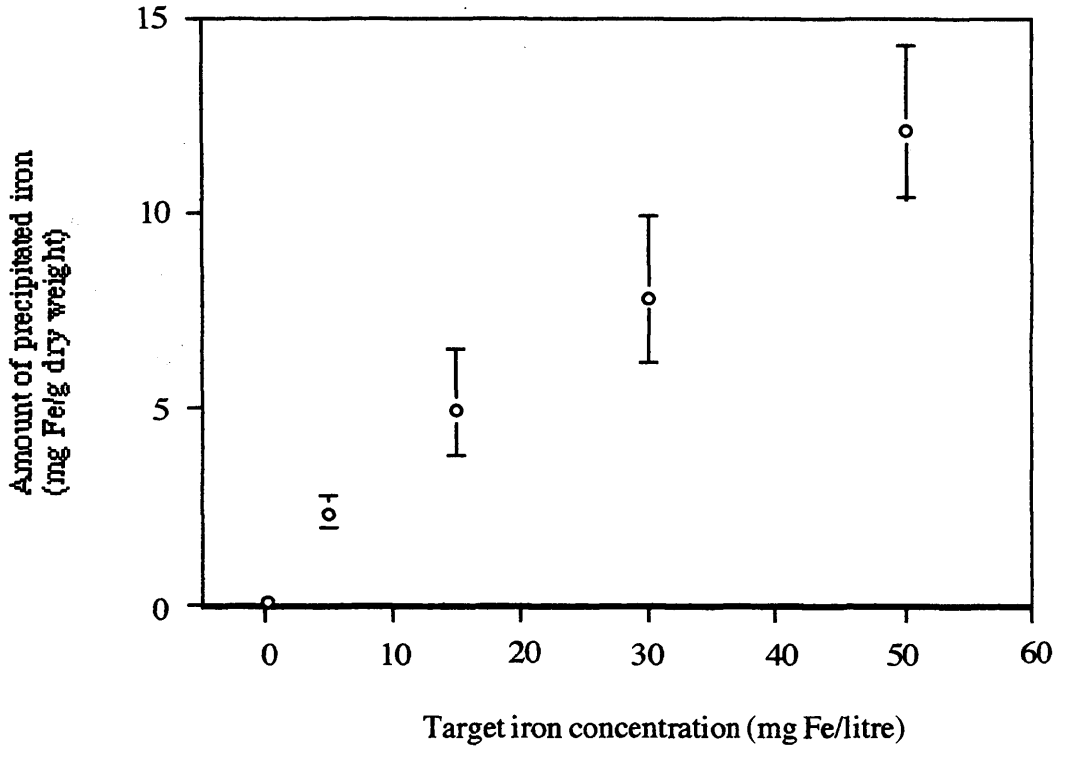

Figure 4.5 *Mean levels of precipitated iron present in target iron treatments. Error bars represent 95% confidence intervals form the Student's t-distribution.*

significant difference to either variable but sample day was significant in both. No significant interaction of treatment and run was present, but the effect of interaction of treatment and day on dissolved/suspended iron levels was significant. The effect of sample day might be explained by the use of replacement tanks half-way through each experimental run. Despite replacement tanks being set up in the same way as original tanks it is inevitable that some differences between tanks will be present.

The pH range of the filtered reservoir water used in these experiments was approximately 8.0 – 8.5 and the water contained high calcium carbonate levels. The iron III sulphate stock solution used, however, was strongly acidic (approx. pH 2) and so pH changes due to the addition of iron III sulphate were monitored (Figure 4.6). The pH of the control treatment remained at about pH 8.0 for the duration of the experiment and target iron treatments upto 15 mg Fe litre^{-1} displayed little reduction in pH (all measurements were above neutral pH). The initial pH (day 0) of the 30 mg Fe litre^{-1} treatment was 4.75 but rapidly rose above neutrality (by day 6). This pattern was repeated in the replacement tank, and was more pronounced in the 50 mg Fe litre^{-1} treatments. Initial pHs were between 2.5 – 3 followed by a gradual increase to 5.5 – 6.5 in this treatment. It is clear that the buffering capacity of the reservoir water is high and that it is capable of correcting severe depression of pH.

Mortality of individuals within the exposure period was divided into four time periods. The first was non-viable or unhatched eggs; second was death of first instar larvae prior to leaving the eggmass (LD1); third was death of larvae after leaving the eggmass but before the first census (LD2) and fourth was larval death between the first census and the second one at the end of the experiment (LD3). Figure 4.7 shows these four mortality timings as percentages of the numbers of individuals entering each time period, a total percentage larval mortality is also included (Total LD). These values were calculated from the total numbers entering the four experiments and accounted for individuals that emerged or were sampled during the experiment (see appendix C.9).

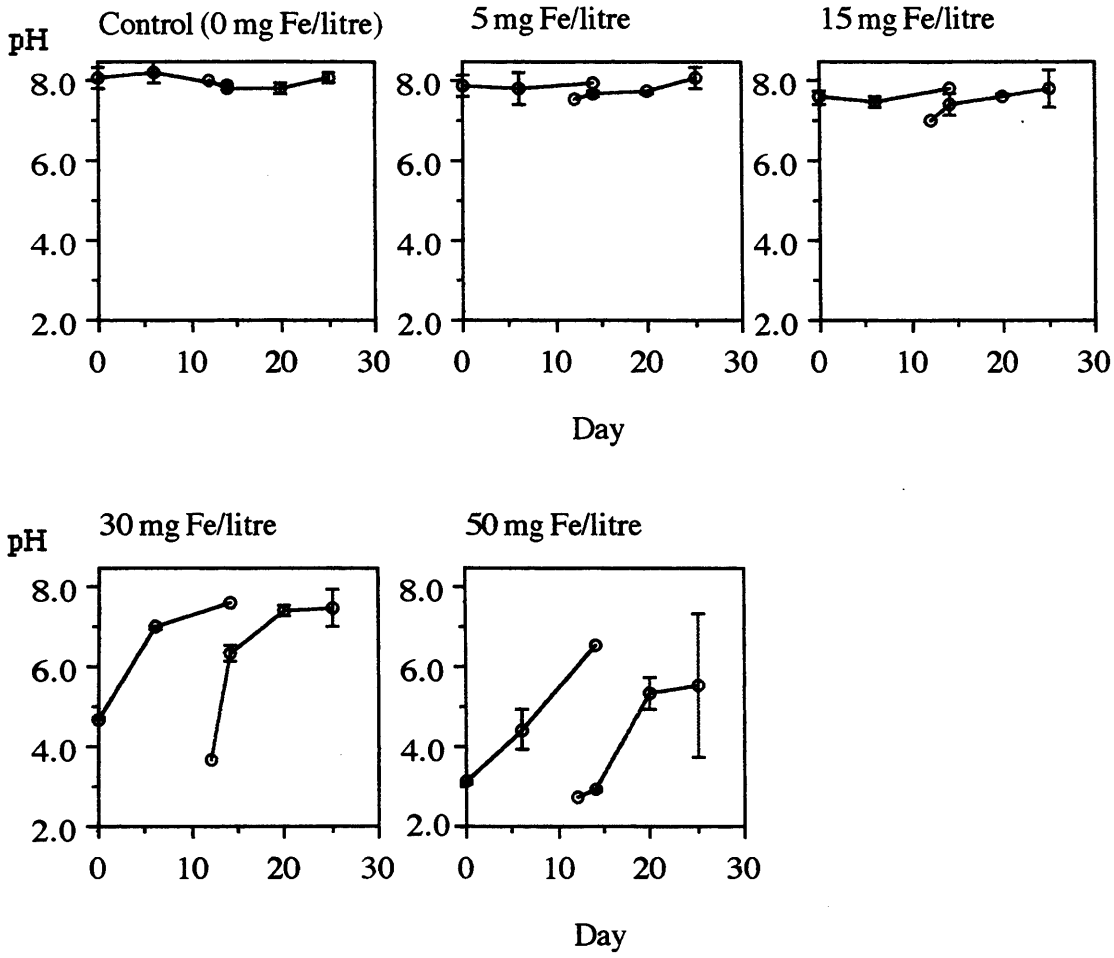

Figure 4.6 *Changes in pH following the addition of iron III sulphate. Error bars represent 95% confidence intervals form the Student's t-distribution.*

Percentage egg non-viability appears not to be affected by increasing target iron concentration. Greater than 80 percent hatch occurred in all treatments. Total larval mortality was elevated in all iron treatments compared to the control (6.6%). A substantial increase in total larval mortality occurred at 50 mg Fe litre^{-1}; 95.97% compared to 30.32% in the 30 mg Fe litre^{-1} treatment. By calculating the percentage mortality of larvae entering each period an estimation of the power of the mortality factor of each period can be assessed. The slight increase in mortality in treatments up to 30 mg Fe litre^{-1} appears to be mainly due to the death of larvae between leaving the eggmass and the first census (LD2). At and above 30 mg Fe litre^{-1} LD3, i.e death between the first and second census, appears more important. At 50 mg Fe litre^{-1} high mortality occurred during each of the

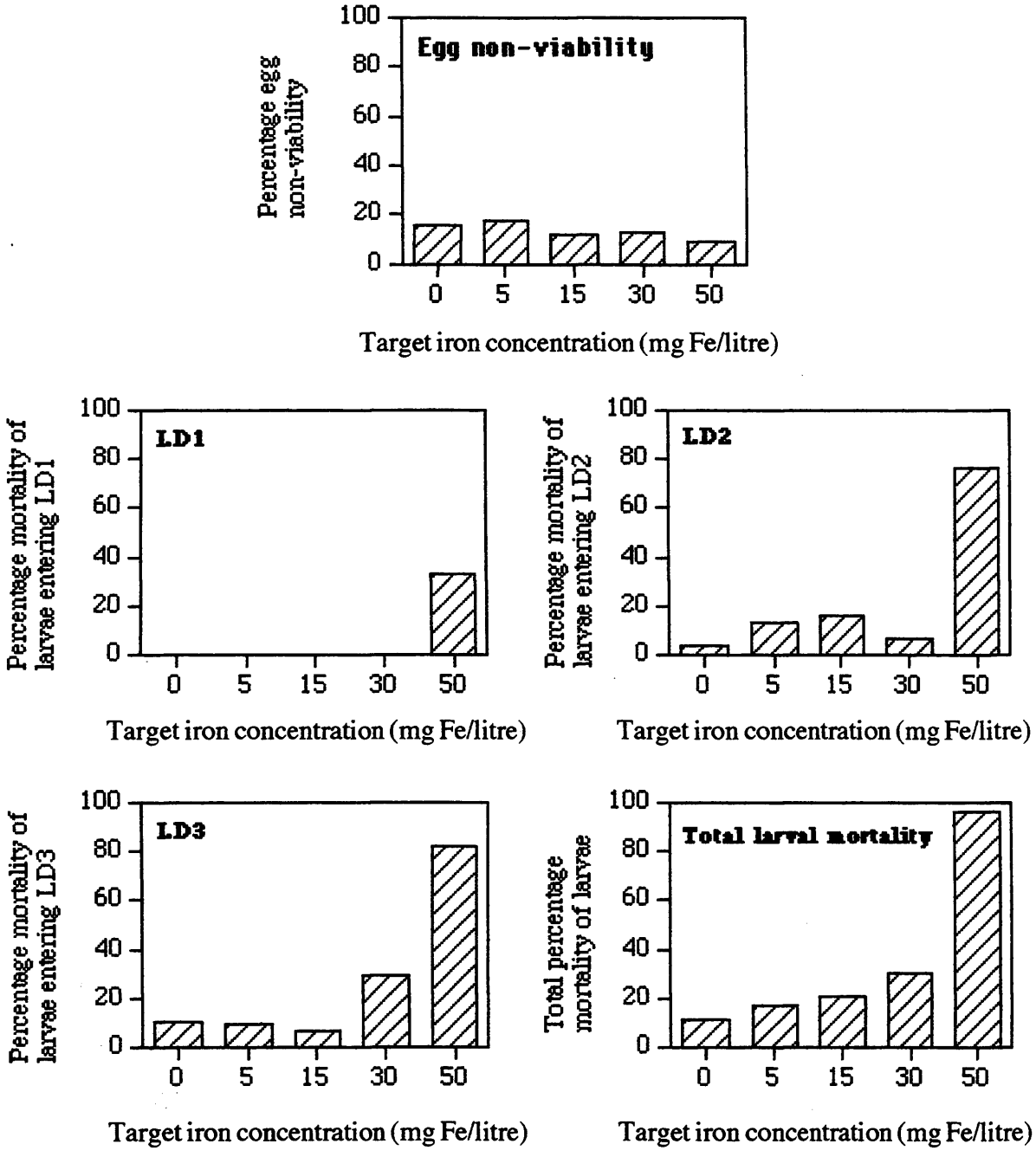

Figure 4.7 *Percentage egg non-viability and mortality of larvae during the test period. Larval mortality is given as the percentage mortality of the larvae entering a particular stage during the test. LD1 refers to larvae that have hatched but die before leaving the eggmass; LD2 refers to larvae that have left the eggmass but die before tank replacement; and LD3 refers to larvae that survived as far as tank replacement on day 14 of the test but did not survive to day 25 of the test. Total larval mortality is a measure of the number of larvae that died before the end of the test as a percentage of the number that hatched.*

three periods, though LD3 remained the strongest. Notably 50 mg Fe litre^{-1} was the only treatment in which larvae died prior to leaving the eggmass (LD1) and this accounted for over 65 percent of successfully hatched larvae.

A two-way GLM was performed for the effects of target iron treatment and experimental run on larval wet weight, the results for each sample day are summarised in Table 4.8. A significant trend of decreasing larval wet weight with increasing target iron concentration was observed (Figure 4.8).

Table 4.8 *Results of two-way GLM"s examining the effects of target iron treatment and experimental run on larval wet weight in 25-day duration tests.*

	Day 14			
	DF	Mean squares	F-statistic	Probability
Target iron concentration	3	3.112	4.79	< 0.005
Experimental Run	3	9.809	15.08	< 0.0005
Interaction	9	4.188	6.44	< 0.0005
Error	134	0.650		
	Day 20			
	DF	Mean squares	F-statistic	Probability
Target iron concentration	3	19.179	10.02	< 0.0005
Experimental Run	3	9.783	5.11	< 0.005
Interaction	9	8.384	4.38	< 0.0005
Error	119	1.913		
	Day 25			
	DF	Mean squares	F-statistic	Probability
Target iron concentration	3	34.137	17.18	< 0.0005
Experimental Run	3	10.162	5.11	< 0.005
Interaction	9	10.291	5.18	< 0.0005
Error	136	1.987		

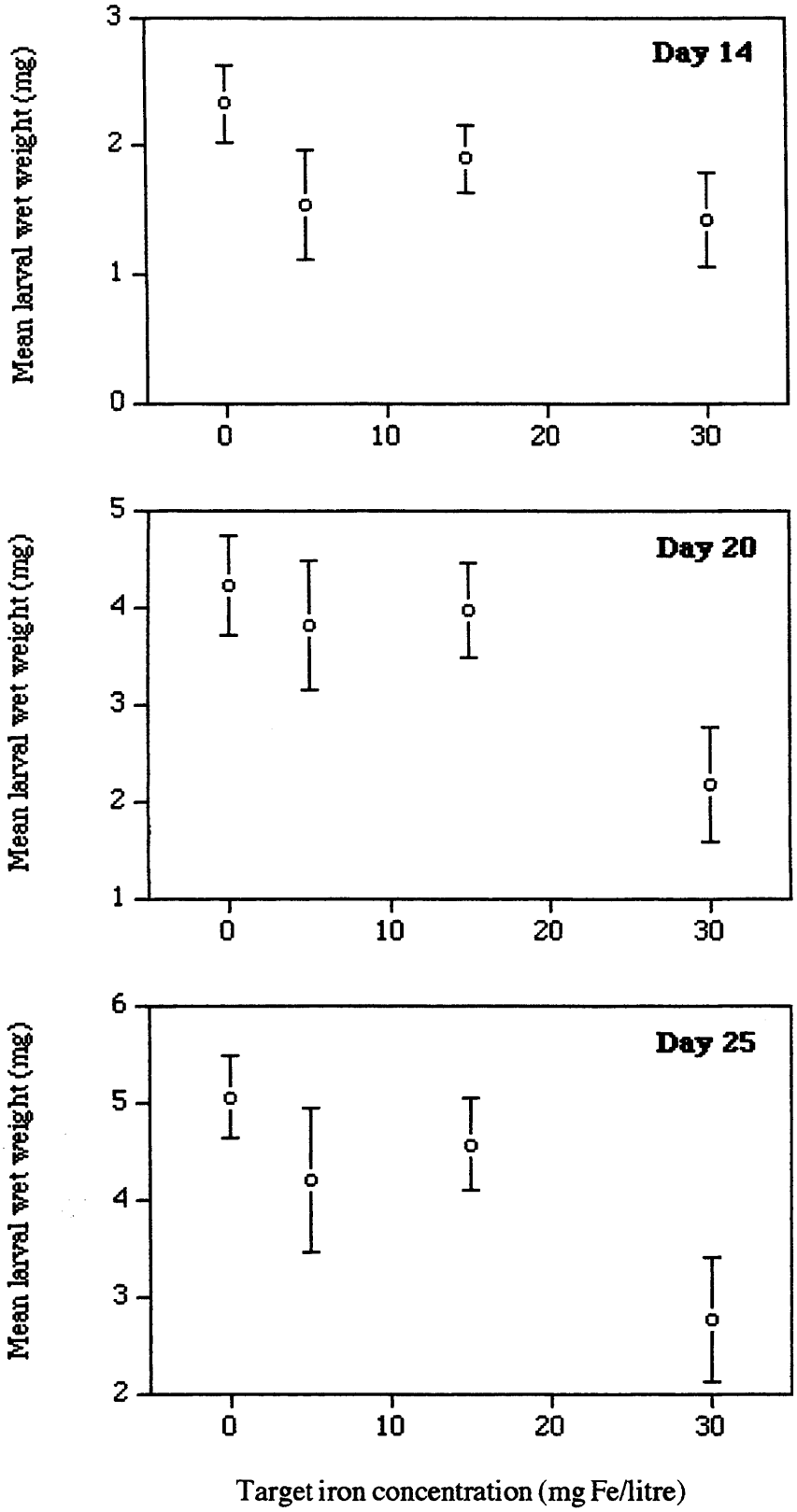

Figure 4.8 *Mean larval wet weight from target iron treatments. Error bars represent 95% confidence intervals from the Student's t-distribution.*

The 95% confidence intervals in Figure 4.8 show that even larvae exposed to 5 mg Fe litre^{-1} had wet weights significantly lower than the control on Day 14. By day 20 wet weights in the 5 and 15 mg Fe litre^{-1} treatments had caught up with the control, but wet weight of larvae in the 30 mg Fe litre^{-1} treatment remained significantly lower than the control. Due to high larval mortality few larvae were recovered from the 50 mg Fe litre^{-1} treatment and so results from this treatment were not included in the analysis. Larval wet weight also varied significantly due to the run of the experiment and there was significant interaction between treatment and experimental run. There are a number of factors that may have affected growth rate and may have contributed to these differences between runs and between treatments from different runs. It is probable, for instance, that the density of larvae will have an effect on growth rate. Bievier (1971) observed delayed larval development in chironomid larvae in higher than optimum density levels. Whilst an effort was made to use eggmasses containing similar numbers of eggs, it was not possible to predict how many of those eggs would hatch and therefore densities will have varied between treatments and runs. Natural genetic variation between and within eggmasses is also a conceivable factor in the growth rate of larvae.

Measurement of head-capsule width indicated that the development of larvae through the four instar stages may have been retarded by increasing target iron concentration (Figure 4.9). Very low numbers of larvae were recovered from the 50 mg Fe litre^{-1} treatment due to high larval mortality in this treatment and are not included in the analysis. G-tests for independence were performed (Sokal and Rohlf, 1981) to examine the effect of target iron treatment and experimental run on larval instar stage. This test was chosen in preference to a χ^2 test for independence as a χ^2 test requires expected values to be ≥ 5, which would not be the case where all larvae might be expected to have reached one stage but larvae from lower stages are observed. G-statistics and probabilities are presented in Table 4.9. The null hypothesis of a G-test states that the parameter (developmental stage of larvae) is independent of the factor under examination (target iron concentration or experimental run). On days 6, 14 and 20 instar stage was

dependent on target iron concentration, whilst on day 25 instar stage was independent of target iron. Instar stage was also found to be dependent on the experimental run on days 6 and 14 but independent of this factor on days 20 and 25 of the test.

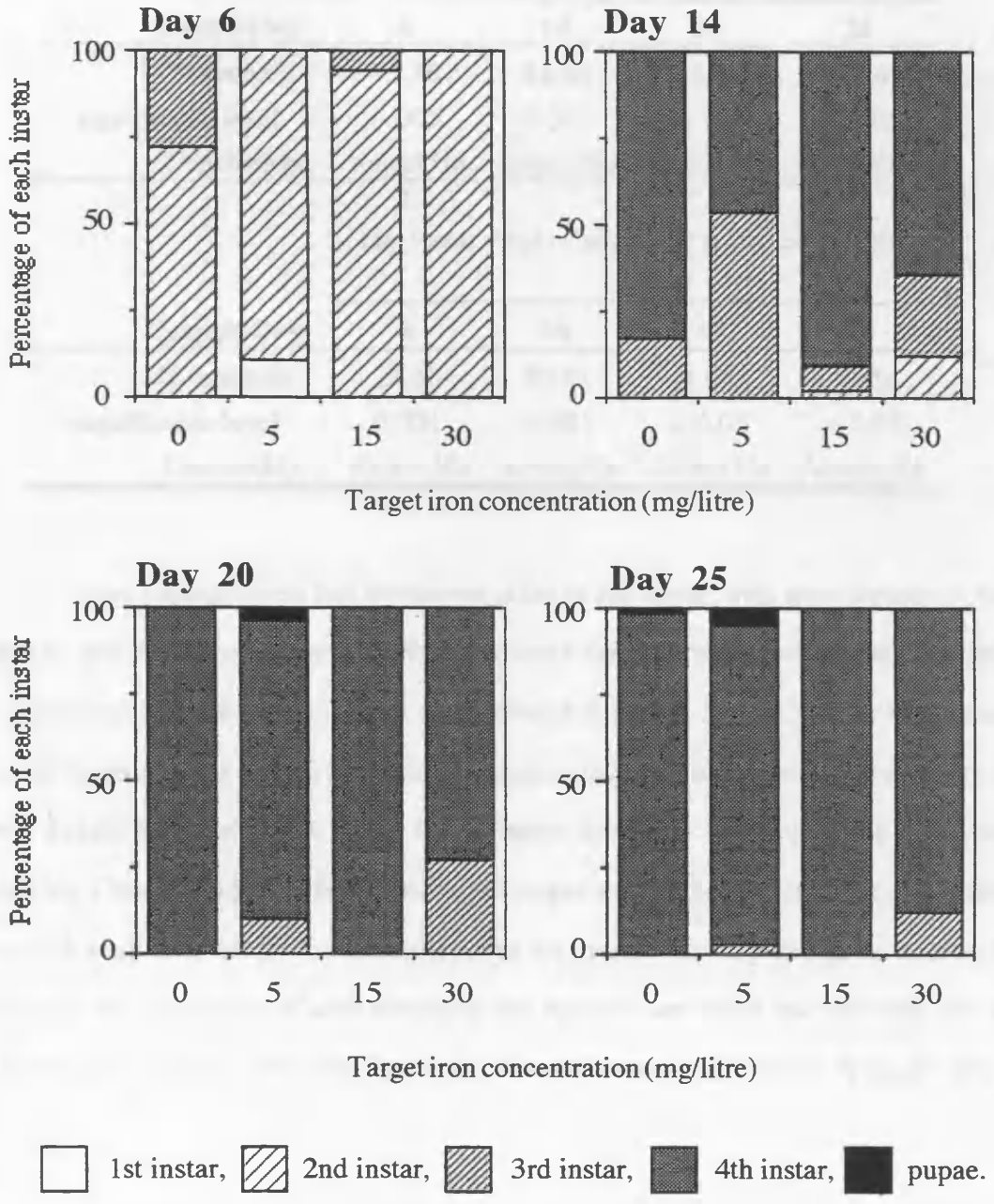

Figure 4.9 *Percentage of each instar stage present within samples from target iron treatments from day 6, 14, 20 and 25 of the tests.*

Table 4.9 G-test for independence results from 25-day experiments.

	Hø: Instar stage is independent of target iron concentration			
Sample Day	**6**	**14**	**20**	**25**
G - statistic	15.38	24.64	23.00	3.74
significance level	0.005	0.001	0.001	> 0.05
Conclusion	Reject Hø	Reject Hø	Reject Hø	Accept Hø

	Hø: Instar stage is independent of experimental run			
Sample Day	**6**	**14**	**20**	**25**
G - statistic	29.13	30.91	4.04	5.89
significance level	0.001	0.001	> 0.05	> 0.05
Conclusion	Reject Hø	Reject Hø	Accept Hø	Accept Hø

Most control larvae had developed as far as 2nd instar, with some already at 3rd instar by day 6. The majority of larvae recovered form all other treatments were still second instars and the small number of recovered from the 50 mg Fe litre^{-1} treatment were all 1st instars. By day 20 fourth instars dominated in all treatments. The majority of larval weight increase occurs in the fourth instar since it is the stage used for over-wintering. Consequently, the first three larval stages are short-lived. It is not surprising, even with a lag in development that most larvae are fourth instars by day 20 of a 25 – 30 day life cycle. Emergence of adult flies from the cultures was noted but was very low in all treatments (generally less than 5 percent). No adults emerged from the 50 mg Fe litre^{-1} treatment.

Measurements of larval iron content were made across all four experimental runs and four of the target iron treatments (the 50 mg Fe litre^{-1} treatment was excluded due to lack of data). A significant trend of increasing total iron content with increasing target iron concentration was found (Figure 4.10). Experimental run and treatment/run

interactions were not significant factors affecting larval iron content (Table 4.10). The data represent the combined data from the different sampling days. Larval iron content was found to vary significantly with sample day (Table 4.10) but not with treatment/sample day interaction. As the larvae develop, their ability to take in iron may have altered e.g. later instar larvae with larger mouth parts may take in significantly more iron precipitate than earlier instar larvae. Thus, the day of sampling may appear to affect the iron content of larvae due to the distribution of larval stages present in the sample. Data were $\log_{10}$ transformed to gain homogeneity of block variances.

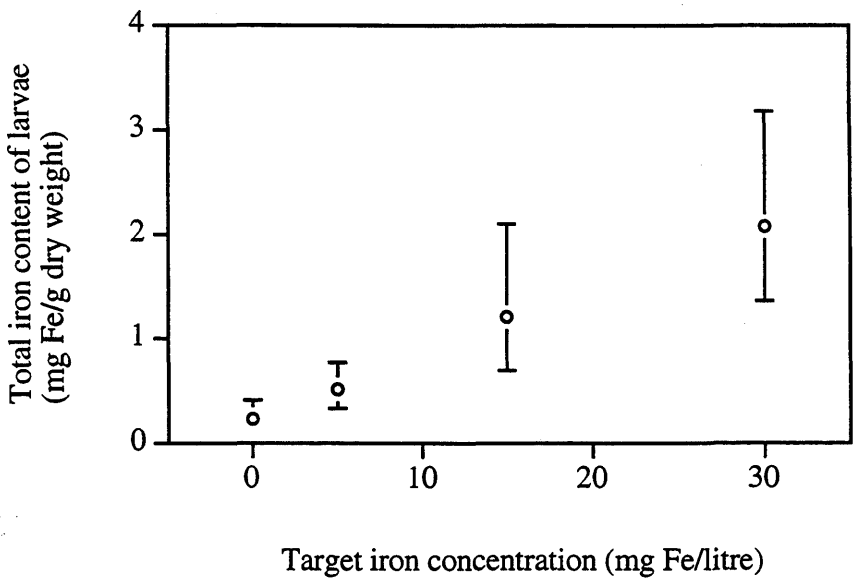

Figure 4.10 *Mean total iron content of chironomid larvae from target iron concentrations. Error bars represent 95% confidence intervals of the Student's t-distribution.*

Table 4.10 Results of two two-way GLM"s examining the effects of target iron treatment, experimental run and sample day on total iron content of larvae from 25-day duration tests.

	Degrees of freedom	Mean squares	F-statistic	Probability
Target iron concentration	3	1.814	15.62	< 0.001
Experimental Run	3	0.133	1.15	> 0.05
Treatment/Run Interaction	9	0.087	0.75	> 0.05
Error	31	0.116		
Target iron concentration	3	1.858	23.56	< 0.001
Sample Day	2	0.640	8.12	> 0.01
Treatment/Day Interaction	6	0.112	1.43	> 0.05
Error	35	0.79		

Observation of larvae from even the lowest iron concentrations revealed orange precipitates within the guts (Figure 4.11), thus suggesting that gut associated iron makes up the bulk of larval iron content. It is likely that ingestion of iron precipitates by chironomid larvae has taken place.

4.4 DISCUSSION

It is clear from the experiments described in this chapter that several chemical and biotic consequences result from the addition of iron III sulphate. In both of the experiment types iron III precipitated from solution rapidly and almost completely after addition. The extent to which this statement is true depends on the exact conditions present in the test chamber. In both experiment types efforts were made to maintain a high oxygen saturation in the test medium in order to keep iron in the iron III form, although no oxygen measurement was made. Determination by titration (manganese dioxide) could not be used since iron interferes with the reaction (Gerhardt 1992) and

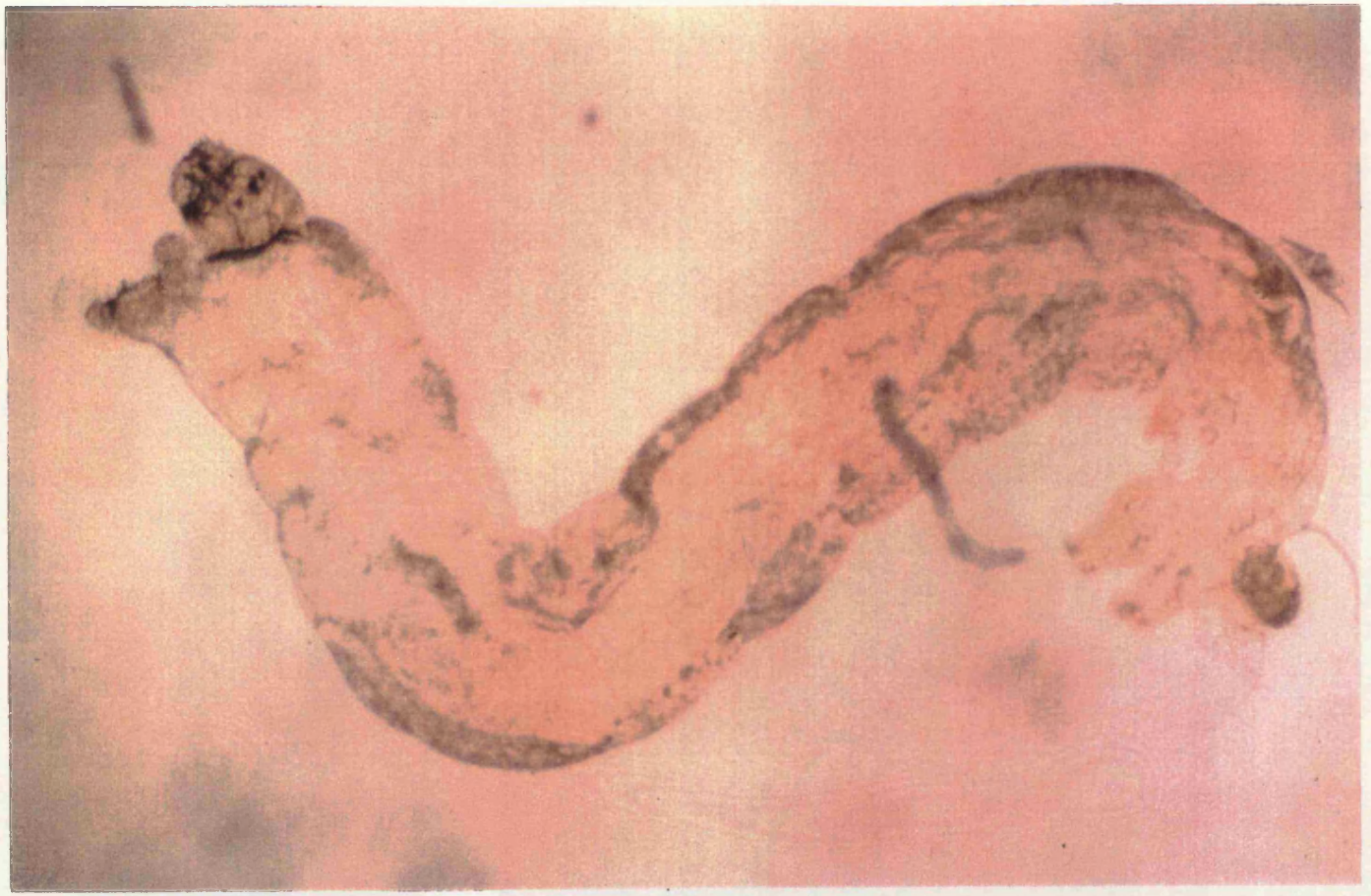

Figure 4.11 *(i)* Chironomus riparius *larva recovered from a control treatment (0 mg Fe litre^{-1}), x40 magnification.*

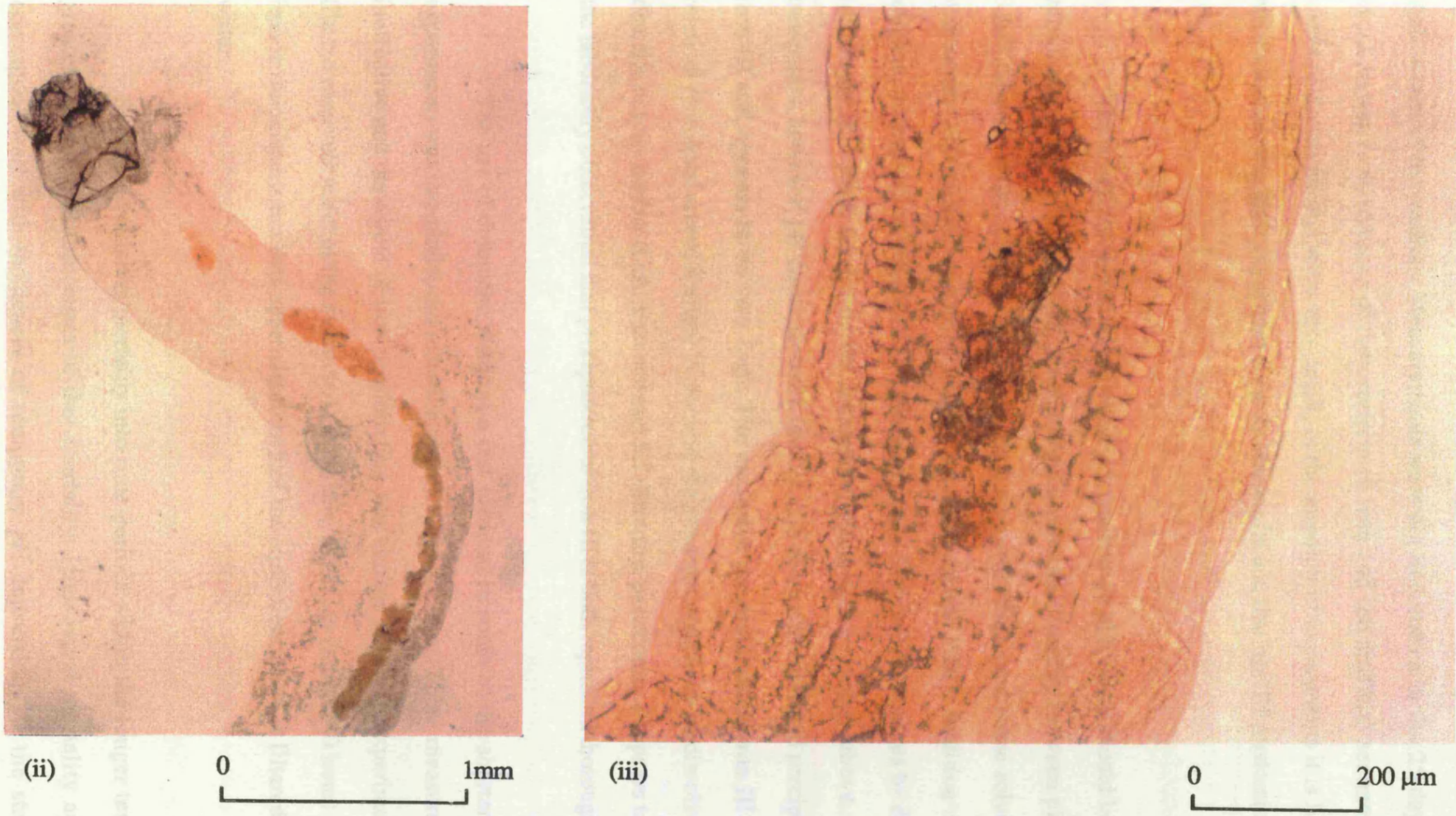

Figure 4.11 *(ii)* C. riparius *larva recovered from a 30 mg Fe litre^{-1} treatment, x40 magnification, showing orange precipitates in the gut (iii) close view (x100 magnification) of precipitates in midgut of larva recovered from a 10 mg Fe litre^{-1} treatment.*

measurement by oxygen electrode could not be made in 10-day experiments due to the shallowness of the medium. Measurements were not undertaken in the 25-day tests to avoid excessive disturbance of the precipitated iron and the artificial sediment. Since levels of dissolved iron were very small, in the majority of experiments it is likely that efforts to maintain high oxygen levels were successful and that iron III predominated.

The solubility of iron III is strongly dependent on pH, which should be close to zero for iron III to be in solution. Precipitation occurs most readily between pH 1.2 and 3.0 (Cotton & Wilkinson 1962; Sholkovitz & Copland 1981) and some solubilisation may occur between pH 3 and pH 11 (Sholkovitz & Copland 1981). Addition of iron III sulphate may have facilitated its own precipitation in high iron treatments by decreasing initial pH to within the limits for maximum precipitation quoted above. In most treatments, however, pH depression did not occur to these extremes and precipitation of iron III still appears to be very high. The extensive precipitation of iron III within a relatively localised area at Rutland Water and the apparent constancy of dissolved iron at that site despite dosing (C.A. Extence and S.J. Brierley, pers comm.) appears to back up the laboratory observation that precipitation of iron III is both rapid and thorough.

The use of deionised water as a test medium in some of the shorter duration experiments was intended to aid retention of iron III in solution. These measures proved ineffective and the extent of iron precipitation was similar to that in experiments using filtered reservoir water as the medium. Despite this, the LC_{50} value (240 hour) for target iron in deionised water was approximately half the equivalent value in filtered reservoir water.

Separation of larval mortality into time periods within the longer tests allows greater resolution of the causes of that mortality. High larval mortality appears to correspond well with incidences of temporary pH depression. At the start of the

experiment, the pH was low in the high target concentrations, but the eggmass' gelatinous coating may have protected the eggs. Several heavy-metal toxicity studies have suggested that the eggmass coating may have this protective role (Powlesland & George, 1986; Williams, *et al.*, 1987). The filtered reservoir water medium appears to have been able to buffer the pH depression to some extent and with time the pH increased towards pre-dosing levels. Where the pH had risen to more tolerable levels by the time the larvae hatched (day 3), then mortality was small. Where the pH was still low high mortality of larvae occurred whilst still in the eggmass (LD1) or soon afterwards (LD2). Perhaps the highest mortality in high target concentrations occurred after transfer of larvae to replacement tanks. In these treatments pH levels only had one day to recover before larvae were added. Bell (1970) found that pH 4.0 was fatal and pH 5.0 inhibited adult emergence of the midge *Tanytarsus dissimilis*. Temporary pH levels as low as pH 2.5 were recorded in this study. It is likely, therefore, that occurrences of high larval mortality were linked to depression of pH by iron III sulphate addition.

No measurement of pH was made in the shorter duration tests but it is certain that similar reductions of pH occurred after iron III addition. Experiments using deionised water as the test medium did not have the buffering capacity of the filtered reservoir water and thus low pH values continued for longer. Greater, more prolonged depression of pH in deionised water medium would appear to explain the increased toxicity of iron III sulphate addition in this medium.

It seems likely that the retardation in larval growth and development observed in both test durations is due fully or in part to pH depression. Some significant results were obtained in treatments where no substantial pH depression took place (see figures 4.8 and 4.9). It is possible, therefore, that a factor other than low pH may have had some effect.

The total iron content of chironomid larvae appears to increase with increasing target iron concentration, as seen in both experiment types. Smock (1983a) classified the

genus *Chironomus* as 'sediment dependent', defined as sediment dwelling, indiscriminately ingesting sediment with detritus. Microscopic observation found orange precipitates in the guts of larvae indicating that chironomids ingest iron precipitates. It is likely that the bulk of iron uptake by larvae is by ingestion. Larvae of the mayfly, *Leptophlebiamarginata*, became constipated during exposure to iron when their midguts became swollen and blocked (Gerhardt 1992). There is no evidence that this was the case with *C. riparius*, since large quantities of faecal pellets were observed in the sediments. Chapter five examines the separate effects of pH and iron precipitates in more detail.

Terminating the exposure at day 25 in the longer term tests did not allow any elucidation of the effects of iron III addition on adult emergence. If retardation of development had occurred this should be reflected by adult emergence. Exposure periods of up to 50 days would be needed to address this question, which is considered in chapter five.

SUMMARY

The impact of iron III sulphate addition on the midge *Chironomus riparius* was investigated in short (10 day) and longer (25 day) tests. Larval growth and development as well as mortality of eggs and larvae were examined.

After addition, precipitation of iron III from solution was both rapid and virtually complete. Temporary depression of pH accompanied iron III sulphate addition. The magnitude of this depression was dependent on the amount of iron III sulphate added. Separation of mortality into time periods has shown that high larval mortality corresponded to these periods of low pH. Eggs appeared to be largely tolerant to low pH (approximately pH 2.5 – 3.0). Significant trends of retarded larval growth and development with increasing target iron concentration were observed but could not easily be divorced from the depression of pH. Ingestion of iron III precipitates by chironomid

larvae could also have affected growth. Guts of larvae exposed to iron III addition were observed to contain orange substances likely to be iron III precipitates. A significant trend of increasing total iron content of larvae was detected with increasing target iron concentration. Larvae appeared to ingest iron III precipitates and it is likely that this is the major mode of uptake of iron.

Chapter Five

Effects of low pH and of iron III precipitates on *Chironomus riparius* (Meigen)

5.1 INTRODUCTION

Chapter four suggested the hypothesis that high larval mortality is due to depression of pH resulting from iron III sulphate addition but that ingestion of iron III precipitates may be a factor in the retardation of larval growth and development. Separate 25-day experiments exposing chironomids to (i) low pH conditions and (ii) to the presence of iron III precipitates in circumneutral conditions have been used to test this explanation. In addition, the 25-day experimental procedure has been modified to examine the impact of iron III precipitates on adult emergence. Is the retardation of larval development reflected in reduced or delayed emergence?

5.2 EXPERIMENTAL PROCEDURE

5.2.1 pH experiments

To allow direct comparison with previous experiments the same 25-day exposure technique was employed. Depression of pH by iron III sulphate addition was simulated by the addition of different amounts of 1% nitric acid to 2 litres of filtered reservoir water. Provision of eggmasses, their sampling and the sampling and analysis of chironomid larvae were undertaken in the manner described in section 3.10. Larval dry

weight was used instead of larval wet weight. Measurement of pH was carried out using a Jenway 3100 microprocessor pH meter with a Gerplas combination electrode.

The intention of these experiments was not to expose chironomids to a variety of constant pH levels but to mimic the pH depression and subsequently rising pH seen in previous tests. Direct comparison between these two sets of tests as to the timing of mortality and the extent of retardation of larval growth and development were then possible. Different amounts of acid were added to 2 litres of filtered reservoir water in each of eight tanks. These amounts were 47, 50, 55, 60, 70, 85, 100, 120 ml 1% nitric acid. A control tank to which no acid was added was also included. Only one set of pH experiments was undertaken.

5.2.2 25-day iron III precipitate experiments

The experiments were performed following the same 25-day procedure used in the previous iron experiments. Larval growth was inferred from larval dry weight. To avoid the problems of pH depression the tanks were allowed to stand, aerated constantly, for 14 days after iron III sulphate addition, prior to the addition of organisms. This permitted the pH in all tanks to rise naturally to circumneutral levels. This method was considered preferable to the addition of other substances e.g. calcium carbonate to adjust the pH artificially. Regular monitoring of pH was made throughout. Replacement tanks were also allowed 14 days for the pH to rise prior to their use.

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 iron III sulphate addition was made was included in each replicate experiment.

5.2.3 Adult emergence experiments

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 iron III 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 iron III 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 after addition of first instars. 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. The method used in this study is very similar to the *Chironomus* adult emergence test recommended by Nebeker *et al.* (1984). Their technique only differs in that larvae are exposed from second instar stage instead of first instars and for 25-day duration instead of 46 as in this study.

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.

5.3 RESULTS

5.3.1 pH experiments

The degree of pH depression and the speed of recovery of the pH towards neutral varied with the amount of nitric acid added (Figure 5.1). Control tanks remained close to pH 8 throughout the test period. The addition of 47 ml 1% nitric acid caused only slight pH depression and showed quick recovery to neutral levels. Fifty to seventy ml of acid gave initial pH levels between 2.09 and 2.78 but the pH recovered quickly reaching

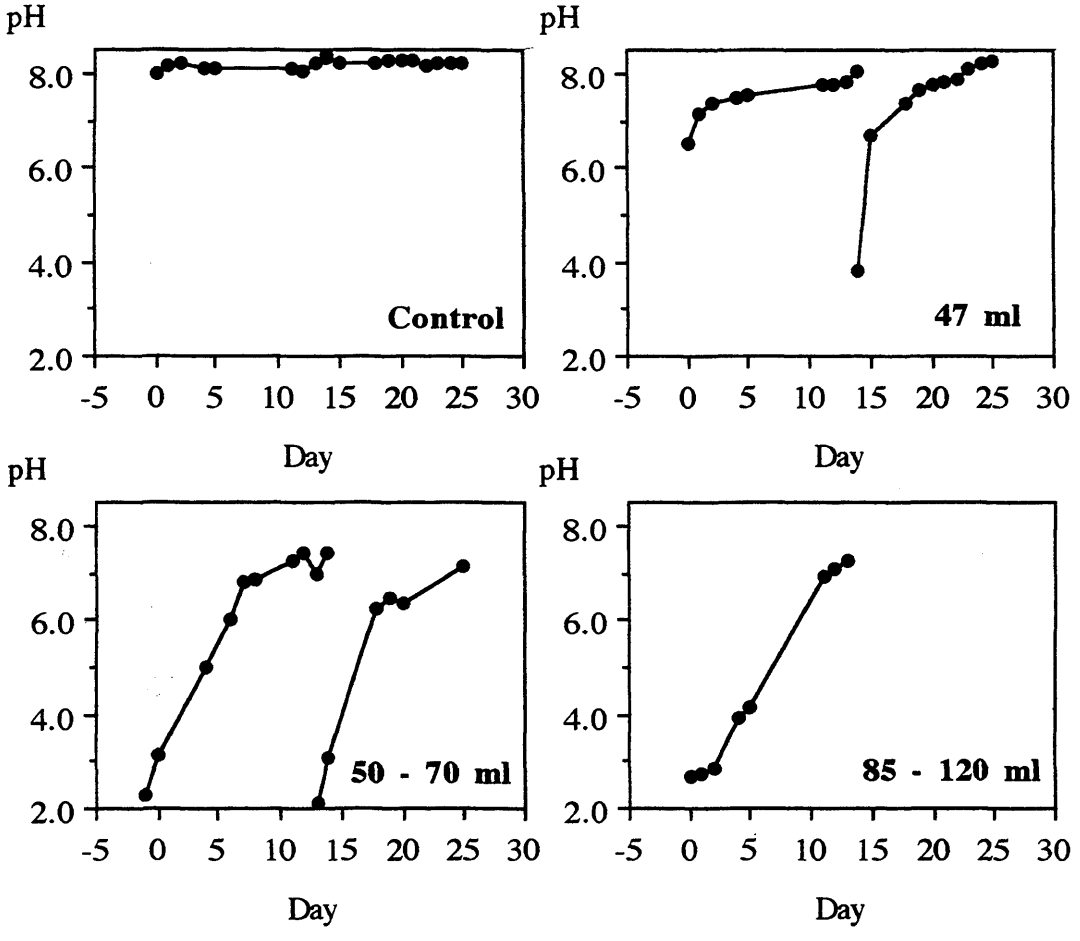

Figure 5.1 *Changes in pH during the experimental period (pH experiments). Graph labels refer to the amounts of 1% nitric added.*

neutrality within 5 to 14 days. Addition of 85 ml of acid or above gave similar initial pH levels (between 2.65 and 2.84) but a three-four day lag period occurred before recovery of pH began.

Separation of egg and larval mortality into four time periods was conducted in the same manner used in Chapter four, i.e:- (i) egg non-viability, (ii) mortality of larvae in eggmass (LD1), (iii) larval mortality after leaving eggmass but before census I (LD2) and (iv) mortality of larvae between census I and the end of the test (LD3). These mortality factors have been plotted against the pH conditions prevalent at the beginning of the mortality period. Egg non-viability is plotted against the initial pH levels i.e. at the start of the experiment; LD1 is plotted against day 4 pH since these conditions are those most likely to affect newly hatched larvae; LD2 is also plotted against day 4 pH since most larvae will leave the eggmass at this time and initial pHs after tank replacement (day 14) were used for LD3 (Figure 6.2). There is no apparent effect of pH on hatch, even below pH 3. By day 4 the pH in most experiments had risen above pH 4 and in these no mortality of larvae before leaving the eggmass (LD1) occurred. Where pH 4 was not reached 100 percent LD1 mortality resulted. LD2 mortality did not vary substantially with pH down to approximately 4.5. After replacement high LD3 mortality only resulted in experiments where the initial pH of replacement tanks was less than pH 3. Above this little mortality occurred.

Larval dry weights and instar stages were compared to the pH conditions prevalent at the beginning of the period of exposure i.e. for larvae sampled on days 6 and 14 the pH on day 4 was used and for larvae sampled on days 20 and 25 the initial pH of the replacement tanks was appropriate. One-way ANOVA found that larval dry weight varied significantly with pH on each of the sample days (Table 5.1) but no clear relationship was present (Figure 5.3). On day 14, for example, 95% confidence intervals show that larvae exposed to pHs 4.98 and 4.61 differed significantly from the control (pH 8.11) but larvae in pH 4.49 did not.

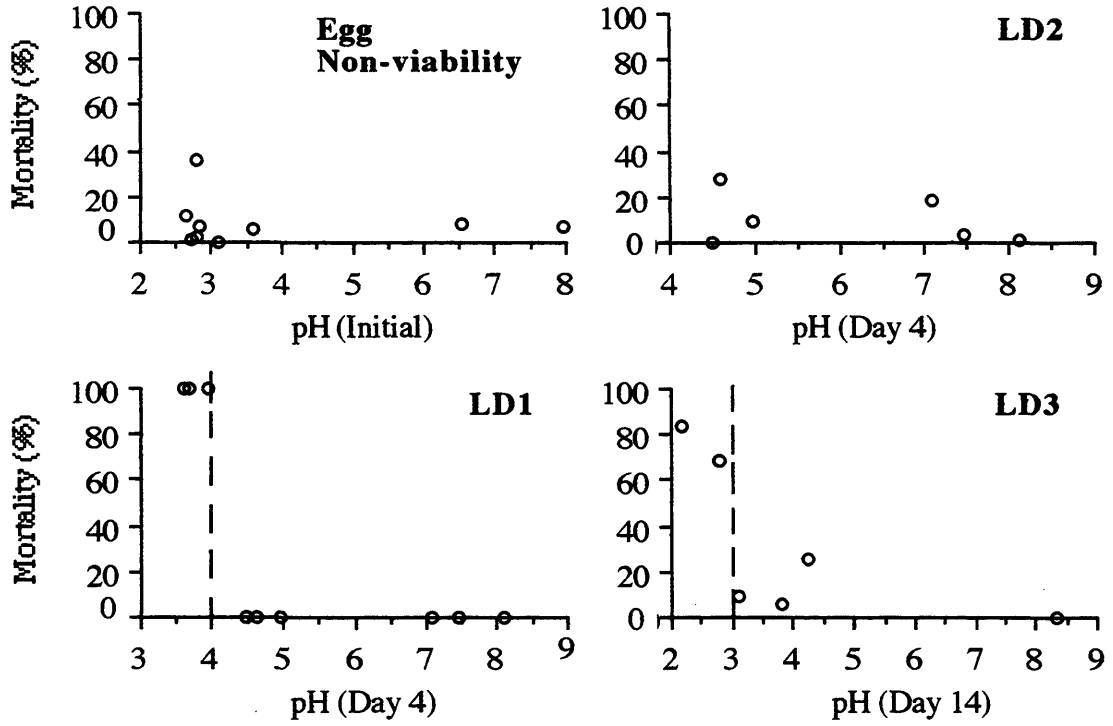

Figure 5.2 *Percentage mortality of chironomid eggs and larvae during exposure to different pH conditions.*

Table 5.1 *One-way ANOVA statistics for differences in larval dry weight between pH treatments.*

Sample		DF	Mean squares	F-statistic	Probabilty
Day 14	Treatment	5	0.148	33.81	< 0.001
	Error	71	0.004		
Day 20	Treatment	4	0.045	3.31	< 0.02
	Error	53	0.014		
Day 25	Treatment	4	0.0243	3.52	< 0.02
	Error	74	0.007		

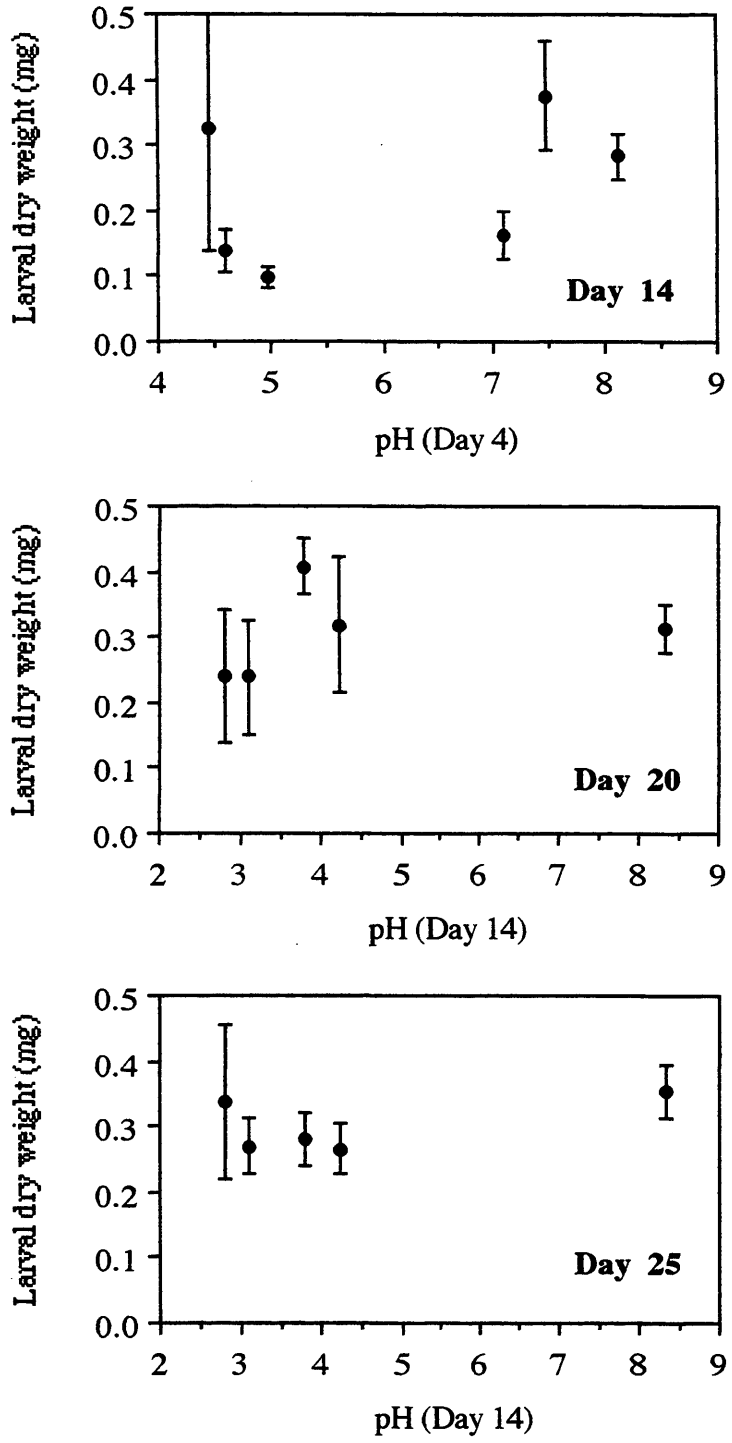

Figure 5.3 *Effect of pH on larval dry weight. Error bars represent 95% confidence intervals from the Student's t-distribution.*

Tests for the independence of developmental stage from pH were performed using the G-test. The effect of pH on larval development is shown in Figure 5.4.

Developmental stage is significantly linked to pH on days 6 and 14 but not on days 20 and 25 (Table 5.2). It appears that larvae which survived the initial low pH levels suffered little further restriction to their growth and development.

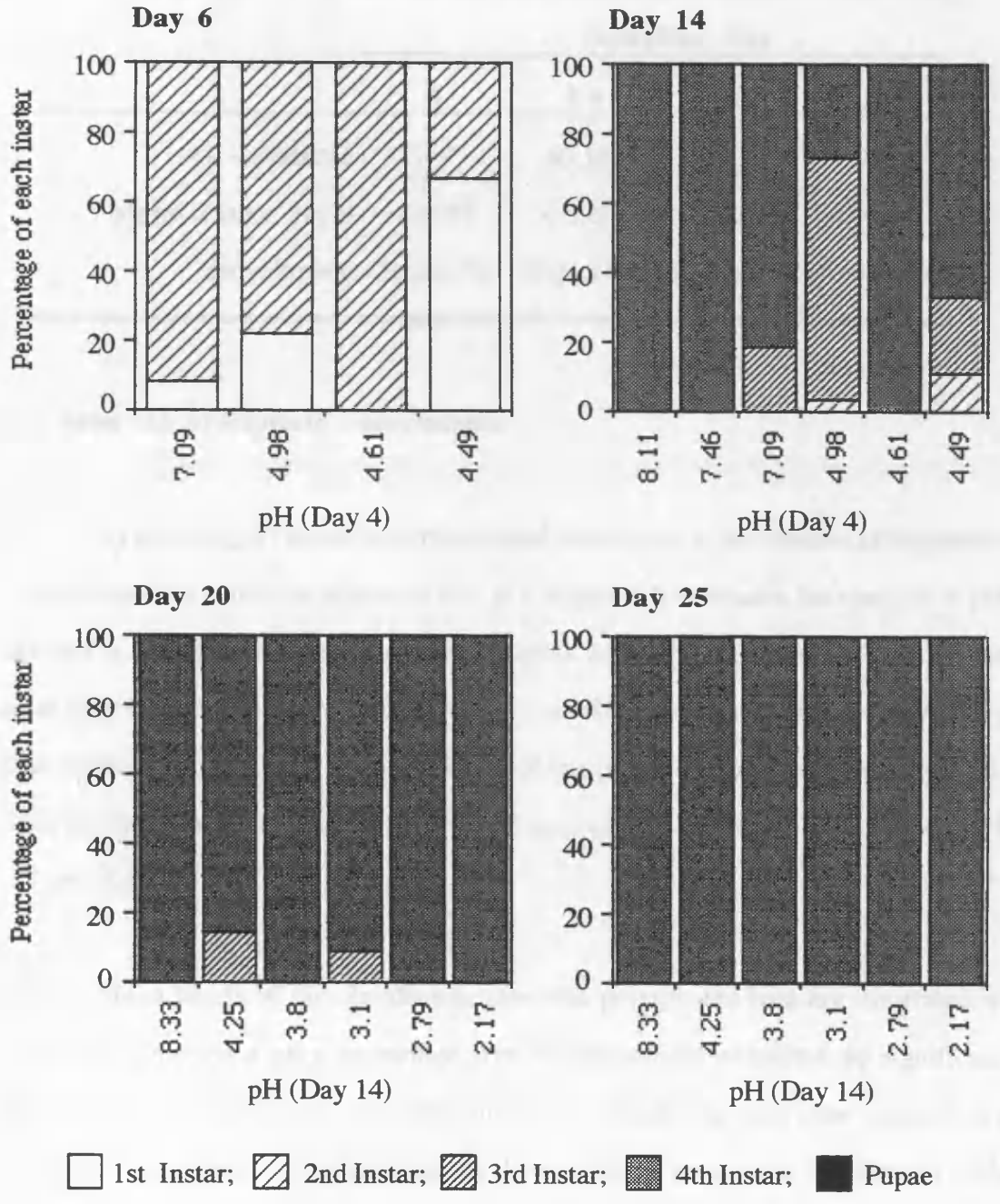

Figure 5.4 *Effect of pH on larval development.*

Table 5.2 G-test for independence results from pH experiments.

Hø : Developmental stage is independent of pH levels.

H1 : Developmental stage is not independent of pH levels

	Sampling Day			
	6	**14**	**20**	**25**
G - statistic	7.997	40.146	5.738	0
significance level	< 0.05	< 0.001	> 0.05	> 0.05
Conclusion	Reject Hø	Reject Hø	Accept Hø	Accept Hø

5.3.2 Iron III precipitate experiments

By allowing pH to rise to circumneutral levels prior to the addition of organisms it was to hoped to avoid the effects of low pH. Figure 5.5 illustrates the changes in pH observed in one of the three replicate experiments and Table 5.3 provides a list of the initial (day 0) and replacement (day 14) pHs for all experiments. Neutral or slightly alkaline conditions prevailed in the majority of treatments during the exposure period, however, initial and replacement pHs in the 75 mg Fe litre^{-1} treatments were between pH 3.67 and 5.67.

Mean levels of dissolved/suspended and precipitated iron are illustrated in Figure 5.6. Dissolved plus suspended iron measurements exhibited no significant difference between treatments, experimental runs, sample day or factor interactions (Table 5.4). In contrast, precipitate iron levels were found to increase significantly with target iron concentration with all treatments significantly different from the control. No significant differences due to experimental run or factor interactions were found but precipitate iron levels varied significantly with sample day. The distribution of precipitate across the surface of the sediment and the distribution of the sediment itself in the tank

Table 5.3 *Initial (day 0) and replacement (day 14) pH's for each replicate iron III 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

was never entirely even and so variation between samples may be expected. This probably accounts for the variation between sample days.

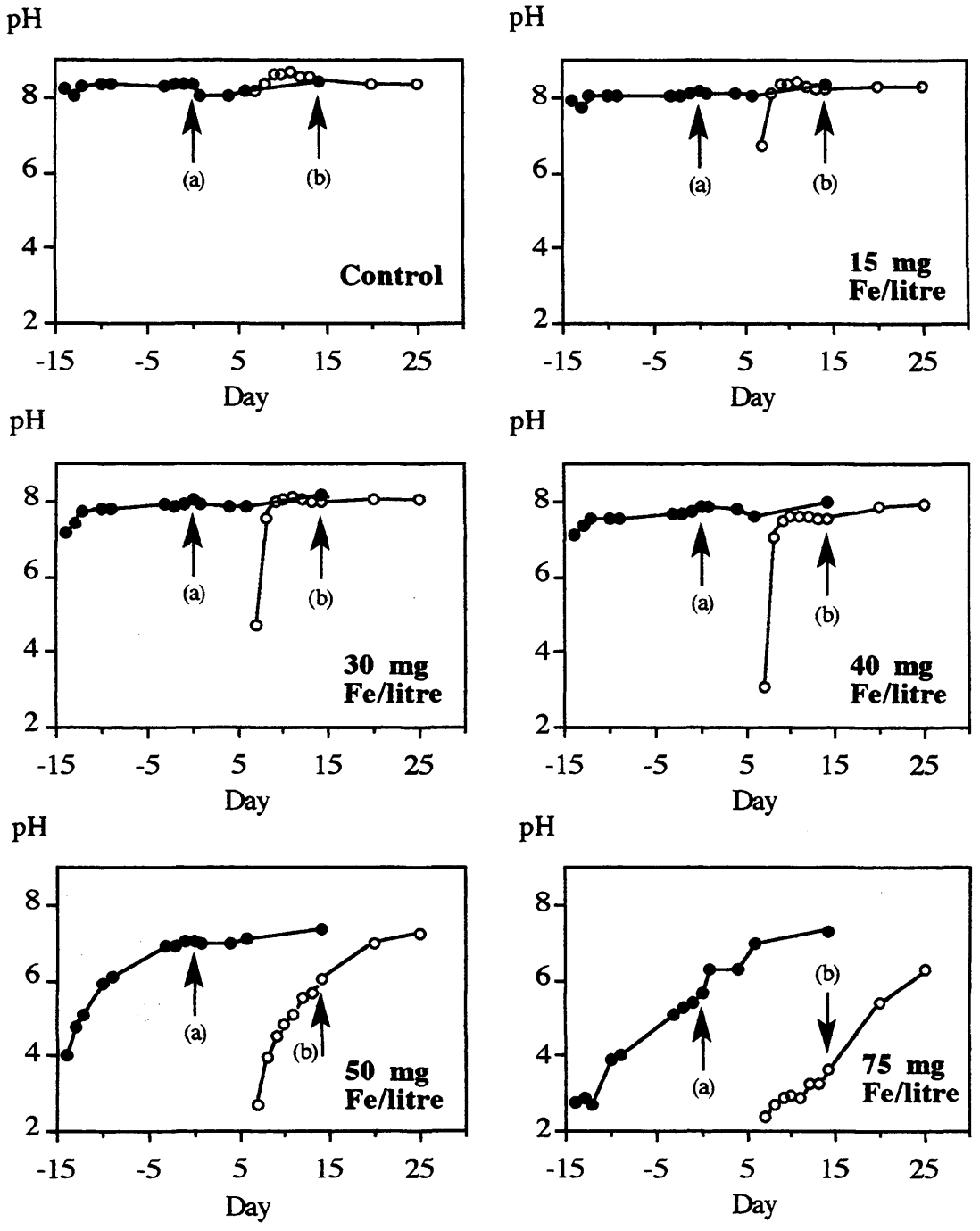

Figure 5.5 *Changes in pH during one replicate iron III precipitate experiment. Arrows indicate the day when animals were added (a) as eggs and (b) as larvae at tank replacement. Changes in pH for the other replicates show similar patterns and are recorded in Appendix D.7.*

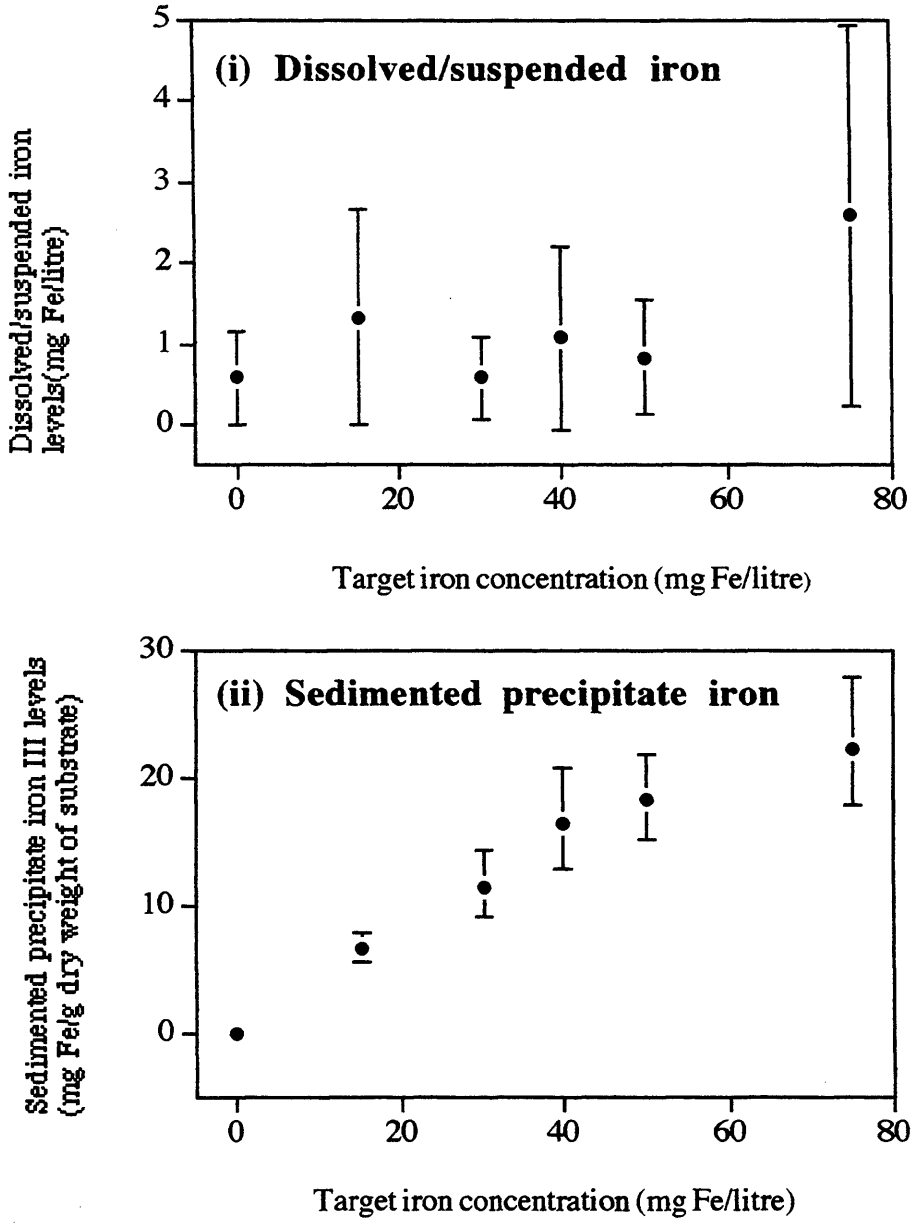

Figure 5.6 *(i) Dissolved/suspended and (ii) precipitated iron levels from target iron and control treatments. Error bars represent 95% confidence intervals from the Student's t-distribution.*

Table 5.4 *Two-way GLM results for dissolved/suspended and precipitate iron levels.*

	Dissolved/suspended iron			
	Degrees of freedom	Mean squares	F-statistic	Probability
Target iron concentration	5	6.800	1.57	> 0.05
Experimental Run	2	5.292	1.22	> 0.05
Treatment/run Interaction	10	1.892	0.44	> 0.05
Error	71	4.343		
Target iron concentration	5	6.800	1.49	> 0.05
Sample Day	3	5.000	1.10	> 0.05
Treatment/day Interaction	15	2.022	0.44	> 0.05
Error	48	4.556		
	Precipitated iron			
	Degrees of freedom	Mean squares	F-statistic	Probability
Target iron concentration	5	11.108	361.68	< 0.001
Experimental Run	2	0.085	2.77	> 0.05
Treatment/run Interaction	10	0.030	0.98	> 0.05
Error	68	0.031		
Target iron concentration	5	11.975	439.85	< 0.001
Sample Day	4	0.114	4.19	<0.01
Treatment/day Interaction	20	0.032	1.16	> 0.05
Error	56	0.027		

As with previous 25 day experiments mortality was split into four time periods (see section 4.3.2). Larval mortality before leaving the eggmass did not occur in any treatment in these experiments. No relationship between mortality and target iron concentration is apparent for the other three time periods (egg non-viability, LD2 and LD3) (Figure 5.7).

A significant trend of decreasing larval dry weight was recorded with increasing target iron concentration (Figure 5.8), similar to that seen in prevoius experiments. On each sample day larval dry weight also significantly varied between experimental runs and with the interaction of treatments and runs (Table 5.5). Using 95% confidence

intervals it is possible to infer that all iron treatments differ significantly from the control on day 14. The 30 and 75 mg Fe litre^{-1} treatments only were significant on day 20 but all treatments above 15 mg Fe litre^{-1} were significant from the control on day 25.

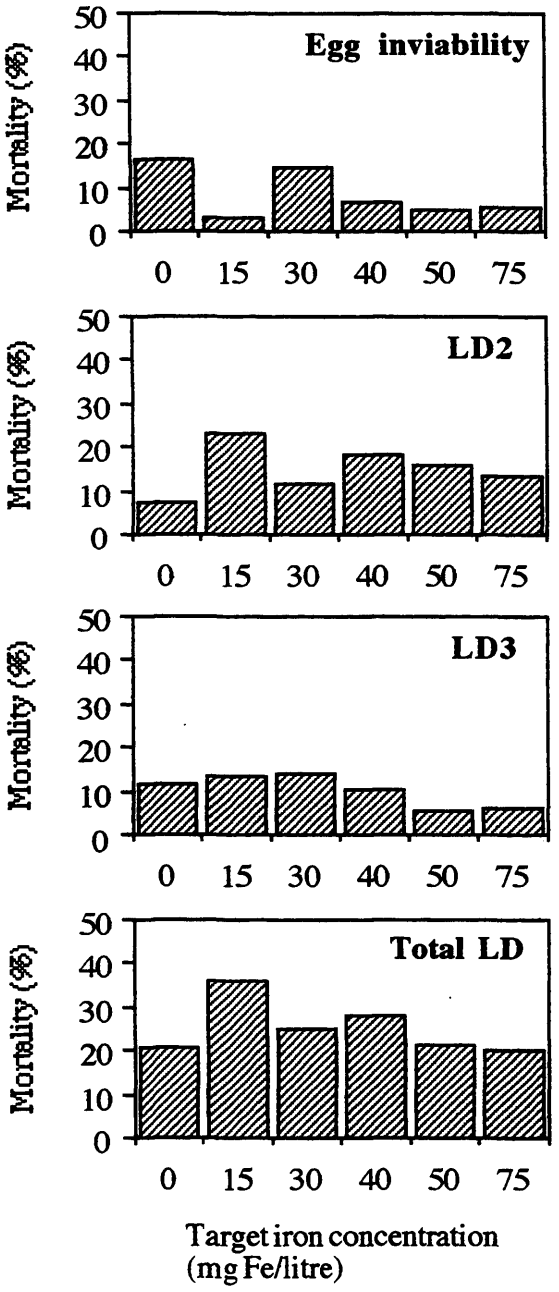

Figure 5.7 *Egg and larval mortality from iron III precipitate experiments. LD2 equals larval mortality after leaving the eggmass but before larval census I; LD3 equals the mortality of larvae between census I and census II and Total LD is the total larval mortality.*

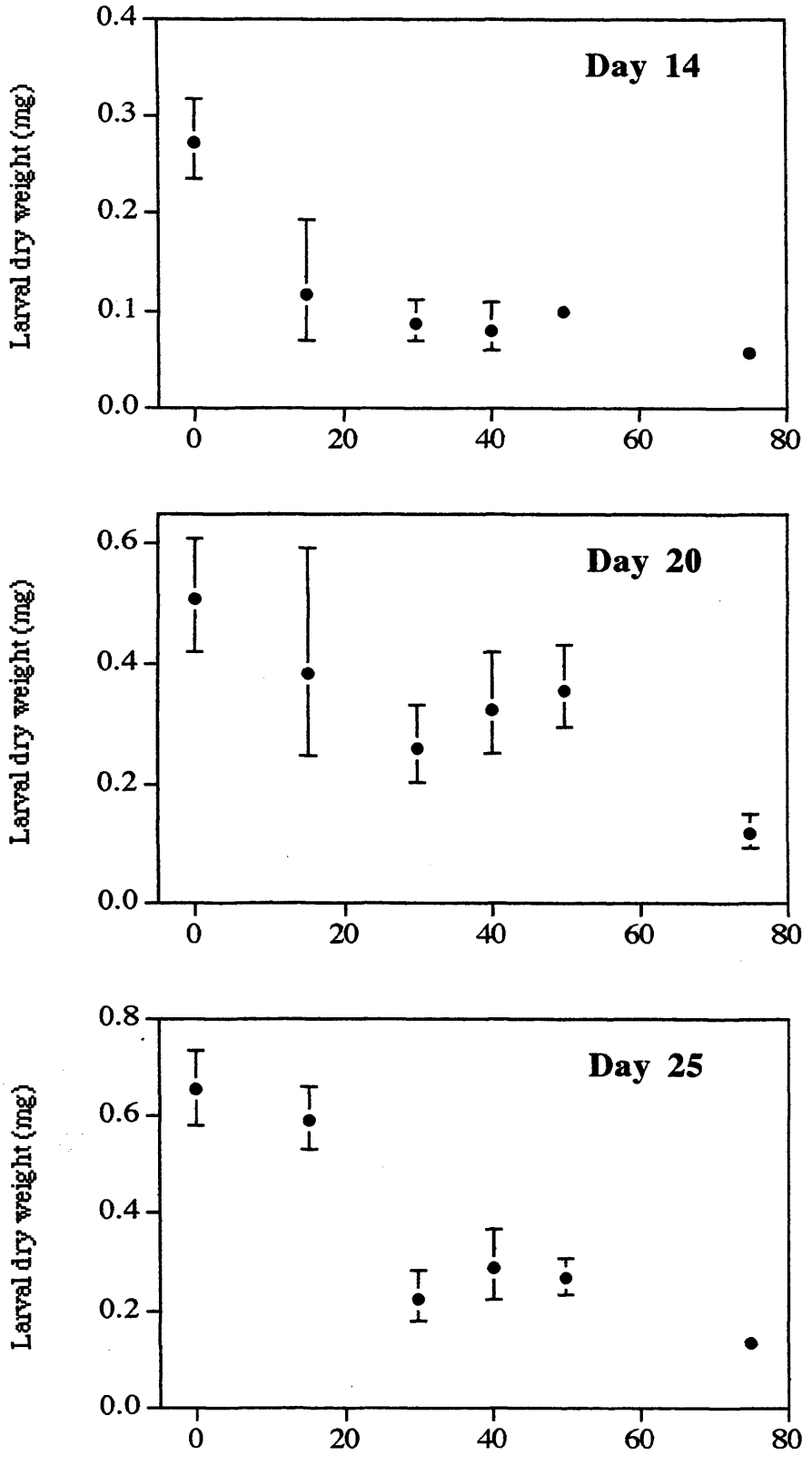

Figure 5.8 *Effect of target iron concentration on larval dry weight. Error bars represent 95% confidence intervals from the Student's t-distribution.*

Table 5.5 *Two-way GLM results for differences in larval dry weight between target iron treatments and experimental runs.*

	Day 14			
	DF	Mean squares	F-statistic	Probability
Target iron concentration	5	1.299	59.70	< 0.001
Experimental Run	2	1.641	75.40	< 0.001
Interaction	10	0.417	19.15	< 0.001
Error	127	0.022		
	Day 20			
	DF	Mean squares	F-statistic	Probability
Target iron concentration	5	1.555	36.59	< 0.001
Experimental Run	2	1.228	28.90	< 0.001
Interaction	10	0.390	9.18	< 0.001
Error	135	0.043		
	Day 25			
	DF	Mean squares	F-statistic	Probability
Target iron concentration	5	1.712	74.83	< 0.001
Experimental Run	2	0.351	15.33	< 0.001
Interaction	10	0.237	10.37	< 0.001
Error	168	0.023		

Retardation of larval development was most noticable in 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 concentration. This and the three other sampling days are illustrated in Figure 5.9. Significant dependence of developmental stage on target iron concentration occurred on days 14, 20 and 25. Dependence on experimental run was also apparent on days 14 and 20, but was independent on day 25. Developmental stage was independent of both target iron concentration and experimental run on day 6. G-test statistics for independence are given in Table 5.6.

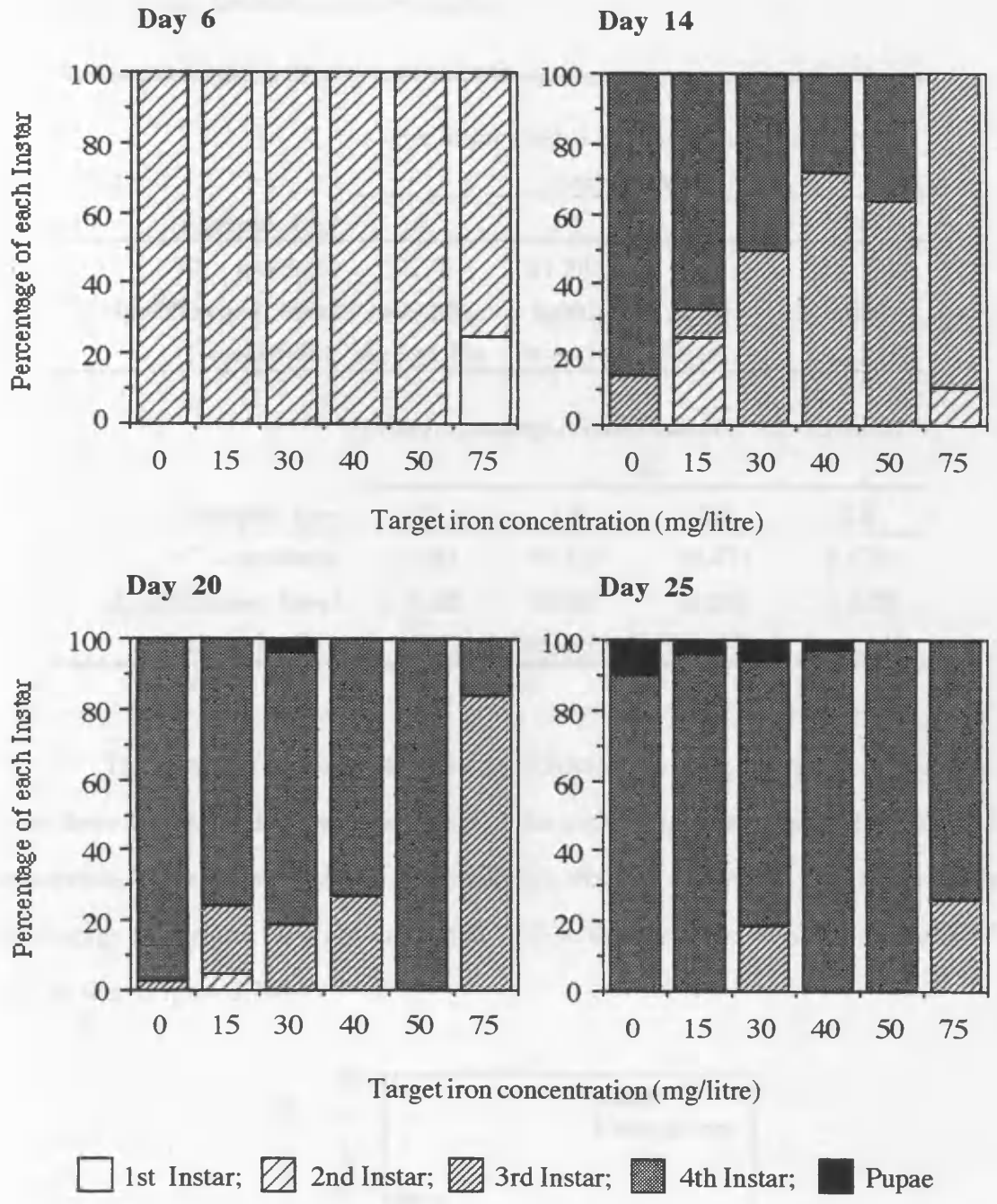

Figure 5.9 *Effect of target iron concentration on* C. riparius *larval development.*

Table 5.6 G-test for independence results.

	Hø: Instar stage is independent of target iron concentration			
Sample Day	**6**	**14**	**20**	**25**
G - statistic	8.738	31.285	72.239	35.638
significance level	> 0.05	0.001	0.001	0.001
Conclusion	Accept Hø	Reject Hø	Reject Hø	Reject Hø

	Hø: Instar stage is independent of experimental run			
Sample Day	**6**	**14**	**20**	**25**
G - statistic	3.180	64.559	25.471	3.678
significance level	> 0.05	0.001	0.001	> 0.05
Conclusion	Accept Hø	Reject Hø	Reject Hø	Accept Hø

The impact of the presence of iron precipitates on adult emergence was unclear from these experiments since termination of the experiment precedes the bulk of adult emergence. There is some indication of a possible effect on emergence since a decrease in percentage emergence from approximately 20% in the control to 0% at 75 mg Fe litre^{-1} can be seen (Figure 5.10).

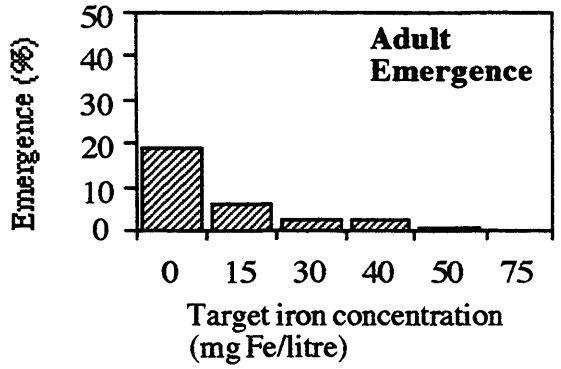

***Figure 5.10** Effect of target iron concentration on percentage adult emergence*

Digestion and atomic absorption analysis for total iron content of larvae has shown a significant trend of increasing total iron content with increasing target iron concentration (Figure 5.11). Larval iron levels also varied significantly with experimental run, but sample day and factor interactions were not significant variables (Table 5.7). Confidence intervals show that the total larval-iron content was significantly different from control larvae at 40 mg Fe litre^{-1} and greater.

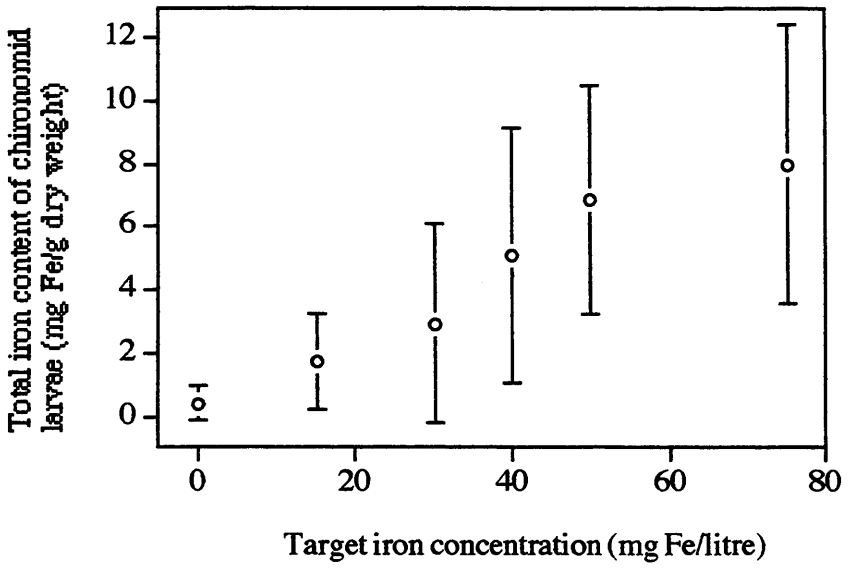

Figure 5.11 *Effect of target iron concentration on total iron content of chironomid larvae. Error bars represent 95% confidence intervals of the Student's t-distribution.*

Table 5.7 *Two-way GLM statistics for variation in total iron content of larvae due to target iron concentration, experimental run and sample day factors and their interactions.*

	DF	Mean squares	F-statistic	Probability
Target iron concentration	5	54.793	16.43	< 0.001
Experimental Run	2	68.481	20.54	< 0.001
Treatment/run Interaction	10	5.139	1.54	> 0.05
Error	17	3.334		
Target iron concentration	5	49.420	4.94	< 0.005
Sample Day	1	0.960	0.10	> 0.05
Treatment/day Interaction	5	2.11	0.21	> 0.05
Error	23	10.01		

5.3.3 Adult emergence experiments

Chemical parameters measured in each treatment are displayed in Table 5.8. The range of values for pH, dissolved/suspended iron and iron in particulate matter in the initial tank and the two replacement tanks for each treatment are given. All tanks in the control and the lower two iron treatments were at least neutral pH and were often slightly alkaline, pH 7.5 – 8.7. Initial and first replacement tanks of the 50 mg Fe litre^{-1} treatments were also approximately neutral pH but some of the second replacement tanks did not reach this level. None of these, however, were below the tolerance level of late instar larvae (pH 3).

Dissolved/suspended iron levels rarely exceeded the detection limit (< 0.056 mg Fe litre^{-1}) of the atomic absorption spectrometer. Variation was significant between target iron treatments, but not between sampling days or the interaction of the two factors (Table 5.9). Precipitated iron levels were below the detection limit in the control and increased significantly with increasing target iron concentration. Precipitated iron levels did not significantly vary with sample day or the interaction between treatment and sample day.

Mean larval mortality is presented in Figure 5.12. Confidence intervals were wide and have been omitted to maintain clarity. High mortality occurred in all treatments including the control, increasing with target iron concentration. These differences between treatments were, however, not significant (Kruskal-Wallis H = 5.821, $p > 0.05$). Most mortality occurred after the first replacement which coincides with the commencement of emergence.

Table 5.8 *pH, dissolved/suspended iron and precipitated iron levels from emergence experiments.*

Treatment		pH	Dissolved/suspended (mM)	Iron in particulate matter (mg Fe g^{-1} dry weight)
Control	Initial tank	8.64 - 8.73	0	< 0.056
	First Replacement	8.60 - 8.66	0	< 0.056
	Second Replacement	8.44 - 8.49	0	< 0.056
15 mg Fe $litre^{-1}$	Initial tank	8.34 - 8.46	0	3.98 - 5.59
	First Replacement	8.20 - 8.34	0	2.57 - 5.89
	Second Replacement	8.09 - 8.18	0	2.72 - 4.42
30 mg Fe $litre^{-1}$	Initial tank	8.08 - 8.19	0	3.08 - 8.79
	First Replacement	7.00 - 7.30	0	6.07 - 8.80
	Second Replacement	7.46 - 7.71	1	3.42 - 7.55
50 mg Fe $litre^{-1}$	Initial tank	7.05 - 7.64	0	10.38 - 12.46
	First Replacement	6.78 - 7.19	1 - 10	6.98 - 9.19
	Second Replacement	4.03 - 6.22	0 - 2	8.19 - 11.67

Table 5.9 *Two-way GLM results for the variation in dissolved/suspended and precipitated iron levels due to target iron concentration and sample day factors.*

	Dissolved/suspended iron			
	DF	Mean squares	F-statistic	Probability
Target iron concentration	3	9.704	5.37	< 0.01
Sample Day	2	5.778	3.20	> 0.05
Treatment/day Interaction	6	6.148	3.41	< 0.02
Error	24	1.806		
	Precipitated iron			
	DF	Mean squares	F-statistic	Probability
Target iron concentration	3	151.108	68.13	< 0.001
Sample Day	2	2.013	0.91	> 0.05
Treatment/day Interaction	6	4.310	1.94	> 0.05
Error	24	2.218		

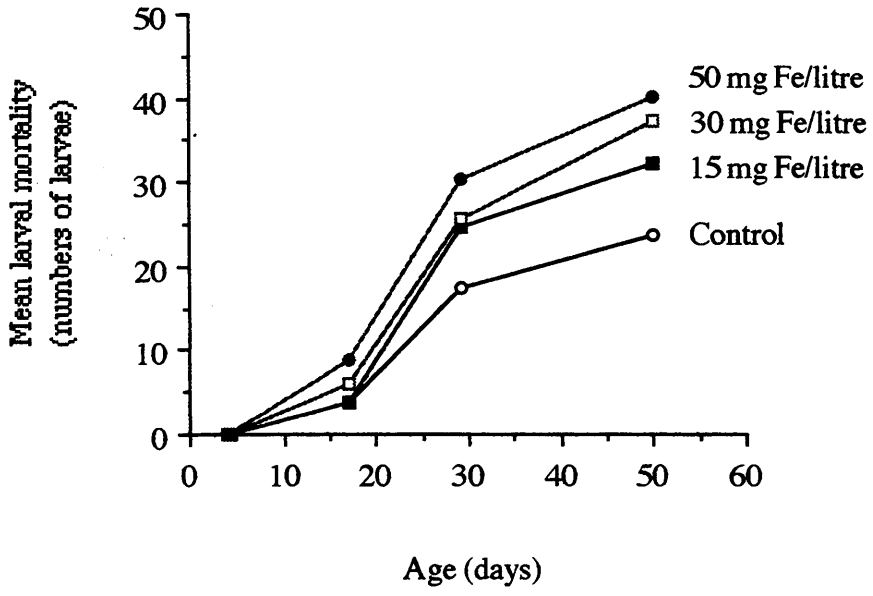

Figure 5.12 *Effect of target iron concentration on larval mortality.*

Cumulative emergence of males and females is illustrated in Figure 5.13, confidence intervals are not shown 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 that 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}. The ratio of males to females of emerged adults ranged from 1.6:1 in the control to 2:1 at 50 mg Fe litre^{-1}, but were independent of target iron concentration (G - statistic, 0.154, $p > 0.05$). The expected ratio of emerged *C. riparius* males to females is 1:1 (Pascoe, 1989). Only the control treatment differed significantly from this expected ratio (χ^2 statistic, 4.154, $p = 0.0415$).

5.4 Discussion

It is apparent from the experiments described in chapter four that the pH depression induced by iron III sulphate addition was responsible for much of the larval mortality detected. The role of pH in the observed effects on larval growth and development was less certain, but it was impossible to conclude that pH did not contribute to these responses. Whether the presence of iron had any bearing on either mortality or the sub-lethal effects was also equivocal. The aim then of the experiments described in this chapter was to clarify the role of pH and iron in the responses indicated.

Larvae were exposed to temporary low pHs followed by gradual pH increase. This simulated the pH response to the addition of iron III sulphate. Exposure of different life stages to a variety of constant pHs may have made analysis easier, but the method used could more effectively be compared to pH levels, mortality and sub-lethal effects observed in the previous iron experiments. This comparison is discussed in the following sections.

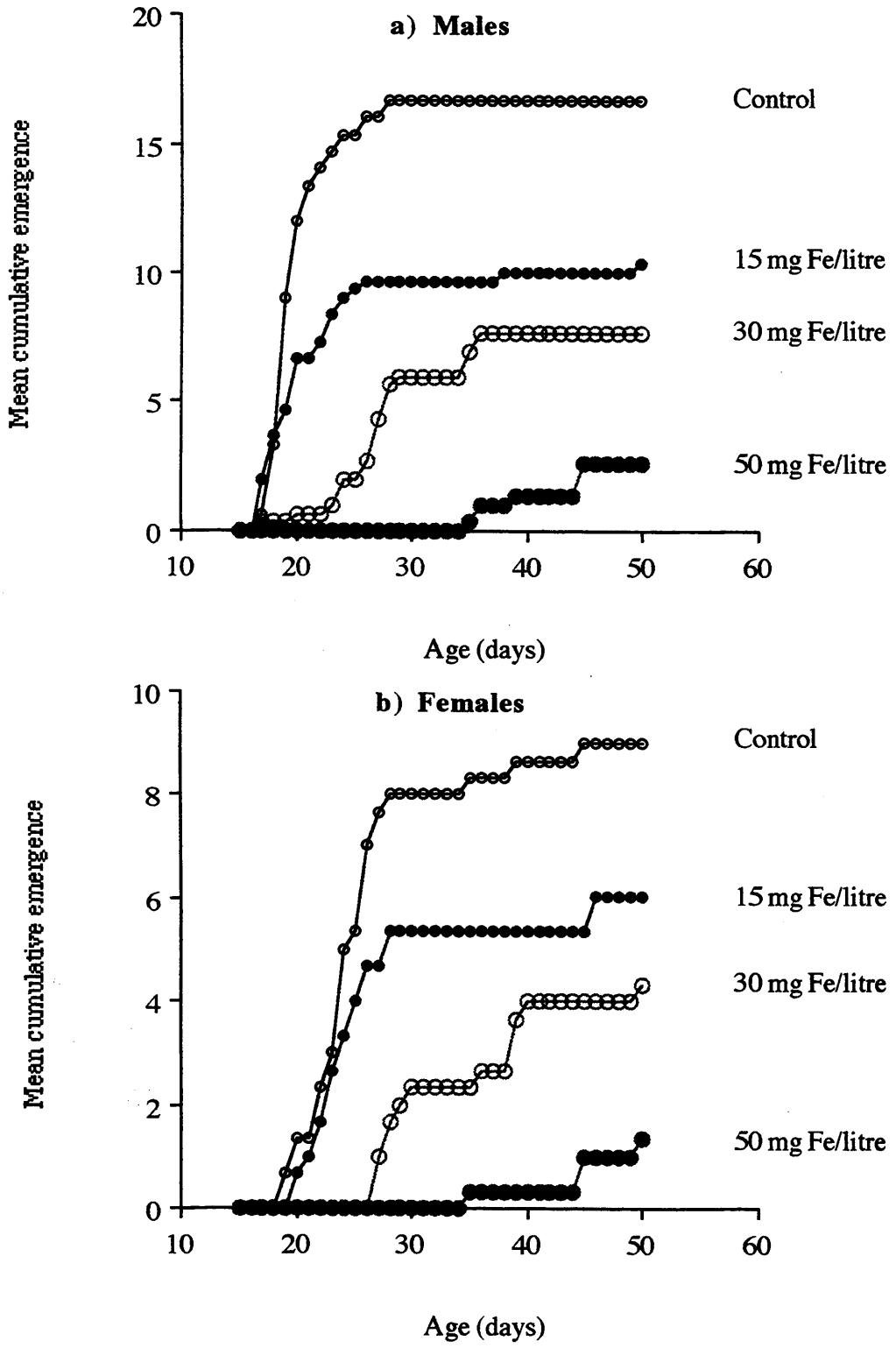

Figure 5.13 *Effect of target iron concentration on adult emergence of a) males and b) females.*

5.4.1 Mortality

The viability of *Chironomus riparius* eggs appears to be largely unaffected by either pH or iron (Figures 4.7, 5.2 and 5.7). Since iron is predominantly in the form of sedimented precipitate it is unsurprising that eggs covered by a gelatinous coating exposed only to the surrounding water (eggmasses were placed on a filter paper) show no detrimental response. Eggs were, however, exposed to extreme low pHs without any apparent increase in egg mortality. The tolerance of eggs to low pH may be attributable to protective properties of the eggmass coating (Gauss, *et al.*, 1985; Powlesland & George, 1986; Williams, *et al.*, 1987). Williams *et al.* (1987) showed that eggmasses oviposited directly into water contaminated with cadmium exhibited reduced hatch compared to eggmases laid first into clean culture and then placed in contaminated media. If in these experiments eggmasses had been oviposited directly into low pH water it is possible that increased egg mortality would have resulted.

After hatching, larvae first become exposed to the surrounding medium when they start to feed on the eggmass coating around them. This corresponds to days 3 and 4 in these tests. By this time the pH levels in most tanks had begun to rise from the initial pH (day 0). From the pH experiments it appears that if by day 4 the pH had increased beyond pH 4 then no LD1 mortality occurred, if it remained below pH 4 then 100 percent mortality of larvae within the eggmass resulted (Figure 5.2). Comparison with iron experiments (chapter four) agrees with this. High LD1 mortality was only recorded in the 50 mg Fe litre^{-1} treatment where the day 4 pH was 3.99.

Mortality of larvae after leaving the eggmass but before the first census (day 14) was not prominent in the pH experiments. During this period pH levels continued to increase and thus larvae that had survived LD1 mortality might be expected to continue to survive at least until tank replacement. High LD2 mortality occurred only in the 50 mg Fe litre^{-1} treatment of the iron experiments (Chapter Four). High iron levels may have

been a factor but it is more likely that early instars died very soon after leaving the eggmass whilst pH levels remained very low (day 4 pH = 3.99).

Replacement of tanks in both the first set of iron experiments (Chapter Four) and the pH experiment renewed the exposure of larvae to extreme low pH. Tank replacement was performed on day 14 of the test by which time most larvae had developed to at least third instar. These late instars of *C. riparius* appear to be highly tolerant of low pH. High LD3 mortality only occurred below pH 3 in the pH experiments. Similarly, in the first set of iron experiments only the 50 mg Fe litre^{-1} treatment had initial replacement tank pH less than pH 6 (pH 2.91). High LD3 only occurred in this iron treatment.

In the second set of iron III precipitate experiments (this chapter) pH was allowed two weeks after iron III sulphate addition to rise to more tolerable levels prior to the addition of organisms. For most treatments this worked well with the majority of initial pHs approximately neutral or slightly alkaline (Table 5.3). In high target iron concentrations pH 7 was not always achieved, however, all day 4 pHs were greater than pH 4 and all initial replacement tank pHs were greater than pH 3. No LD1 mortality and little LD2 or LD3 mortality was observed in these experiments.

From this evidence it seems that little if any of the larval mortality seen in the iron experiments described in chapter four can be attributed to the presence of iron precipitates. Depression of pH as a consequence of iron III sulphate addition is clearly the major cause. *C. riparius* appears to be extremely tolerant of low pH. Early instars are only sensitive below pH 4 and late instars can endure down to pH 3. Eggs appear to have still greater tolerance, down below pH 3. Jernelöv (1981) reported that *C. riparius* larvae from Smoking Hills, Canada survived (95%) 7-day exposure to pH 3.5, though it is not clear which instar(s) were used. In the same study *C. riparius* larvae from Uppsala in Sweden only showed 75% survival at pH 3.5. Jernelöv proposed that haemoglobin in the

haemolymph of these larvae provided buffering to allow tolerance of low pH and found that the Canadian specimens had twice as much haemoglobin as those from Sweden. In general, it is noted that aquatic insects are tolerant of low pH particularly over short periods. Certain caddis flies have been shown to tolerate pHs as low as 1.5 for 96 hours (Bell, 1970).

5.4.2 Larval growth

The iron experiments described in Chapter Four produced a significant trend of decreasing larval wet weight with increasing target iron concentration. The contribution of pH to this phenomenon could not be ascertained. Despite significant differences in larval dry weight between pH treatments (Table 5.1), differences were not always with the control treatment and no pattern was obvious (Figure 5.3). Why some treatments should have significantly lower larval weights than others is unclear. Similar relationships between target iron concentration and larval dry weight were apparent in both sets of iron experiments; if anything the relationship is more pronounced in the second set. It is possible that high mortality in the first set due to low pH selected fitter larvae and so larval growth restriction by iron was less pronounced. The mean dry weights of larvae recovered from circumneutral pH iron experiments on day 14 were significantly lower than the control even in the lowest target iron concentration (15 mg Fe $litre^{-1}$). Mean larval dry weight from 30 mg Fe $litre^{-1}$ was significantly different from the control throughout the 25-day period.

Mean larval dry weight also varied significantly with experimental run and the interaction of treatment and run. As discussed earlier in Chapter Four eggmasses used in the experiments did not contain exactly the same numbers of eggs, percentage hatch will not have been exactly the same for each eggmass and the numbers surviving each test will also have varied. Due to these factors larval density will have varied between like treatment tanks in different experimental runs and between different treatments in

different runs (interaction). Delayed larval development has been observed due to variation in chironomid larval densities (Bievier, 1971). In addition, larvae from different eggmasses and even larvae from the same eggmass won't necessarily grow and develop at the same rate even in favourable conditions (Pinder, 1986). These differences could conceivably be a factor in the variation described above.

5.4.3 Larval development

A clear relationship between increasing target iron concentration and greater retardation of larval development is apparent in the iron experiments presented in Chapter Four and here (Figures 4.9 and 5.9). A similar relationship was not apparent with low pH (Figure 5.4). The clear conclusion is that the retardation of larval growth and development shown in the initial iron experiments is almost entirely explained by increasing target iron concentration. This is obvious from the second set of iron experiments in which the influence of low pH was eliminated. That this retardation could be reflected in adult emergence was examined in separate experiments described in this chapter. 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 III precipitates is uncertain due to high mortality observed. The cause of this mortality 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 affected all treatments approximately equally since no significant difference in mortality between treatments was found. It is likely, however, that the delay in emergence is a reflection of the retardation of larval growth and development by iron observed in earlier results.

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) and 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. Pascoe, *et al.*, (1989) reported that equal numbers of both sexes emerged from all cadmium treatments. In general, ratios of emerged males to females in this study did not differ significantly from this expected ratio and variations between treatments were independent of target iron concentration. Overall iron III precipitates seem 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 iron III 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). 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 iron III 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.

Few previous studies have exposed test organisms to iron precipitates. Fifty percent mortality of coupled *Gammarus minus* adults exposed to suspended iron II 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 iron II sulphate solution. Iron II was the dominant dissolved iron form but some precipitation of iron, presumably iron III, was observed. Reduced growth of fish species e.g. brook trout (*Salvelinus fontinalis*) and coho salmon (*Oncorhynchus kisutch*) in the presence of suspended iron III precipitates was ascribed to impaired visibility effecting food consumption (Smith and Sykora, 1976), but once settled iron III 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 iron III iron precipitates.

It is clear from these experiments and those discussed in earlier chapters that contamination of artificial sediment with iron III precipitates has potentially serious effects on the growth and development of *Chironomus riparius*. 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 (Figures 4.10 and 5.11). The feeding habits of *C. riparius* (Smock, 1983) indicate that ingestion of these precipitates is likely to be the main method of uptake but the mode of action of iron cannot be commented on from these results. Further discussion of the mode of iron toxicity in these experiments will be made in Chapter Six.

SUMMARY

Iron III sulphate addition to aerated filtered reservoir water resulted in temporary pH depression as well as the production of iron III precipitates. Separate exposure of *Chironomus riparius* (Meigen) to (i) conditions of low pH and (ii) iron III precipitates in circumneutral conditions were used to clarify the influence of these two contaminants. In both cases modified versions of the 25-day test procedure described in earlier chapters were used. A dilute solution of nitric acid (1%) was used to simulate pH depression by iron III sulphate. After addition pH gradually increased. Iron treatments were allowed two weeks for pH to naturally increase to circumneutral before eggmasses were introduced to the containers. To assess the effect of iron precipitates on adult emergence first instar *Chironomus riparius* larvae were exposed to iron III precipitates at circumneutral pH using a static-with-replacement system and allowed development to adulthood. The numbers and sex of emerged adults were recorded daily.

Eggs of *C. riparius* were found to be tolerant of pH between 2.5 and 3. Newly hatched larvae died within the eggmass at pH < 4, none died at pH > 4. Larvae were again exposed to extreme lows of pH at tank replacement (day 14). Late instar larvae were tolerant of pH > 3 but high mortality occurred below this pH. These results imitated closely those of earlier 25-day iron experiments (Chapter Four) and it is clear that the larval mortality observed in those experiments was directly attributable to extreme low pH conditions. Where pH was allowed to increase before organisms were exposed little mortality occurred indicating that the target iron concentrations used caused little fatality.

Significant retardation of larval growth and development was observed with increasing target iron concentration. Mean larval dry weight was significantly lower than the control at 30 mg Fe litre^{-1} throughout the test. Development of larvae lagged behind the control in all iron treatments ($\leq$ 15 mg Fe litre^{-1}) to day 14 and continued to be

retarded in the highest concentration (75 mg Fe litre^{-1}) until the end of the test (day 25). These trends were not matched by exposure of *C. riparius* to different pH conditions.

Emergence was reduced in all iron treatments compared to the control. The larval mortality that this results from could not be traced, with any certainty, to iron III precipitates. Peak emergence in the control occurred at age 19 days (after oviposition). Emergence was delayed 6 days and 18 days at 30 and 50 mg Fe litre^{-1} respectively. It is likely that this is a reflection of the retardation of larval growth and development shown in earlier experiments. Males and females appeared to be affected equally by iron III contamination. The delayed emergence shown represents a severe deviation from normal life history patterns. Such deviation could result in reduced winter survivorship and reduced success of swarming and mating.

Increasing total iron content of larvae with increasing target iron concentration and the sub-lethal effects described above are likely to be linked to the ingestion of iron III precipitates by larvae. The precise mode of action of ingested iron was not clear from these experiments.

Chapter Six

Intake of iron by *Chironomus riparius* (Meigen) from iron III precipitates and reservoir sediment sources.

6.1 INTRODUCTION

The methods and extent of contaminant intake by organisms and its resultant effects will be influenced both by the nature of the contaminant and by the physical attributes and habits of the exposed organisms. Dissolved contaminants are generally considered to be more toxic than particulate forms but the relatively high contaminant concentrations associated with particulate matter can mean that organisms take in more contaminant from this source (Reynoldson, 1987). Smock (1983a) observed that the feeding habits of organisms and, in particular, the degree of discrimination against ingesting inorganic particles affected intake of metals. Sediment-dwelling invertebrates which indiscriminately ingested sediment along with detritus (sediment-dependent) had the highest metal body burdens, followed in order by filter-feeders, invertebrates with some ability to select against sediment (sediment-associated e.g. shredder species) and carnivores. Organism size can also be of importance. Relatively large organisms may take in less contaminant per unit size than relatively smaller organisms if surface adsorption is the major form of loading (Krantzberg, 1989; Smock, 1980; Smock, 1983b).

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, *et al.*, 1989). Further partitioning of contaminant load between different tissues can be examined for some organisms. Mason & Simkiss (1983) found that copper and magnesium were associated with specific connective tissue cells, group A metals were accumulated by the digestive gland and group B metals were concentrated in the kidney of the gastropod *Littorina littorea*. 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.

In earlier chapters of, this study, larvae of *Chironomus riparius* were exposed to sedimented precipitate iron. Larvae reared in iron-dosed treatments exhibited higher total iron content than control larvae. Levels increased as target iron concentration rose. 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 intake. Neither the extent of internal absorption of ingested iron nor the contribution of surface-adsorbed iron could be 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.

6.2 EXPERIMENTAL PROCEDURE

Eggmasses collected within eighteen hours of oviposition were reared in a separate culture for ten days. Fifty larvae were then randomly allocated to each of the 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. Inclusion of this treatment enabled comparison of iron intake and effects from natural sediment sources with the intake and effects of precipitated iron. 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 then 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 6.1. 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 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 of 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.

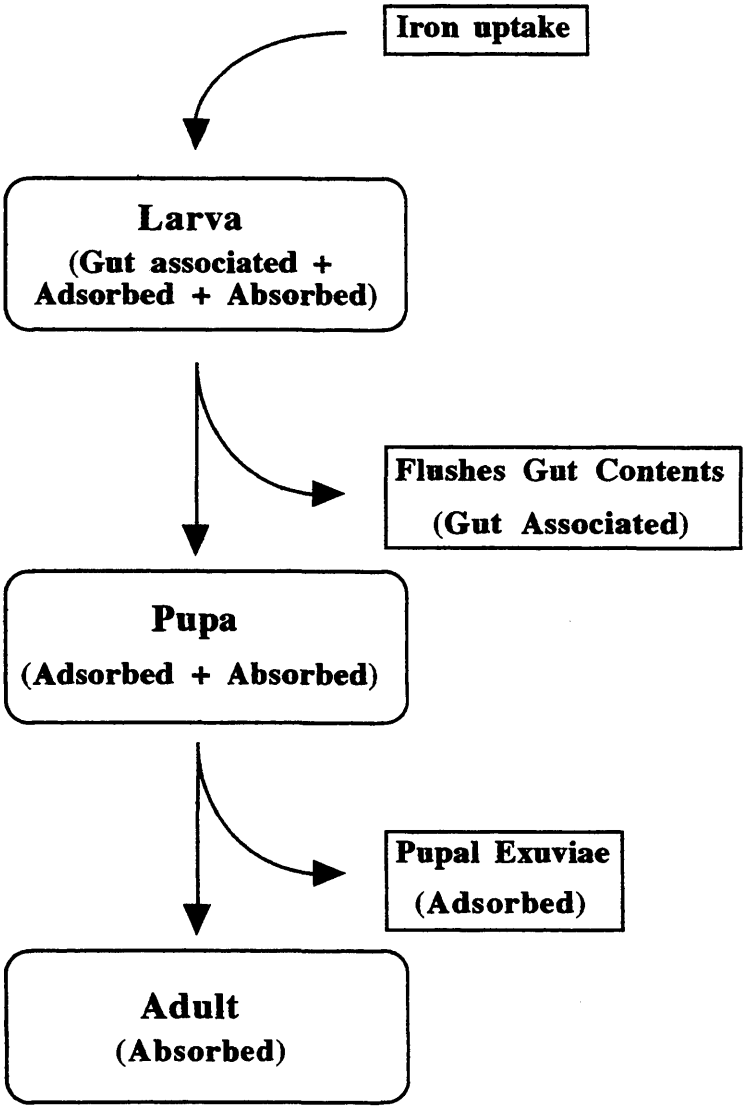

Figure 6.1 *Partitioning of total iron content in the developmental stages of* Chironomus riparius.

Working with a species of *Tipula*, Elwood *et al.* (1976) removed gut contents by dissection in order to evaluate the contribution they made to the total metal body burden. A number of chironomid larvae from each treatment in this study were similarly dissected in order to provide an alternative measure of gut-associated iron. It was found, however, that the relatively small size of *C. riparius* larvae made this task difficult and inaccurate so this method was abandoned.

Measurements of iron in particulate matter, dissolved/suspended iron and pH were made at the beginning of the experiment (just prior to larval addition) using the methods described in earlier chapters.

6.3 RESULTS

Measurements of iron in dissolved/suspended and particulate matter forms and of pH are given in Table 6.1. Dissolved/suspended iron was below detection limits (1mM Fe) 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* (see chapter five) in all treatments.

Table 6.1 *Chemical parameters in control, iron-dosed and natural sediment treatments.*

Treatment	Iron in particulate matter (mg Fe g^{-1} dry wt.)	Dissolved/suspended Iron (mM)	pH
Control	.055	0	8.61
15 mg Fe $litre^{-1}$	2.192	0	8.31
30 mg Fe $litre^{-1}$	7.236	0	8.11
50 mg Fe $litre^{-1}$	8.069	0	7.83
75 mg Fe $litre^{-1}$	14.226	0	5.14
Natural Sediment	27.124	0	8.57

Survivorship was 94% or greater in all treatments. Numbers of larvae, pupae and adults recovered by the end of the test were counted (see Appendix E.1) and a G-test for independence performed. Life stage was dependent on treatment (G-statistic, 32.61,

$p < 0.001$). Larval dry weight significantly decreased (Kruskal-Wallis H statistic, 26.37, $p < 0.0005$) as target iron concentration increased as in previous experiments (Figure 6.2). Parametric analysis was not used due to heterogeneity of block variances in the data.

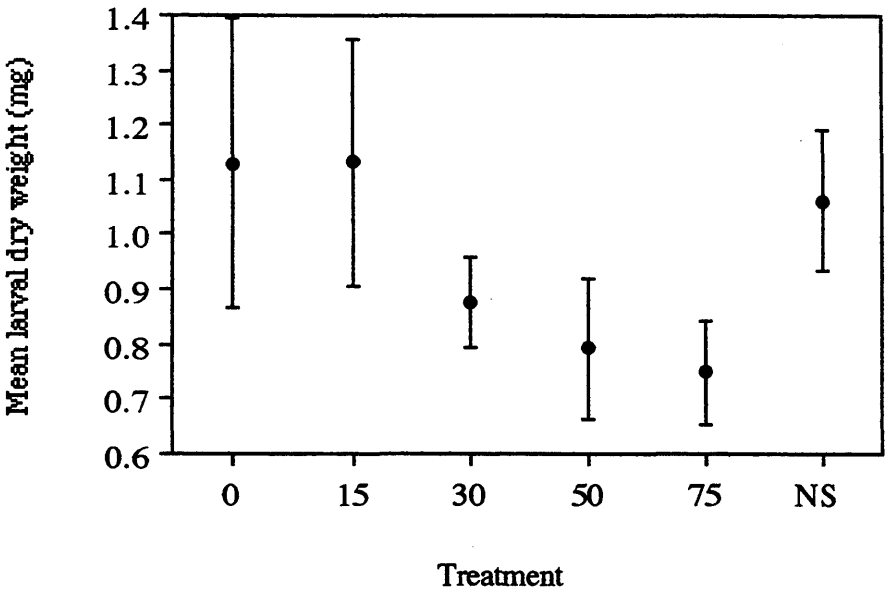

Figure 6.2 *Effect of target iron concentration and natural sediment (NS) on larval dry weight.*

Figure 6.3 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, whilst pupal and adult iron contents did not significantly vary with target iron (Table 6.2). This indicates that only gut-associated iron varies with target iron concentration since larvae are the only stage to include this fraction. If iron is accumulated in the tissues of the larvae then significant variation in iron levels should be observed in the pupal and adult digests (unless, in the latter, iron is moved to the pupal case at metamorphosis). This suggests that iron is regulated by the larvae before pupation.

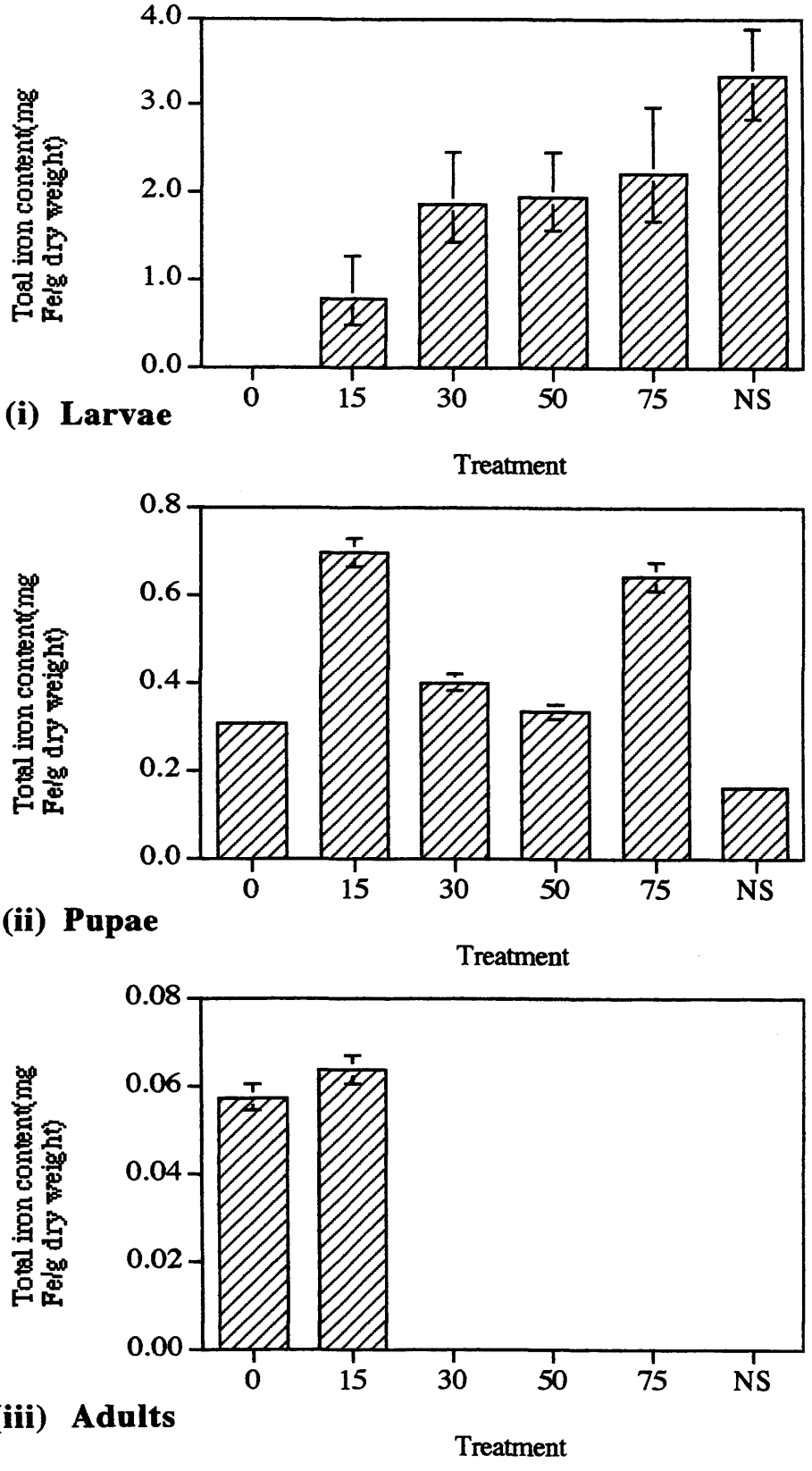

Figure 6.3 *Total iron content from digests of (i) larvae, (ii) pupae and (iii) adult* Chironomus riparius *reared in various target iron (0 – 75 mg Fe litre^{-1}) and natural sediment (NS) treatments.*

Table 6.2 *One-way ANOVA statistics for variation in total iron content of larvae, pupae and adult* Chironomus riparius.

Life stage		DF	Mean squares	F-statistic	Probability
Larvae	Treatment	5	3.424	38.40	< 0.001
	Error	139	0.089		
Pupae	Treatment	5	0.328	1.28	> 0.05
	Error	47	0.257		
Adults	Treatment	3	0.004	0.68	> 0.05
	Error	13	0.006		

Estimates of the partitioning of iron between gut-associated, surface-adsorbed and internally-absorbed fractions using the scheme represented in Figure 6.1 are given in Table 6.3. 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 6.3 *Estimates of the partitioning of the total iron body burden of* Chironomus riparius *reared in target iron and natural sediment treatments. Values given in mg Fe g^{-1} dry weight.*

Treatment	Gut associated	Surface Adsorbed	Internally Absorbed
Control	< 0.056	0.249	0.058
15 mg Fe litre^{-1}	0.344	0.634	0.064
30 mg Fe litre^{-1}	1.869	0.403	< 0.056
50 mg Fe litre^{-1}	1.966	0.334	< 0.056
75 mg Fe litre^{-1}	2.375	0.642	–
Natural Sediment	3.489	0.161	< 0.056

Figure 6.4 represents the percentage of the total iron concentration of *C. riparius* 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.

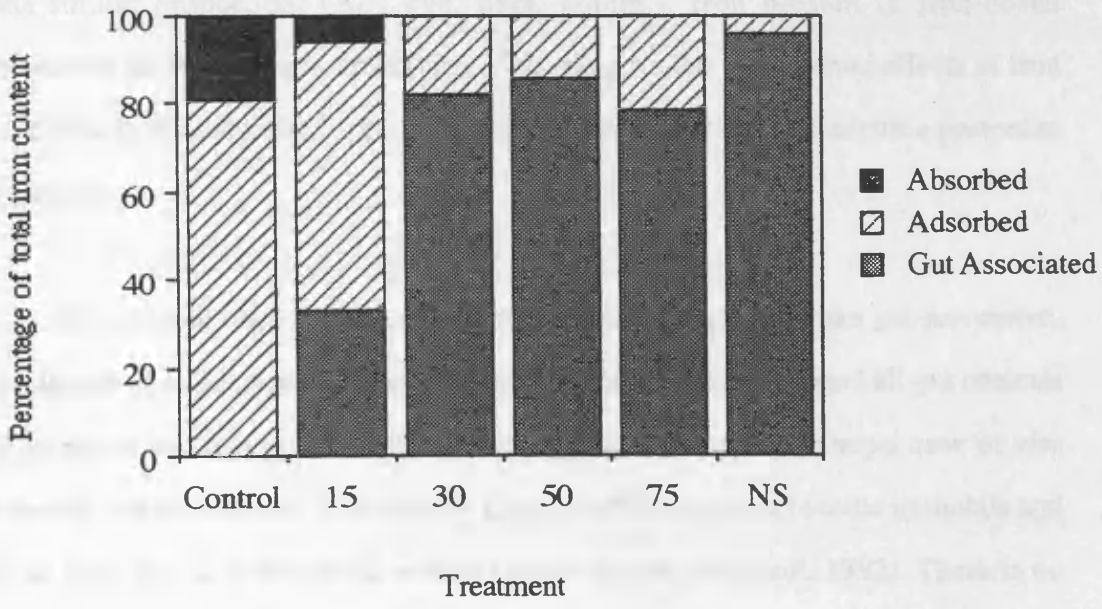

Figure 6.4 *Percentage of total iron concentration that is gut-associated, surface-adsorbed or internally-absorbed in* Chironomus riparius *reared in various target iron (0 - 75 mg Fe litre^{-1}) and natural sediment (NS) treatments.*

6.4 DISCUSSION

Larvae of *Chironomus riparius* reared in natural sediments were exposed to higher levels of iron per unit weight than larvae reared in iron-dosed treatments. No apparent retardation of larval growth was observed from the natural sediment treatment despite these high background levels of iron. Larvae reared in iron-dosed treatments exhibited significantly retarded larval growth with increasing target iron concentration as in previous experiments (chapters four and five). A number of authors have indicated that between 80 – 85% of the total iron concentration of uncontaminated lake sediments is strongly bound in crystalline lattices (Tipping, *et al.*, 1982; Davison & 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 (A. Love, pers. comm.). Iron present in iron-dosed treatments was all in the amorphous form. 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.

The scheme used to estimate the partitioning of iron between gut-associated, surface-adsorbed and internally-absorbed iron assumes that larvae expel all gut contents before pupation and that no internally-absorbed iron is moved to the pupal case or *vice versa* during metamorphosis. The mayfly *Leptophlebia marginata* became immobile and ceased to feed due to constipation when exposed to iron (Gerhardt, 1992). There is no evidence that the same is true of *Chironomus riparius* as larvae unable to expel gut contents would be unlikely to pupate. Pupae were recovered from all treatments. Exclusion of trace metals from new tissues and their loss to the pupal case during metamorphosis has been observed by a number of authors. Timmermans and Walker (1989) noted that *ca.* 90% of cadmium and copper was eliminated by *Chironomus riparius* and *Stictochronomus histrio* (cadmium only) between larval and adult stages. Using radionuclides Harvey (1971) found that 98% of ^{54}Mn and 88% of ^{59}Fe and ^{57}Co

was lost on emergence by *Chironomus plumosus*. Roughly 80% of cadmium was left behind in the pupal case of the blowfly *Luciliasericata* on adult emergence (Simkiss *et al.*, 1993). Loss of iron to the pupal case during metamorphosis would mean that internally-absorbed iron was underestimated and surface-adsorbed iron overestimated in the scheme used in this study. This may be indicated by the dominance of surface-adsorbed over internally-absorbed iron in control organisms. Control organisms were presented with negligible sources of iron thus little could be expected to be surface-adsorbed. Internally absorbed iron would have been present within haemoglobin contained in the larva's haemolymph.

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 mechanism by which iron was taken up both from dosed artificial sediment and from natural sediment. Surface adsorption of iron appeared, however, to be of greater importance in iron III dosed treatments than in natural sediment.

Ingestion of precipitate iron has led to retardation of growth and development, ingestion of iron bound in crystalline lattices from natural sediment has not. 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. Gerhardt (1994) exposed the mayfly *Leptophlebia marginata* to iron II at two different pHs. Survival of the mayfly and its ability to perform escape behaviour were impaired at ≥ 50 mg Fe litre^{-1}. Gerhardt suggested that these effects were caused by crusts of iron forming on the body surface of the mayfly, preventing free movement and possibly oxygen/ion uptake. It was suggested that cytotoxic effects of iron II could not be responsible for the effects observed and that the mayfly probably regulated any iron absorbed through the gut. 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.

SUMMARY

Larvae were reared in a number of target iron treatments (artificial sediment) and a natural sediment treatment to which no iron addition was made. Increasing target iron concentration led to an increase in total iron concentration in *Chironomus riparius* larvae. This iron load was partitioned between that which was associated with gut contents, that which was surface-adsorbed and that which was internally-absorbed. Estimates of these parameters have been inferred from measurements of larval, pupal and adult iron concentrations.

Larval growth was retarded in iron treatments above 30 mg Fe litre^{-1}. No retardation was seen in the natural sediment treatment despite total particulate iron concentrations greater than in the highest target iron treatment. The form of iron to which larvae are exposed is therefore of importance, since the majority of iron in natural sediment is likely to be present in crystalline lattices rather than as precipitates. Iron uptake was predominately by ingestion in both types of sediment. Gut associated iron contributed *ca.* 82% of the total in iron-dosed treatments and *ca.* 95% from the natural sediment. Gut associated iron was the only fraction which increased with increasing iron concentration, although surface adsorption was of greater importance in iron-dosed treatments than in natural sediment. That little iron was internally-absorbed and that no variance of this fraction with external iron concentration was seen suggests that the mode of action is physical rather than directly toxic. Ingestion of iron precipitates reduced the organic food intake of the larvae requiring them to feed longer to gain enough energy to

develop. These effects were made more severe by increasing iron-precipitate presence. Natural sediments contain lower amounts of precipitated iron and higher organic content and so no growth retardation was seen.

Chapter Seven

General Discussion

7.1 INTRODUCTION

Little information concerning the impact of deposited iron precipitates on aquatic ecosystems was available prior to this study. Iron III dosing of water-supply reservoirs has been carried out by Anglian Water since 1989, and concern about the impact of this activity had prompted the present study. Growth and development of *Chironomus riparius* (Meigen) in laboratory tests was inhibited by deposited iron. Retarded development resulted in delayed adult emergence, representing a departure from normal life-history patterns. The effects of deposited iron on *C. riparius* are therefore clearly deleterious. The purpose of this final chapter is to discuss the extent to which the laboratory study relates to the effect of iron III dosing in the field.

7.2 RELATION OF THE LABORATORY STUDY TO THE EFFECT OF IRON III IN THE FIELD.

One of the main advantages of laboratory-based ecotoxicological studies is their reproducibility. Organisms are tested in near-constant environments controlling as many biotic and abiotic factors as possible. As in this study, laboratory populations are often used. These have been reared for many generations resulting in a greater degree of genetic uniformity. This coupled with the shorter time-span of laboratory testing compared to field-based studies has encouraged their widespread use (Macriorowski & Clarke, 1980). It is necessary, however, to relate laboratory studies to the field situation.

The same factors that increase reproducibility decrease the reliability of extrapolation to the field. Seitz and Ratte (1991) discussed these problems in relation to aquatic ecosystems. Field populations require a high level of genetic variability to be able to adapt successfully to environmental fluctuations on a seasonal and for some species daily basis. Further problems occur if the extrapolation is to be taken to the community level. Laboratory studies on a single species provide little or no information of the effect of a toxicant on interactions within the community. Modification of competition and predator-prey interactions can be caused by a toxicant in many ways. Any impact on competitive strength, predator effectiveness or the vulnerability of prey species is likely to affect the fine balance between populations and therefore affects community structure.

Extrapolation of this study to population and community level effects at Rutland Water and other dosed reservoirs suffers from the drawbacks discussed above and a number of additional problems. The majority of this study involved exposure of a laboratory-cultured species to iron III precipitates in standardised conditions. A constant temperature of 20 °C was used to decrease generation time of the species to allow whole life cycle exposure in just 25 days. Field populations of this species would experience generation times of a year. Temperatures would remain much lower than 20 °C for much, if not all of the year and fluctuations in temperature would be much greater than in the laboratory. The midge species used in the culture, *Chironomus riparius*, has been identified in samples from Rutland Water (Brown, 1981) but it is a littoral species and so is probably not directly affected by the iron-dosing scheme. Related species e.g. *Chironomus plumosus*, *Tanytarsus* spp. and *Polypedilum* occur in the profundal zone of the reservoir and are more likely to encounter the presence of iron III precipitates. A single discrete iron dose was used in each laboratory test and was applied prior to the addition of the test species. Dosing at Rutland Water has been virtually continuous since 1990 resulting in a build up of iron III precipitates (Champion *et al.*, 1991). Exposure of existing benthic communities to the constant deposition of iron precipitates onto their habitat may cause many effects not recognised by the laboratory study. Examples may

include reduced ability of visual feeders e.g benthivorous fish to find food or inability of burrowing species to build tubes within the precipitate layer. In the laboratory tests the dosant was added to provide a target concentration from which a certain amount of precipitate would be produced forming a layer over the entire sediment in the tank. Rapid precipitation of iron after mixing with inlet waters at Rutland Water has resulted in a localised build-up of precipitates close to the inlet. The concentration of dosant in the inlet water therefore bears little relation to the concentration of dosant in the whole reservoir and to the amount of precipitate overlying the sediment surface. In this study growth and development of *C. riparius* were retarded at target iron concentrations $\geq$ 30 mg litre^{-1}. Temporary effects were observed at 15 mg Fe litre^{-1}. These target-iron concentrations cannot easily be transferred to the field situation. 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.

Extrapolation from laboratory experiments at the individual and population levels to the field situation requires extreme caution. Observations of community effects in the field or manipulations of the field situation are required for any extrapolation to be possible (Seitz and Ratte, 1991). A study of chironomid-community structure and distribution at Rutland Water was presented in Chapter Two. This and other survey work by the National Rivers Authority (Champion *et al.*, 1991 and Extence *et al.*, 1992) can be used to help provide limited extrapolation of the laboratory studies to the field situation. Impoverished benthic-invertebrate communities coincident with high iron precipitate deposition were observed in each of these Rutland Water studies. 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 (see Chapter Two). Sediment-dependent chironomid taxa such as *Polypedilum*, *Cryptochironomus* and the Tanytarsini tribe, filter feeding genera such as *Glyptotendipes* (Rasmussen, 1984) and the largely carnivorous genus *Procladius* were also absent from iron-contaminated sites. Only sediment-dependent oligochaetes and *Chironomus* sp.[1] remained in affected areas. *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) reported 240-h LC_{50} values for cadmium and copper of 3.8 and 16.3 μg litre^{-1} respectively for embryo to 3rd instar *Tanytarsus dissimilis*. By comparison, the 96-h LC_{50} for second instar *Chironomus riparius* larvae exposed to cadmium was 13,000 μg litre^{-1} (Williams *et al.*, 1986) and the 48-h LC_{50} for fourth instar *C. decorus* for copper was 739 μg litre^{-1} (Kosalwat and Knight, 1987). Oligochaetes and *Chironomus* sp. 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 nymphs (Plecoptera).

Laboratory observations in this study have shown that *Chironomus riparius* larvae exposed to deposited iron III precipitates exhibit reduced growth and development. Smock (1983a) described *C. riparius*' feeding habits as sediment dependent, which he defined as 'deposit feeders generally living within the sediment and indiscriminately ingesting this along with detritus'. The present study showed that *C. riparius* larvae ingest iron precipitates. Since the precipitates are inorganic it is probable that this leads to a reduced energy intake per unit time. The observed growth and development effects could be a direct consequence of this. In summary, if *C. riparius* larvae are presented

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

with a sedimented food source of acceptable particle size range they will feed, regardless of quality.

Smock (1983a) examined the effect of feeding habit on accumulation of seven metals, including iron. Of sediment-dwelling invertebrates, sediment-dependent species had the highest concentrations of most metals (Cr, Fe, Na, Sb, Sc), filter feeders the next highest, followed by sediment-associated species ('species which to some degree selectively ingest detritus and periphyton') and lastly carnivores. A major proportion of the total metal concentration was often associated with the gut material. It is likely therefore, that the sensitivity of other benthic invertebrates to deposited iron precipitates is largely dependent on their feeding habits.

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 iron III-dosing is often flocculent in nature (Extence, *et al.*, 1992). Sediment-dwelling filter feeders are also likely therefore to be presented with iron III 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 may be less affected depending on their ability selectively to ingest detritus or periphyton. However, the thin layer of iron precipitates commonly deposited on stones or vegetation, in streams affected by mine drainage closely resembles the biofilm. 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).

These observations lend credence to the argument that deposited iron precipitates may adversely affect organisms of different feeding habits. To understand the exact interaction between iron precipitates and the benthic community would require much more evidence than is presented in this study. Further examination of Rutland Water and other dosed sites would be required to be able to understand fully the effects of iron-dosing on reservoir ecosystems. However, evidence provided by this study indicates that iron-dosing is likely to be detrimental to individual benthic species and potentially to benthic communities in both shallow and deep lake/reservoir systems. What effect iron-dosing may have on other communities within such systems cannot be extrapolated from this study.

7.3 EFFECTIVENESS OF IRON-DOSING

Whether iron-dosing is effective at reducing phytoplankton (particularly cyanobacteria) biomass has not been tested in this study, but forms part of the NRA and Anglian Water surveys. If iron-dosing is effective in this respect then its advantages as a management tool may be seen to outweigh any deleterious effects. However, it seems unlikely that iron-dosing alone will achieve phytoplankton population control at Rutland Water. Incoming phosphates are precipitated to the sediment and the layer of precipitate formed may act as a barrier to internal loading of phosphorus. At Rutland Water this barrier is very localised around the inlet, covering just 10% of the sediment surface (Extence *et al.*, 1992). Phosphorus loading from exposed sediments is therefore possible. Internal loading is considered to be a more critical problem in shallow systems than deeper ones (Moss, 1990). In Rutland Water, removal of nutrients from inlet waters by phosphate stripping at sewage plants and use of engineering techniques such as hypolimnetic aeration (already present in the main basin at Rutland Water) may provide

the best results. Possible top-down control methods should also be considered, particularly since Rutland Water has a large area of shallows in its two arms.

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Appendix A

Raw data from Chapter Two

Appendix A.1 Numbers of each chironomid taxa retrieved from modified Ekman grab samples from the NRA's grid survey, December 1991.

LF = Lodge Farm transect; SW = Slipway transect; HI = Howell's Inlet; NB = North Buoy and ST = Secondary Tower.

	TANYPODINAE	CHIRONOMINAE								
		Chironomini						Tanytarsini		
SITE	*Procladius*	*Chironomus*	*Polypedilum*	*Micro-tendipes*	*Glypto-tendipes*	*Crypto-chironomus*	*Chironomus/ Polypedilum*	*Tanytarsus*	*Tanytarsus/ Micropsectra*	TOTALS
LF1a	40	7	2					6	20	75
LF1b	38	11						6	14	69
LF2a	29	10	6	1				4		50
LF2b	21	13						2	9	45
LF3a	14	12				1			2	29
LF3b	25	5	1					11	13	55
SW1a		19								19
SW1b		27								27
SW2a	12	15	1					1		29
SW2b		20								20
SW3a	4	6						2		12
SW3b	7	29	3						1	40
Inlet										
HI1a	49	13						11	15	88
HI1b	21	9						3	2	35
HI2a	35	22	2					1	9	69
HI2b	13	30	1						1	45
HI3a	17	17	3	1	1		1		14	54
HI3b	42	17	1					6	4	70
NB1a	46	9	1		1			10	16	83
NB1b	38	7				1		4	23	73
STa	22	11	1					10	10	54
STb	15	13				1		2	13	44

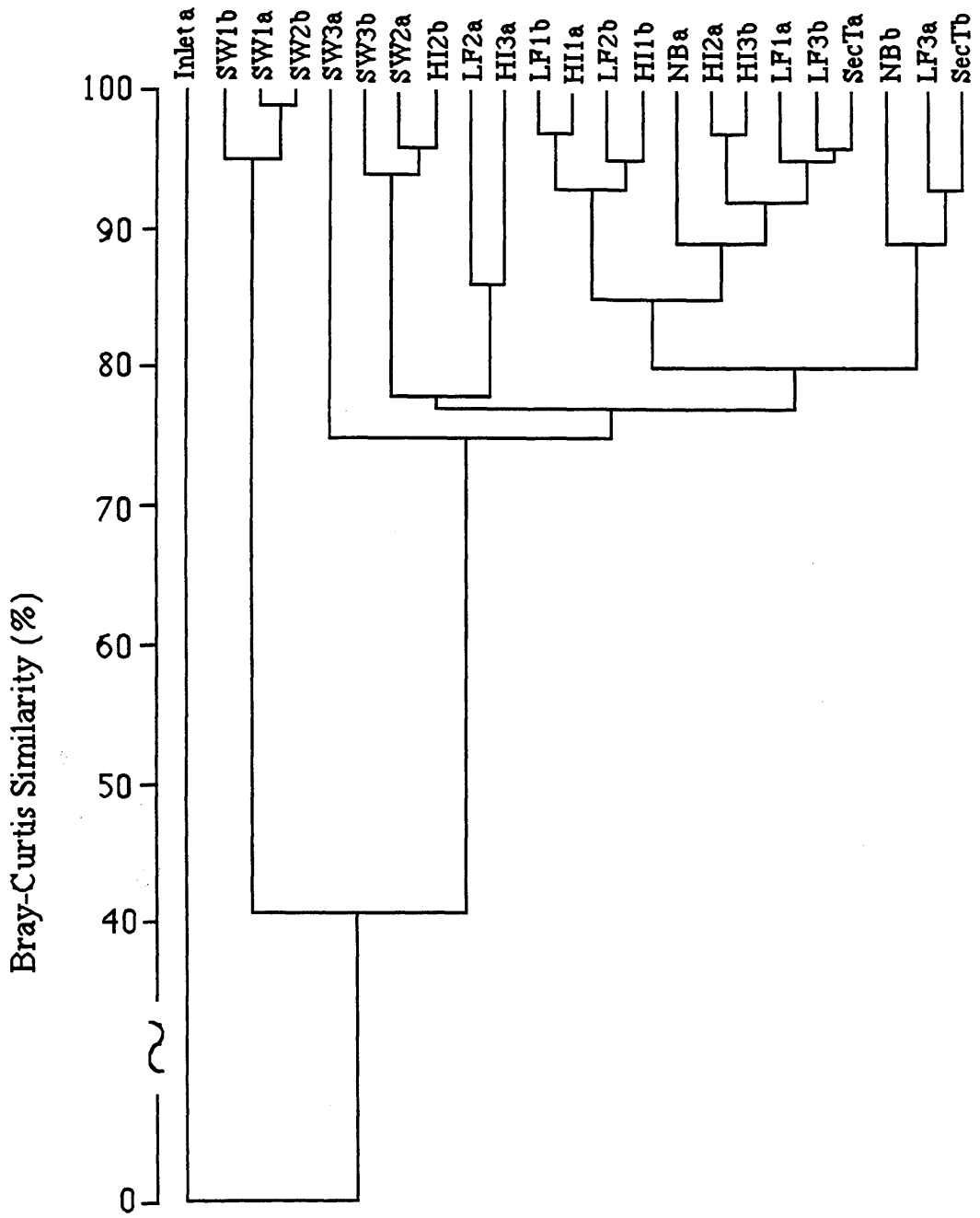
Appendix A.2 Numbers of four chironomid taxa retrieved from modified Ekman grab samples taken by the NRA at grid survey sites (February - November 1992).

LF = Lodge Farm transect; SW = Slipway transect; HI = Howell's Inlet transect; NB = North Buoy; ST = Secondary Tower.

		Site																					
Date	**Taxa**	LF1a	LF1b	LF2a	LF2b	LF3a	LF3b	SW1a	SW1b	SW2a	SW2b	SW3a	SW3b	Inlet a	Inlet b	HI1a	HI1b	HI2a	HI2b	HI3a	HI3b	NBa	NBb	STa	STb
	Chironomus	5		8		14		2		25		9			4	16		13		10		9		11	
	Procladius	53		37		21		16		1		2			1	34		15		25		23		43	
07:02:92	Tanytarsini	39		23		7		8				1			1	12		18		16		14		71	
	Others	1		1		4						1						1		3				5	
	Totals	**98**		**69**		**46**		**26**		**26**		**13**			**6**	**62**		**47**		**54**		**46**		**130**	
	Chironomus	9	14	12	7	12	9	12	10	14	21	7	5			28	38	18	3	5		6	6	9	
	Procladius	30	32	44	25	28	26	3	5	7		3	3		3	12	10	13	67	19	21	65	45	33	
25:03:92	Tanytarsini	16	16	48	9	10	39			2						1		5	8	18	9	32	15	53	
	Others	1	1	7		4	1					2					1		2	1	1	7	14	2	
	Totals	**56**	**63**	**111**	**41**	**54**	**75**	**15**	**15**	**23**	**21**	**12**	**8**		**3**	**41**	**49**	**36**	**80**	**44**	**31**	**110**	**80**	**97**	
	Chironomus	1	1	2	4	2		6	3	4	2		1	1			2	5	7		1				
	Procladius	16	17	20	19	29	29		1	2		2	7		1	25	13	34	18	30	26	15	25	22	28
19:05:92	Tanytarsini	10	9	6	7	6	8									10		5		1	1	4		2	4
	Others	3	1		1	4	4									1			2	1	1	1		2	1
	Totals	**30**	**28**	**28**	**31**	**41**	**41**	**6**	**4**	**6**	**2**	**2**	**8**	**1**	**1**	**36**	**15**	**44**	**27**	**32**	**29**	**20**	**25**	**26**	**33**
	Chironomus	13	3	5	3	2		1	3				3	1	1	2		1	2	1		4	11	8	6
	Procladius	16	16	21	20	38			1	1		6	3		2	49	9	45	63	37	29	43	43	39	67
16:07:92	Tanytarsini	4		6	1	3									1	7		4	3	10	5	6	2	4	19
	Others	1	2	6	1	3		1				1				2	2	18	11	4	2	1	4	2	2
	Totals	**34**	**21**	**38**	**25**	**46**		**2**	**4**	**1**		**7**	**6**	**1**	**4**	**60**	**11**	**68**	**79**	**52**	**36**	**54**	**60**	**53**	**94**
	Chironomus	5	6	6	6	6	3	2	2	3		1		3		7	2				3	9	10	5	8
	Procladius	33	30	15	21	33	36		1			7				24	2	25	5	24	37	80	36	46	33
08:09:92	Tanytarsini	12	10	8	22	19	26					2		1		10	7	8	15	38	20	97	38	80	27
	Others	1	1		1	1			1					7	1		1	3	11	4	4	2	11	4	1
	Totals	**51**	**47**	**29**	**50**	**59**	**65**	**2**	**4**	**3**		**10**		**11**	**1**	**41**	**12**	**36**	**31**	**66**	**64**	**188**	**95**	**135**	**69**
	Chironomus	2	1	1	6			1		1	1			10	9							5	4		1
	Procladius	19	25	20	21	17	20	1	2	3		10	5	8	15	14	17	29	43	29	6	22	27	33	27
04:11:92	Tanytarsini	7	6	35	12	42	30					2					2	6	5	21	2	25	9	41	59
	Others	2	2	2	6	39	17		1	3	4				1	6	2	2	5	7	4	1	2	29	
	Totals	**30**	**34**	**58**	**45**	**98**	**67**	**2**	**3**	**7**	**5**	**12**	**5**	**18**	**25**	**20**	**21**	**37**	**53**	**57**	**12**	**53**	**42**	**103**	**87**

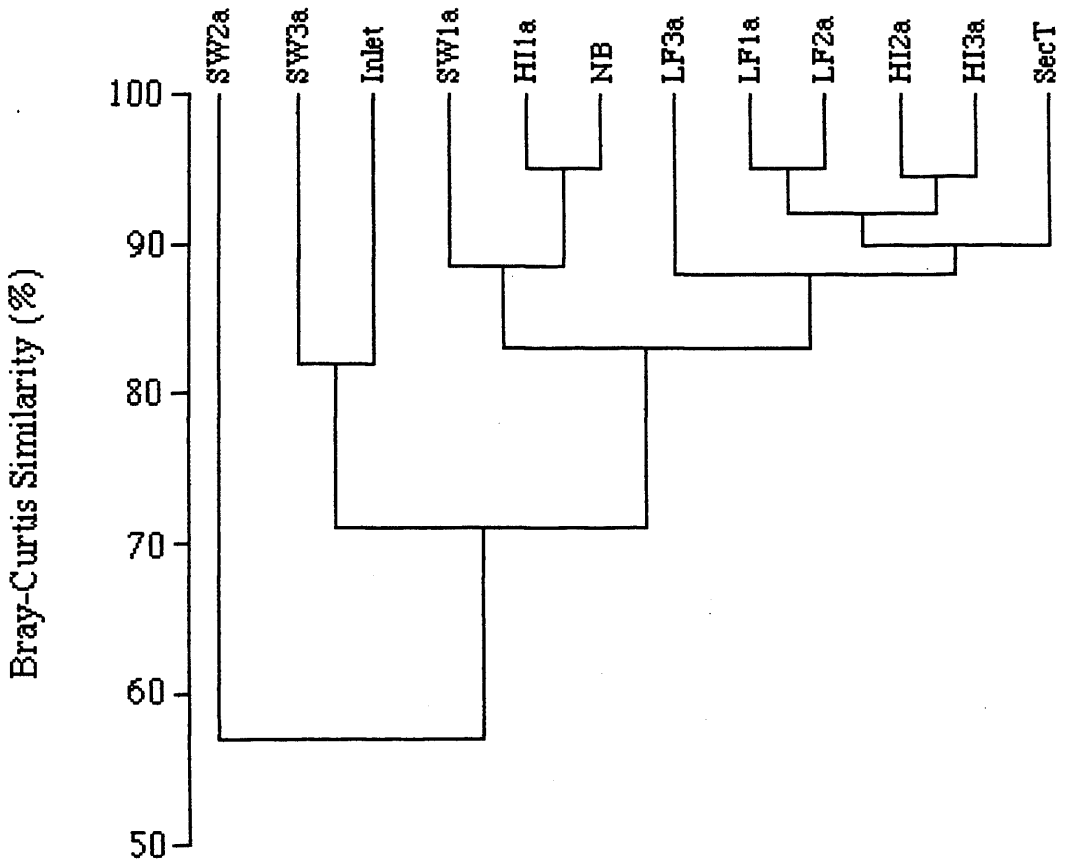
Appendix A.3 Dendrograms of simularity between NRA grid survey sites produced using the PRIMER system (see text for details). HI = Howell's Inlet, LF = Lodge Farm, NB = North Buoy, SecT = Secondary Tower and SW = Slipway.

(i) December 1991



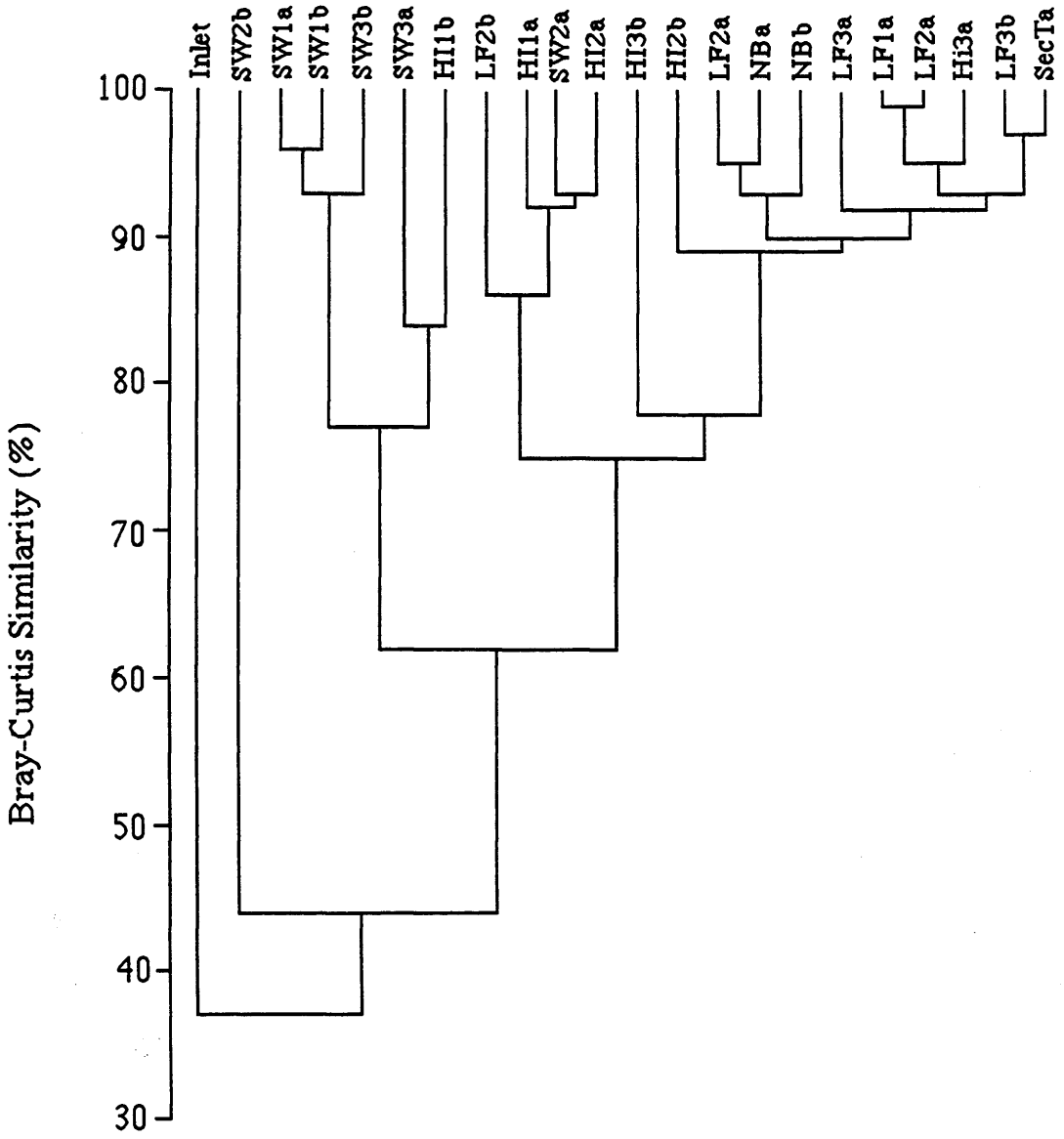
Appendix A.3 continued...

(ii) February 1992



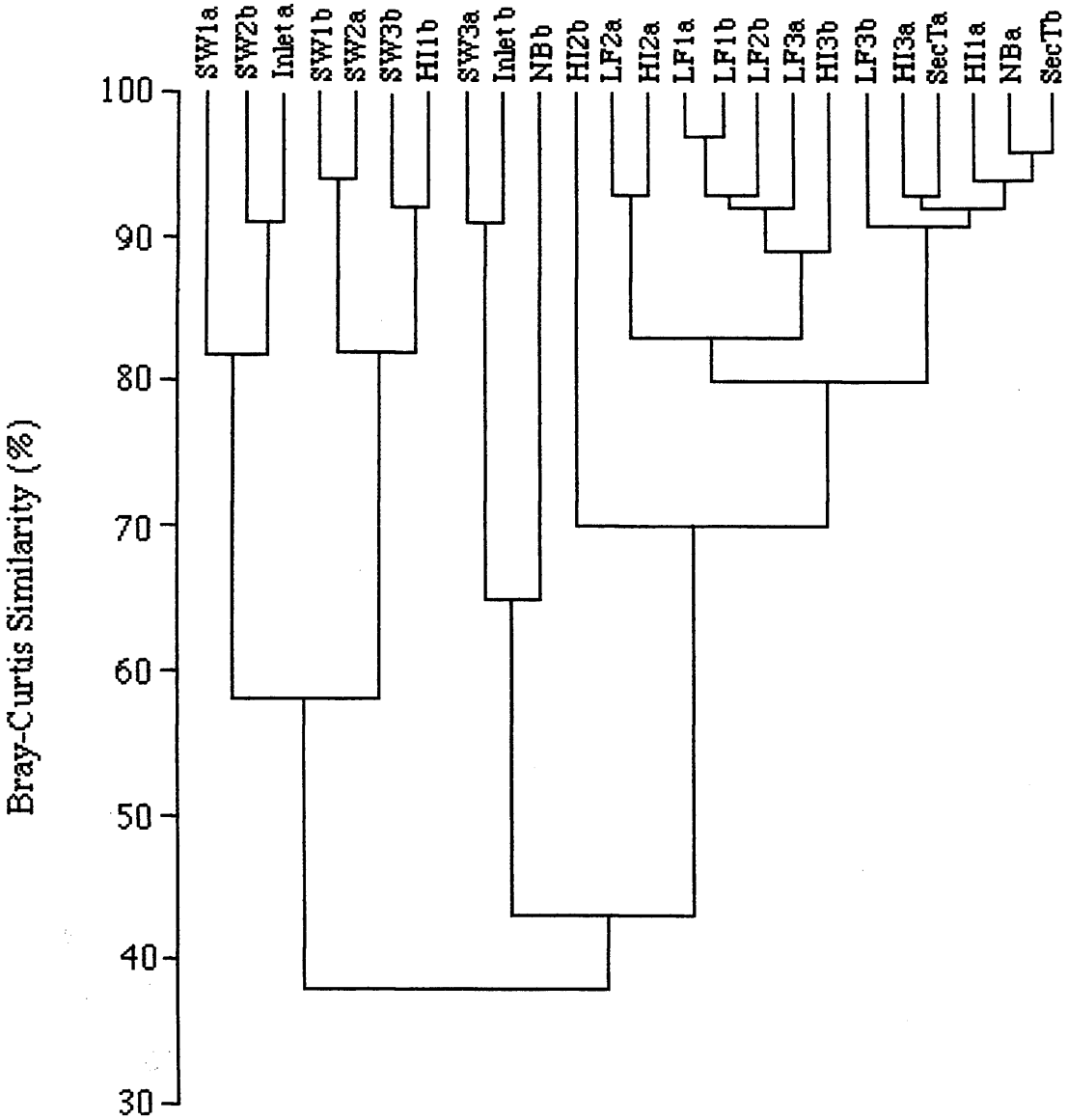
Appendix A.3 continued...

(iii) March 1992



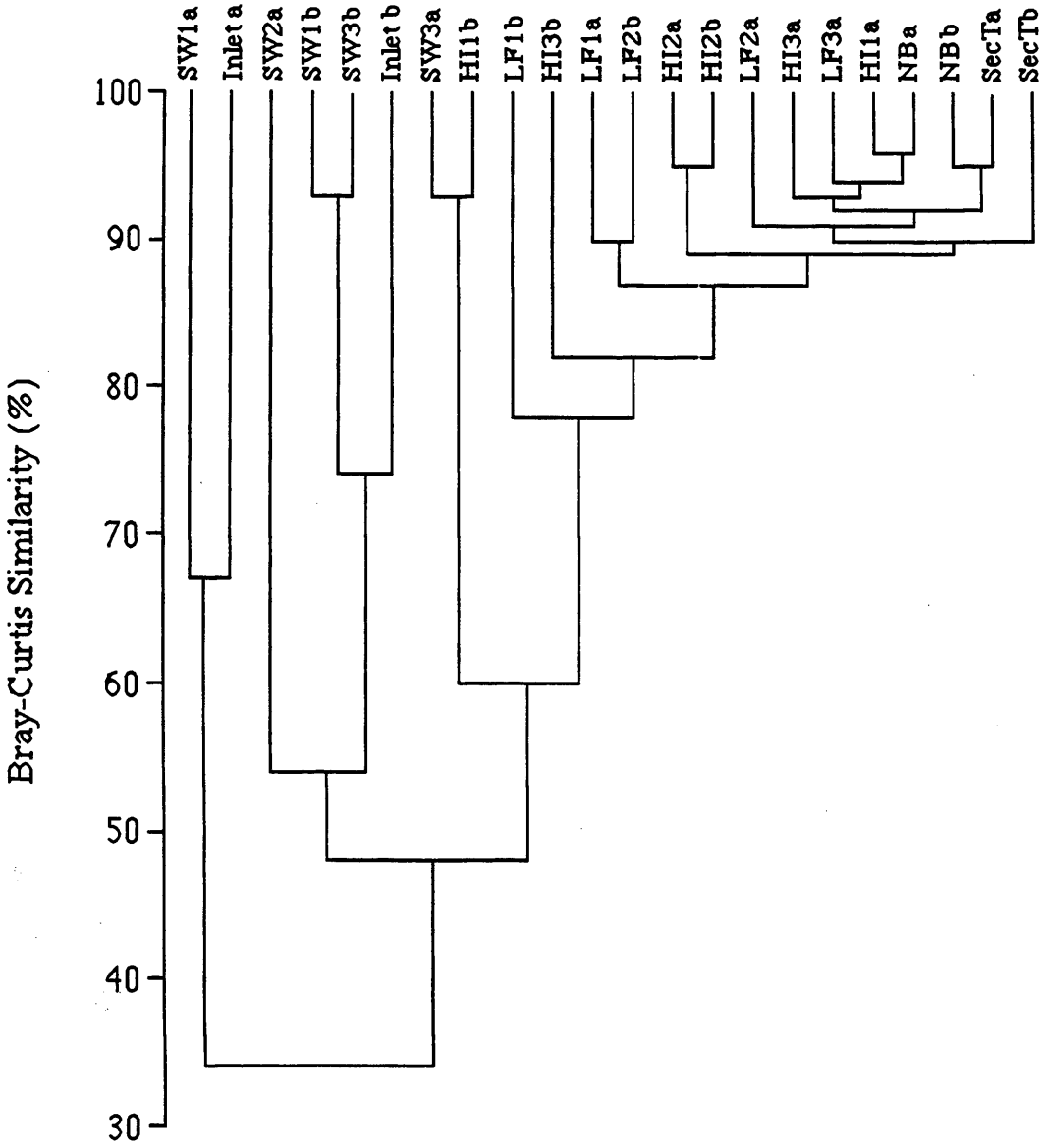
Appendix A.3 continued...

(iv) May 1992



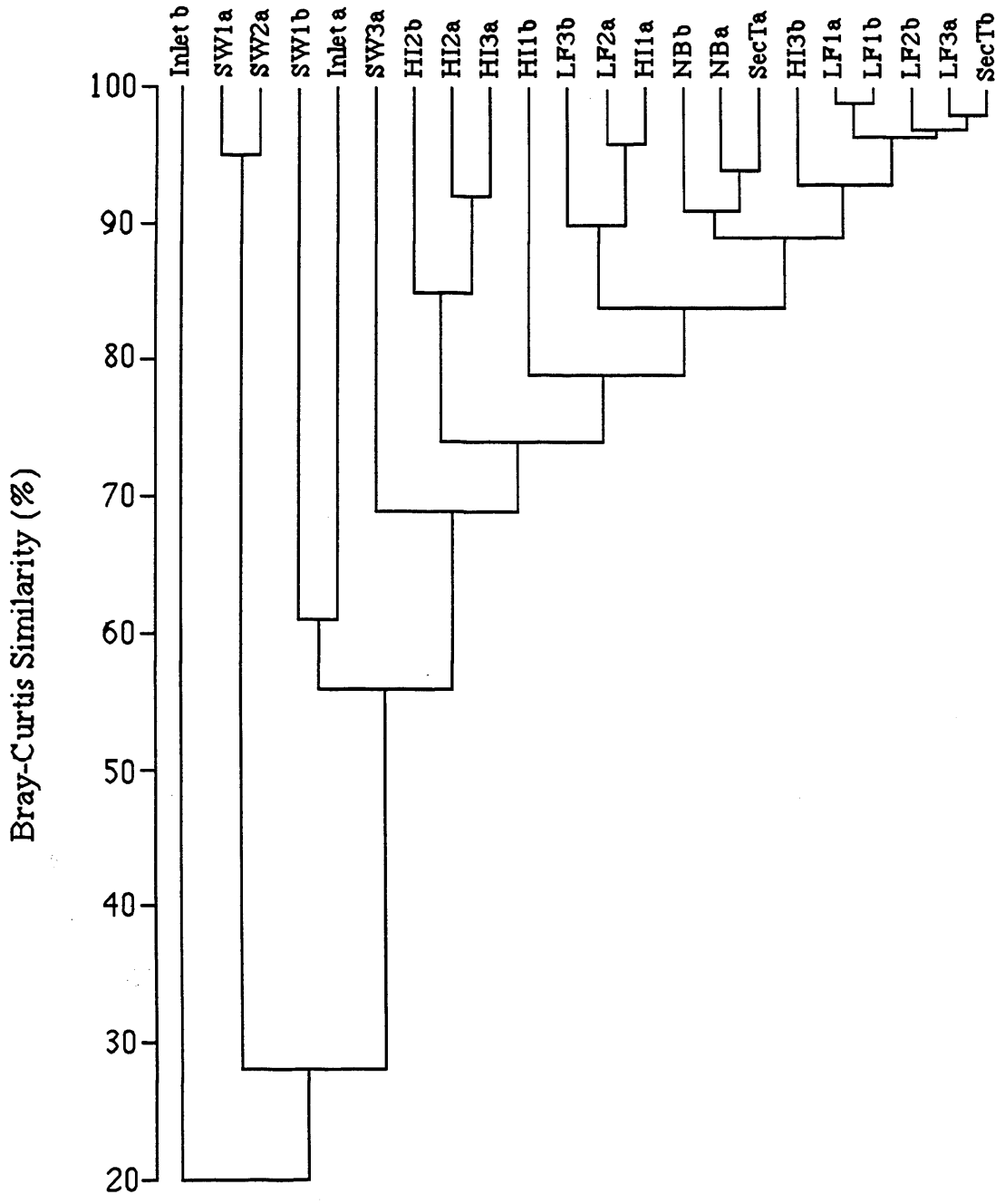
Appendix A.3 continued...

(v) July 1992



Appendix A.3 continued...

(vi) September 1992



Appendix A.4 Chemical data from 0 – 5 m water samples taken and analysed by the National Rivers Authority between 05:05:92 and 07:09:92 from two sites at Rutland Water. ND equals no data.

	Secondary Tower			Inlet		
Date	Dissolved Iron (mg Fe/litre)	Total Iron (mg Fe/litre)	pH	Dissolved Iron (mg Fe/litre)	Total Iron (mg Fe/litre)	pH
05:05	.010	.023	8.21	.010	1.880	7.93
11:05	.013	.040	8.19	.016	.819	7.92
18:05	.012	.017	8.41	.010	.059	8.17
26:05	.013	.012	8.25	.010	.021	8.35
01:06	.010	.023	8.20	.010	.102	8.20
09:06	.010	.039	8.20	.017	2.120	7.88
15:06	.010	.010	8.22	ND	ND	ND
22:06	.032	.010	8.21	ND	ND	ND
29:06	.010	.013	8.22	ND	ND	ND
06:07	.010	.031	8.19	.069	4.330	7.82
13:07	.010	.014	8.29	.030	5.950	7.75
20:07	.010	.010	8.33	.010	1.680	7.94
27:07	.010	.012	8.34	.010	.128	8.13
03:08	.010	.044	8.21	.010	.079	8.21
10:08	.010	.010	8.25	.010	.058	8.18
17:08	.010	.051	8.28	.010	.274	8.15
24:08	.010	.010	8.21	.010	.055	8.18
01:09	.010	.010	8.07	.010	.176	8.13
07:09	ND	ND	8.26	ND	ND	7.93

Appendix B

Raw data from Chapter Three

Appendix B.1 Counts of eggs per eggmass from laboratory culture of *Chironomus riparius*.

309	338	354	281	272	575	380	331	346
376	289	297	276	265	480	380	331	282
311	194	210	259	243	372	504	425	372
386	519	211	446	364	248	383	311	349
275	245	228	429	287	223	214	193	254
328	324	313	273	266	375	400	286	362
423	305	252	530	464	347	255	375	293
452	343	302	269	431	388	552	280	407

Appendix B.2 Measurements of (i) conductivity (µS/cm), (ii) alkalinity $CaCO_3$ (mg/l) and (iii) pH from 0 – 5m water samples taken and analysed by the National Rivers Authority from the North Arm of Rutland Water on a weekly basis between 29:05:90 and 07:09:92. Conductivity measurements were not made on every occassion.

(i) Conductivity (µS/mm)

784	822	716	743	643	649	780	593
689	726	757	711	711	764	623	846
830	753	772	823	757	816	837	821
824	1270	920	869	886	828	837	880

Appendix B.2 continued...

(ii) Alkalinity $CaCO_3$ (mg/l)

173	171	162	169	138	140	135	130	135	135
150	145	130	120	140	120	125	125	130	140
140	130	130	130	130	110	130	135	135	140
125	130	130	150	130	120	125	120	130	125
120	120	135	125	130	135	130	118	110	118
117	118	117	110	111	114	120	108	98	100
108	108	107	110	111	111	102	101	107	114
97	101	122	108	115	115	124	121	123	117
106	114	102	115	113	115	116	115	116	114
106	112	117	114	112	112	120	118	115	113
109	111	108	113	110	110	104	110	109	109

(iii) pH

8.39	8.21	7.89	8.16	8.26	8.00	7.72	7.88	7.82	7.92
7.73	7.87	8.13	8.10	7.90	8.03	8.21	8.16	8.20	8.16
8.14	8.13	8.20	8.17	8.06	8.16	8.17	7.96	8.10	8.07
8.30	8.20	8.06	7.94	8.00	8.14	8.22	8.21	8.44	8.26
8.32	8.29	8.22	8.20	8.16	7.98	7.91	8.19	8.32	8.15
8.18	7.49	8.13	8.30	8.40	8.14	8.32	8.26	8.35	8.25
8.24	8.28	8.16	7.96	8.02	8.05	7.96	8.07	7.89	8.24
7.97	8.03	7.97	7.76	8.02	8.11	8.10	8.11	8.08	7.92
7.85	8.08	8.04	8.03	8.10	8.19	8.19	8.16	8.14	8.16
8.23	8.17	8.16	8.44	8.22	8.16	8.20	8.25	8.21	8.35
8.18	8.25	8.28	8.28	8.11	8.25	8.35	8.06	7.86	8.28

Appendix B.3 Head capsule width (H.c.w) and body length (B.l.) measurements of *Chironomus riparius* larvae (n = 162).

H.c.w. (μm)	B.l (mm)	H.c.w. (μm)	B.l (mm)	H.c.w. (μm)	B.l (mm)	H.c.w. (μm)	B.l (mm)	H.c.w. (μm)	B.l (mm)
90	0.55	350	3.36	300	3.60	575	14.00	525	9.00
80	0.53	350	3.60	325	4.10	600	16.00	550	14.00
95	0.54	325	4.80	375	5.64	625	11.00	600	13.00
90	0.55	300	4.56	200	2.88	550	9.00	575	16.00
100	0.60	350	4.32	190	2.76	550	13.00	625	8.00
90	0.54	325	3.48	200	2.40	600	13.00	600	14.00
100	0.55	300	4.80	200	2.40	550	13.00	625	15.00
100	0.60	600	17.00	190	2.50	500	10.00	625	10.00
90	0.54	350	6.72	180	2.85	525	11.00	500	4.80
90	0.53	300	4.56	325	4.56	550	11.00	575	10.00
110	0.49	300	4.20	375	4.32	575	10.00	500	8.00
100	0.60	300	6.12	350	6.00	500	8.00	525	8.00
110	0.70	275	2.64	375	4.32	575	13.00	375	5.64
110	0.54	575	16.00	190	2.09	625	17.00	325	5.28
100	0.46	525	8.00	350	3.60	600	14.00	550	6.00
90	0.51	525	9.00	350	4.32	600	12.00	575	12.00
100	0.54	525	6.84	210	3.12	525	16.00	600	14.00
100	0.51	600	16.00	550	13.00	550	9.00	600	12.00
100	0.52	500	9.00	325	4.80	575	11.00	625	11.00
100	0.55	575	11.00	375	4.51	575	11.00	600	16.00
110	0.54	325	3.48	375	3.84	575	14.00	625	12.00
100	0.51	325	5.40	350	6.72	550	8.00	600	9.50
105	0.56	325	4.03	375	6.36	525	6.84	325	6.00
110	0.56	325	4.80	350	3.72	550	13.00	325	5.04
110	0.57	180	1.80	350	3.24	625	11.00	325	4.56
110	0.55	190	2.88	200	2.16	600	6.00	325	5.16
325	4.10	180	2.75	300	4.08	600	12.00	325	3.24
350	6.00	180	2.85	575	11.00	575	11.00	375	4.32
300	4.80	210	4.32	550	8.00	600	12.00	200	3.12
300	4.08	300	5.04	625	8.00	600	13.00	325	4.10
325	4.80	300	4.08	575	11.00	525	16.00		
300	3.60	325	4.80	550	6.00	525	6.00		
210	4.32	180	2.88	190	2.76	190	2.64		

Appendix B.4 Iron concentration of stock solutions. Stock solutions were diluted by 100 times using 10% nitric acid and measured using atomic absorbtion spectrophotometry. This gives readings as mMolar which are converted to mg Fe litre^{-1} by multiplying by the atomic weight of iron (56 g).

Iron concentration		
AAS reading (mM Fe)	* Dilution factor (100)	mg Fe litre^{-1}
0.089	89.0	498.4
0.089	89.0	498.4
0.089	89.0	498.4
0.091	91.0	509.6
0.090	90.0	504.0
0.089	89.0	498.4
0.087	87.0	487.2
0.087	87.0	487.2

Appendix B.5 Total weight of ferric iron precipitates and total iron content of those precipitates formed at different target iron concentrations.

Target iron concentration (mg Fe litre^{-1})	Total weight of precipitate (mg)	Total iron content of precipitate (mg Fe litre^{-1})
0	0.0	–
5	38.0	152.990
15	193.1	168.652
30	269.4	196.719
30		189.520
40	340.2	187.525
40		181.641
50	490.4	150.240
50		174.389
75	538.1	153.656
75		195.040

Appendix B.6 Weight of cellulose nitrate filter membranes (mg).

84.0	84.3	84.0	83.3	83.2	83.0	83.7	83.2	83.3	83.3

Appendix C

Raw data from Chapter Four

Appendix C.1 Levels of dissolved iron and iron in particulate matter from 10-day tests using deionised water as the test medium. Dry weight correction of particulate matter levels were not made in these 10-day tests.

Experiment	Target iron concentration (mg Fe litre^{-1})	Replicate	Dissolved iron (mM)	Iron in particulate matter (mM)
A	0	a	0	4
	0	b	0	4
	0.5	a	1	11
	0.5	b	1	12
	5	a	2	95
	5	b	2	106
	25	a	2	557
	25	b	1	554
	25	c	3	547
	50	a	113	993
	50	b	81	3670
	125	a	574	3770
	125	b	562	4019
B	0	a	1	18
	0	b	1	13
	0	c	1	3
	25	a	2	445
	25	b	12	305
	25	c	3	515
	32.5	a	43	714
	32.5	b	62	681
	32.5	c	9	793
	42.5	a	68	756
	42.5	b	80	707
	42.5	c	8	896
	50	a	78	2583
	50	b	60	2423
	50	c	79	2354

Appendix C.2 Levels of dissolved iron and iron in particulate matter from 10-day tests using filtered reservoir water as the test medium. Dry weight correction of particulate matter levels were not made in these 10-day tests.

Experiment	Target iron concentration (mg Fe litre^{-1})	Replicate	Dissolved iron (mg Fe litre^{-1})	Iron in particulate matter (mg Fe litre^{-1})
C	0	a	1	18
	0	b	2	15
	0.5	a	2	27
	0.5	b	2	30
	5	a	5	86
	5	b	2	88
	25	a	11	329
	25	b	7	457
	50	a	5	2214
	50	b	6	3361
	125	a	349	3910
	125	b	287	4249
D	0	a	2	9
	0	b	1	4
	0	c	1	6
	25	a	32	405
	25	b	16	417
	25	c	15	415
	32.5	a	20	515
	32.5	b	25	590
	32.5	c	26	509
	42.5	a	2	886
	42.5	b	20	640
	42.5	c	50	710
	50	a	0	1127
	50	b	2	1123
	50	c	1	1111
E	0	a	0	4
	25	a	6	220
	32.5	a	11	173
	42.5	a	5	339
	45	a	7	176
	47.5	a	0	181
	50	a	3	181
	55	a	1	251

Appendix C.3 Mortality of larvae (numbers of dead larvae) from each replicate of the 10-day duration tests. Deionised water was used as the medium in experiments A and B, filtered reservoir water was used in experiments C, D and E.

Experiment	Target iron concentration (mg Fe litre^{-1})										
(replicate)	0	0.5	5	25	32.5	42.5	45	47.5	50	55	125
A (a)	0	1	0	3					10		10
(b)	0	0	1	3					10		10
(c)	0	0	0	1					10		10
(d)	0	0	1	10					10		10
(e)	0	0	0	0					10		10
B (a)	0			1	10	10			10		
(b)	0			0	10	10			10		
(c)	0			2	10	10			10		
C (a)	2	1	0	0					7		10
(b)	0	0	0	0					7		10
(c)	1	0	0	0					8		10
(d)	1	2	2	0					4		10
(e)	0	0	1	1					2		10
D (a)	1			0	0	0			3		
(b)	0			0	2	0			6		
(c)	2			2	0	0			7		
E (a)	0			0	0	0	0	0	0	10	
(b)	0			0	2	0	0	1	1	10	
(c)	0			0	0	0	1	0	0	3	

Appendix C.4 Average wet weight (mg) of larvae recovered from each replicate of the 10-day duration tests. For these experiments larvae recovered from each replicate were weighed together and an average weight calculated. Deionised water was used as the medium in experiments A and B, filtered reservoir water was used in experiments C, D and E. Where total mortality occurred a dash (–) is recorded.

Experiment	Target iron concentration (mg Fe litre^{-1})										
(replicate)	0	0.5	5	25	32.5	42.5	45	47.5	50	55	125
A (a)	7.85	8.74	8.00	7.56					–		–
(b)	8.05	8.42	9.63	6.61					–		–
(c)	7.65	8.69	8.16	7.61					–		–
(d)	7.45	8.25	9.11	6.16					–		–
(e)	7.20	8.82	8.75	–					–		–
B (a)	8.69			7.63	–	–			–		
(b)	9.64			7.58	–	–			–		
(c)	8.83			7.84	–	–			–		
C (a)	7.36	7.26	7.39	7.50					3.10		–
(b)	5.50	7.32	6.79	6.53					4.40		–
(c)	7.49	7.34	9.03	7.41					4.45		–
(d)	7.10	7.94	7.11	8.02					4.57		–
(e)	6.94	7.63	8.49	7.10					6.55		–
D (a)	7.89			7.26	8.66	7.31			6.61		
(b)	7.77			8.68	7.10	9.09			4.98		
(c)	8.06			7.69	7.72	6.67			5.47		
E (a)	6.98			7.67	7.29	7.93	8.04	6.14	7.11	–	
(b)	8.27			8.22	7.70	6.63	8.88	6.14	7.34	–	
(c)	5.24			7.54	7.46	8.08	7.30	6.13	6.61	5.36	

Appendix C.5 Iron content of chironomid larvae from 10-day test, experiment E.

Target concentration (mg Fe litre^{-1})	Iron content (μg g^{-1} wet weight)
0.0	41.0
25.0	153.0
32.5	200.0
42.5	370.0
45.0	333.0
47.5	659.0
50.0	453.0
55.0	968.0

Appendix C.6 Dissolved iron levels from 25-day duration iron tests described in Chapter Four. Values are given in mg Fe litre^{-1}. The symbol 'R' denotes samples taken from replacement tanks.

	Sample	Target concentration (mg Fe litre^{-1})				
Test	day	0	5	15	30	50
A	0	0	2	1	2	64
	6	6	3	3	2	8
	13R	5	8	1	4	77
	14	0	0	0	0	34
	20R	2	0	2	0	19
	25R	1	1	1	0	29
B	0	4	2	2	3	23
	6	2	4	1	2	12
	13R	0	5	5	8	72
	14	13	0	0	0	21
	20R	4	3	3	4	10
	25R	0	0	0	0	2
C	0	0	1	0	2	24
	6	1	2	2	3	6
	13R	2	6	6	10	83
	14	2	2	2	2	21
	20R	4	2	3	3	14
	25R	2	2	3	4	6
D	0	0	1	2	2	20
	6	0	0	1	1	9
	13R	1	5	4	9	77
	14	1	0	0	0	0
	20R	0	2	0	0	5
	25R	2	2	2	2	22

Appendix C.7 Iron in particulate matter levels from 25-day duration iron tests described in Chapter Four. Values are given in mg Fe g^{-1} dry weight of particulate matter. Multiplication by the atomic weight of iron (56 g) was used to convert mM Fe readings from atomic absorption spectrophotometry to mg Fe $litre^{-1}$. This figure was then corrected for dry weight using the calculation described in section 3.12. The symbol 'R' denotes samples taken from replacement tanks.

	Sample	Target concentration (mg Fe $litre^{-1}$)				
Test	day	0	5	15	30	50
A	0	0.000	2.401	6.656	6.442	8.541
	6	0.089	1.865	6.977	5.547	14.660
	13R	0.106	1.282	2.639	14.443	10.327
	14	0.104	2.048	2.436	12.400	7.211
	20R	0.039	3.398	6.368	9.047	8.147
	25R	0.074	3.433	5.681	13.580	8.366
B	0	0.063	1.869	3.432	6.366	10.594
	6	0.087	1.294	3.509	2.668	10.546
	13R	0.041	1.604	1.410	4.686	12.023
	14	0.048	1,297	4.312	6.508	9.797
	20R	0.097	2.131	4.222	10.108	19.280
	25R	0.040	3.020	3.705	7.860	11.325
C	0	0.000	1.366	6.574	13.049	15.592
	6	0.076	1.527	2.720	6.211	13.646
	13R	0.030	3.003	8.253	8.322	7.423
	14	0.000	2.155	7.361	9.683	15.625
	20R	0.093	2.266	8.806	14.538	13.341
	25R	0.077	3.805	9.550	14.798	19.530
D	0	0.057	2.595	1.109	8.730	5.917
	6	0.076	1.817	3.169	4.288	10.636
	13R	0.047	3.585	11.111	1.416	12.968
	14	0.061	3.492	8.923	9.993	18.129
	20R	0.070	4.179	11.031	11.493	21.709
	25R	0.043	4.112	11.662	10.394	26.482

Appendix C.8 pH measurements from iron tests described in Chapter Four, (i) replicate 3 and (ii) replicate 4. Tank numbers refer to initial (Tank 1) and replacement (Tank 2) tanks.

(i)

		Treatment (mg Fe litre^{-1})											
		0		5		15		30		40		50	
Day	Tank	1	2	1	2	1	2	1	2	1	2	1	2
0		8.28		8.07		7.74		4.67		3.54		3.12	
6		8.39		7.55		7.38		7.02		5.81		4.79	
12			8.03		7.59		7.02		3.67		3.02		2.76
14			7.81		7.66		7.61		6.51		3.29		2.86
20			7.73		7.75		7.67		7.34		6.98		5.09
25			7.99		7.92		7.52		7.18		6.81		4.30

(ii)

		Treatment (mg Fe litre^{-1})											
		0		5		15		30		40		50	
Day	Tank	1	2	1	2	1	2	1	2	1	2	1	2
0		7.91		7.70		7.49		4.75		3.14		3.10	
6		8.01		8.10		7.58		6.98		4.04		4.07	
14		7.88	7.87	7.98	7.72	7.80	7.21	7.61	6.20	7.13	3.30	6.58	2.95
20			7.95		7.71		7.65		7.50		7.33		5.66
25			8.19		8.27		8.16		7.83		7.38		6.85

Appendix C.9 Raw data from 25-day tests described in Chapter Four. Census refers to counts of total larval numbers

Experiment	A					B				
Treatments (mg Fe litre^{-1})										
Counts	0	5	15	30	50	0	5	15	30	50
Total eggs	297	289	346	276	380	282	259	243	380	311
Non-viable eggs	11	7	44	31	51	93	68	50	61	37
Larvae dead in eggmass	0	0	0	0	247	0	0	0	0	153
Sampled before census I	2	3	10	2	0	7	7	1	2	0
Census I	273	256	263	217	0	166	130	176	291	0
Sampled between census I & II	47	26	24	16	-	25	18	15	20	-
Emerged adults	24	9	5	36	-	10	60	17	1	-
Census II	198	208	219	110	-	117	40	131	254	-

Experiment	C					D				
Treatments (mg Fe litre^{-1})										
Counts	0	5	15	30	50	0	5	15	30	50
Total eggs	503	364	372	519	386	275	349	245	287	383
Non-viable eggs	0	2	55	43	11	73	145	0	47	33
Larvae dead in eggmass	0	0	0	0	0	0	0	0	0	0
Sampled before census I	10	6	0	7	13	12	19	20	8	4
Census I	487	333	181	458	252	186	165	216	218	91
Sampled between census I & II	38	38	36	27	0	25	21	24	14	2
Emerged adults	0	0	8	1	0	6	24	2	0	0
Census II	427	295	120	419	11	115	107	188	49	28

Appendix C.10 Individual wet weight of larvae sampled during the 25-day iron tests described in chapter four; (i) Day 14, (ii) Day 20 and (iii) Day 25. Values are given in mg.

(i) Day 14

	Target iron concentration (mg litre^{-1})				
No.	0	5	15	30	50
1	2.5	0.5	1.5	0.1	0.1
2	1.8	0.1	1.7	0.1	0.1
3	3.3	1.3	1.1	0.1	0.1
4	2.2	2.1	2.0	1.3	0.1
5	3.0	3.5	1.7	3.1	
6	3.2	1.9	1.1	0.6	
7	2.8	0.5	1.1	1.2	
8	2.5	0.4	3.0	2.3	
9	1.7	4.1	2.6	2.5	
10	3.2	3.0	2.3	2.5	
11	2.8	4.3	1.1	1.5	
12	3.5	5.0	2.4	1.8	
13	2.1	1.4	3.3	2.7	
14	2.9	1.1	2.6	1.2	
15	2.5	0.9	1.7	1.8	
16	3.1	0.9	3.3	2.7	
17	3.3	1.9	2.8	2.2	
18	4.0	1.2	3.1	0.9	
19	2.4	0.1	2.5	1.9	
20	3.3	0.8	2.4	1.0	
21	4.2	1.0	3.4	1.0	
22	3.6	0.9	1.2	0.9	
23	2.5	0.1	2.0	1.1	
24	2.7	1.0	0,1	1.1	
25	1.0	0.6	0.1	0.1	
26	3.8	1.0	3.6		
27	3.3	1.2	2.9		
28	3.8	0.9	2.0		
29	2.1	0.8	1.0		
30	1.5	3.5	2.0		
31	1.0	2.5	1.5		
32	0.1	2.1	2.0		
33	1.0	2.6	2.4		
34	2.4	1.5	1.8		
35	1.0	0.5	1.8		
36	1.0	0.4	1.8		
37	0.7		1.5		
38	1.1		1.2		
39	0.9		1.9		
40	0.9		1.1		
41	2.6		1.7		
42	1.8		0.9		
43	2.2		0.9		
44	0.9				
45	2.8				
46	2.1				

Appendix C.10 continued...

(ii) Day 20

	Target iron concentration (mg litre^{-1})				
No.	0	5	15	30	50
1	4.5	3.4	1.9	0.1	1.2
2	5.5	4.3	3.0	4.7	1.3
3	4.1	5.8	4.2	4.0	
4	5.7	5.9	4.8	1.1	
5	5.1	1.0	3.0	2.4	
6	6.4	4.2	5.4	5.2	
7	4.0	4.2	5.0	4.4	
8	4.6	1.6	5.3	3.8	
9	6.0	4.6	3.8	4.1	
10	4.2	1.0	2.2	4.1	
11	4.3	6.6	1.7	4.1	
12	5.4	4.2	5.4	1.8	
13	3.2	3.9	1.9	3.0	
14	5.7	3.9	5.0	3.3	
15	6.1	3.8	5.0	2.2	
16	2.8	2.9	2.3	0.1	
17	4.0	3.1	4.0	1.3	
18	2.7	2.4	3.4	4.9	
19	2.3	4.7	5.4	0.6	
20	2.0	2.4	3.7	4.3	
21	2.6	2.1	4.6	0.4	
22	1.7	2.3	4.6	1.0	
23	1.6	5.4	1.6	0.6	
24	1.2	6.8	6.0	0.7	
25	1.6	0.1	6.6	1.8	
26	5.3	5.5	3.2	1.1	
27	5.8	6.4	5.9	1.5	
28	4.8	5.1	2.9	1.1	
29	5.9	3.3	5.8	1.2	
30	5.8		3.2	0.8	
31	4.0		5.2	1.4	
32	4.9		1.9	0.9	
33	5.7		4.9	0.1	
34	5.3		4.8		
35	4.8		5.2		
36	3.4		2.0		
37			2.2		

Appendix C.10 continued...

(iii) Day 25

	Target iron concentration (mg litre^{-1})				
No.	0		5	15	30
1	5.2	2.4	6.0	3.9	4.4
2	7.0	5.0	1.0	4.2	4.1
3	6.6	4.0	5.1	4.3	3.1
4	4.6	4.0	1.1	4.7	0.4
5	5.1	4.1	4.2	4.0	3.2
6	5.9	6.3	3.5	2.4	3.0
7	4.9	7.4	1.6	5.6	3.4
8	6.6	4.8	4.7	3.1	4.3
9	5.9	8.8	5.0	5.2	3.6
10	4.6	8.0	7.1	5.1	4.1
11	5.0	8.3	6.8	3.2	4.5
12	6.2	9.0	3.3	5.0	5.8
13	4.7	6.2	4.3	3.8	4.3
14	5.9	5.1	3.2	5.5	3.4
15	6.1	4.0	4.6	4.5	2.3
16	3.7	8.3	2.4	6.9	3.6
17	4.8	5.3	2.1	7.8	3.4
18	5.9	5.4	1.7	6.6	2.3
19	5.1	4.4	3.1	3.7	0.9
20	4.6		4.0	3.5	1.0
21	4.8		2.1	2.2	1.4
22	4.4		3.9	2.0	1.6
23	3.6		2.3	5.3	0.1
24	5.1		0.7	5.8	0.6
25	6.3		7.5	6.4	0.6
26	6.0		8.3	4.2	
27	3.8		6.6	6.1	
28	4.8		6.6	3.4	
29	4.6		7.3	3.7	
30	2.3		4.5	3.7	
31	1.4		6.7	5.6	
32	3.9		2.8	4.7	
33	2.8		3.1	5.1	
34	2.2		2.6		
35	4.5		7.7		
36	3.7		8.4		
37	3.3		2.4		
38	4.3				
39	2.8				

Appendix C.11 Instar stages of larvae sampled during the 25-day iron experiments described in chapter four; (i) Day 6, (ii) Day 14, (iii) Day 20 and (iv) Day 25.

(i) Day 6

Test	Target iron concentration (mg litre^{-1})	Instar stage		
		First	Second	Third
A	0	–	2	–
	5	–	3	–
	15	–	10	–
	30	–	2	–
	50	No survivors past day 4		
B	0	–	–	7
	5	3	4	–
	15	–	–	1
	30	–	2	–
	50	No survivors past day 4		
C	0	–	10	–
	5	–	6	–
	15	–	–	–
	30	–	7	–
	50	11	–	–
D	0	–	6	–
	5	–	12	–
	15	–	6	–
	30	–	2	–
	50	–	–	–

Appendix C.11 continued...

(ii) Day 14

Test	Target iron concentration (mg litre^{-1})	Instar stage		
		Second	Third	Fourth
A	0	–	–	20
	5	–	4	5
	15	–	1	6
	30	3	1	4
B	0	–	–	9
	5	–	1	7
	15	–	–	8
	30	–	–	6
C	0	–	7	4
	5	–	15	2
	15	1	2	11
	30	–	1	4
	50	–	–	–
D	0	–	1	5
	5	–	2	5
	15	–	–	14
	30	–	4	2
	50	1	3	–

Appendix C.11 continued...

(iii) Day 20

Test	Target iron concentration (mg litre^{-1})	Instar stage		
		Third	Fourth	Pupae
A	0	–	8	–
	5	2	8	–
	15	–	7	–
	30	1	3	–
B	0	–	7	–
	5	–	3	1
	15	–	4	–
	30	–	5	–
C	0	–	10	–
	5	–	9	–
	15	–	13	–
	30	5	9	–
	50	–	–	–
D	0	–	11	–
	5	1	6	–
	15	–	13	–
	30	3	7	–
	50	–	2	–

Appendix C.11 continued...

(iv) Day 25

Test	Target iron concentration (mg litre^{-1})	Instar stage		
		Third	Fourth	Pupae
A	0	–	19	–
	5	–	7	–
	15	–	10	–
	30	1	3	–
B	0	–	8	1
	5	–	5	1
	15	–	3	–
	30	–	9	–
C	0	–	17	–
	5	1	11	–
	15	–	9	–
	30	–	8	–
	50	–	–	–
D	0	–	14	–
	5	–	13	1
	15	–	11	–
	30	2	2	–
	50	–	–	–

Appendix C.12 Total iron content of chironomid larvae from 25-day iron tests described in chapter four. The procedure for larval digestion is described in section 3.11. Values are given in mg Fe g^{-1} dry weight of larvae. Multiplication by the atomic weight of iron (56 g) was used to convert mM Fe readings from atomic absorption spectrophotometry to mg Fe $litre^{-1}$. This figure was then corrected for dry weight using the calculation described in section 3.12.

	Sample	Target iron concentration (mg $litre^{-1}$)			
Test	Day	0	5	15	30
A	14	0.499	1.167	3.569	4.455
	20	0.212	0.467	0.821	3.111
	25	0.081	0.373	0.593	1.400
B	14	0.207	0.455	1.916	6.426
	20	0.161	0.575	0.646	0.844
	25	0.073	0.312	2.100	0.696
C	14	–	2.127	1.934	2.063
	20	0.622	0.406	0.582	1.237
	25	0.464	0.406	7.318	1.830
D	14	0.675	1.065	0.992	2.683
	20	0.451	0.257	0.467	1.842
	25	0.092	0.225	0.568	3.848

Appendix D

Raw data from Chapter Five

Appendix D.1 pH measurements from pH tests described in Chapter Five. Tank numbers refer to initial (Tank 1) and replacement (Tank 2) tanks. Replacement tanks were not used in the 85, 100 and 120 ml 1% nitric acid treatments since all larvae died prior to replacement.

		Treatment (ml 1% nitric acid)							
		0		47		50		55	
Day	Tank	1	2	1	2	1	2	1	2
-1						2.46		2.26	
0		7.98		6.53		3.58		3.11	
1		8.14		7.14					
2		8.22		7.36					
3									
4		8.11		7.46		7.09		4.98	
5		8.10		7.56					
6						6.50		5.99	
7						6.89		6.78	
8						6.83		6.87	
9									
10									
11		8.12		7.76		7.32		7.25	
12		8.05		7.74		7.45		7.41	
13		8.19		7.84		7.21	2.30	7.00	2.11
14		8.41	8.33	8.07	3.80	7.49	4.25	7.41	3.10
15			8.24		6.71				
16									
17									
18			8.22		4.40		7.30		6.26
19			8.29		5.26		7.44		6.47
20			8.30		6.48		7.71		6.33
21			8.26		6.88				
22			8.18		7.03				
23			8.22		7.33				
24			8.24		7.51				
25			8.21		7.57		7.83		7.14

continued....

Appendix D.1 continued...

		Treatment (ml 1% nitric acid)						
		60		70		85	100	120
Day	Tank	1	2	1	2	1	1	1
-1				2.09				
0		2.78		2.79		2.84	2.65	2.70
1						2.95	2.69	2.76
2						2.87	2.62	2.84
3		4.29						
4		4.61		4.49		3.69	3.61	3.94
5		5.26				3.70	3.67	4.15
6		5.67		4.96				
7				4.94				
8				5.03				
9								
10		6.70						
11		6.83		6.60		6.44	6.03	6.92
12				6.90		6.86	6.50	7.11
13				6.70	2.09	7.17	6.88	7.28
14		7.03		6.99	2.79			
15			2.17					
16								
17								
18			3.24		5.54			
19					6.47			
20			5.03		6.54			
21								
22								
23								
24								
25			5.99		6.90			

Appendix D.2 Raw data from pH tests described in Chapter Five. Census refers to counts of total larval numbers.

Treatments (ml 1% nitric acid) Counts	0	47	50	55	60	70	85	100	120
Total eggs	445	338	400	530	193	252	379	433	436
Non-viable eggs	30	29	25	1	69	6	27	50	6
Larvae dead in eggmass	0	0	0	0	0	0	352	383	430
Sampled before census I	10	8	29	40	7	15	–	–	–
Census I	402	290	280	441	84	240	–	–	–
Sampled between census I & II	35	30	32	33	4	9	–	–	–
Emerged adults	1	0	0	0	7	0	–	–	–
Census II	364	246	185	368	12	72	–	–	–

Appendix D.3 Individual dry weight of larvae sampled during the pH tests described in chapter five; (i) Day 14, (ii) Day 20 and (iii) Day 25. Values are given in mg.

(i) Day 14

	Treatment (ml 1% nitric acid added)					
No.	0	47	50	55	60	70
1	0.26	0.31	0.34	0.02	0.22	0.17
2	0.35	0.50	0.15	0.14	0.11	0.21
3	0.27	0.37	0.12	0.07	0.11	0.53
4	0.22	0.37	0.13	0.04	0.11	0.43
5	0.35	0.31	0.20	0.01	0.14	0.31
6	0.23	0.49	0.14	0.10	0.14	
7	0.27	0.21	0.19	0.14	0.14	
8	0.26	0.44	0.15	0.11	0.16	
9	0.34		0.18	0.06		
10	0.29		0.18	0.15		
11			0.02	0.05		
12			0.19	0.09		
13			0.21	0.12		
14			0.08	0.09		
15			0.16	0.13		
16			0.15	0.06		
17				0.12		
18				0.08		
19				0.12		
20				0.07		
21				0.11		
22				0.04		
23				0.10		
24				0.16		
25				0.17		
26				0.09		
27				0.16		
28				0.14		
29				0.10		
30				0.13		

Appendix D.3 continued...

(ii) Day 20

	Treatment (ml 1% nitric acid added)				
No.	0	.46	50	55	70
1	0.36	0.48	0.40	0.21	0.29
2	0.35	0.47	0.53	0.21	0.30
3	0.23	0.25	0.34	0.22	0.17
4	0.40	0.41	0.04	0.16	0.20
5	0.36	0.39	0.18	0.10	
6	0.34	0.40	0.39	0.36	
7	0.28	0.37	0.37	0.17	
8	0.35	0.35	0.03	0.35	
9	0.20	0.49	0.27	0.16	
10	0.30	0.48	0.16	0.54	
11	0.22	0.42	0.70	0.14	
12	0.41	0.33	0.42		
13	0.23		0.33		
14	0.28		0.29		
15	0.36				
16	0.34				
17	0.31				

(ii) Day 25

	Treatment (ml 1% nitric acid added)				
No.	0	47	50	55	70
1	0.37	0.26	0.20	0.21	0.29
2	0.26	0.28	0.10	0.32	0.28
3	0.31	0.40	0.28	0.27	0.43
4	0.32	0.23	0.21	0.19	0.24
5	0.31	0.19	0.28	0.27	0.45
6	0.35	0.24	0.28	0.33	
7	0.41	0.33	0.29	0.50	
8	0.45	0.29	0.47	0.19	
9	0.38	0.34	0.26	0.19	
10	0.57	0.35	0.21	0.20	
11	0.30	0.26	0.29	0.17	
12	0.41	0.24	0.29	0.26	
13	0.23	0.35	0.24	0.39	
14	0.36	0.28	0.32	0.30	
15	0.27	0.42	0.35	0.44	
16	0.33	0.15	0.27	0.28	
17	0.35	0.16	0.19	0.25	
18			0.25	0.26	
19				0.34	
20				0.27	
21				0.12	
22				0.18	

Appendix D.4 Instar stages of larvae sampled during the pH experiments described in chapter five; (i) Day 6, (ii) Day 14, (iii) Day 20, (iv) Day 25

(i) Day 6

Treatment (ml 1% nitric acid)	Instar stage	
	First	Second
0	No data	
47	No data	
50	1	11
55	2	7
60	–	2
70	4	2

(ii) Day 14

Treatment (ml 1% nitric acid)	Instar stage		
	Second	Third	Fourth
0	–	–	10
47	–	–	8
50	–	3	13
55	1	21	8
60	–	–	5
70	1	2	6

(iii) Day 20

Treatment (ml 1% nitric acid)	Instar stage	
	Third	Fourth
0	–	17
47	–	13
50	2	12
55	1	10
60	–	2
70	–	4

(iv) Day 25

Treatment (ml 1% nitric acid)	Instar stage
	Fourth
0	18
47	17
50	18
55	22
60	2
70	5

Appendix D.5 Dissolved iron levels from 25-day duration iron tests described in chapter five. Values are given in mM Fe. The symbol 'R' denotes samples taken from replacement tanks.

	Sample	Target concentration (mg Fe litre^{-1})					
Test	day	0	15	30	40	50	75
A	0	1	1	1	1	2	4
	14	2	3	2	1	0	0
	20R	0	0	0	0	0	0
	25R	0	1	1	1	1	12
B	0	2	3	2	6	2	7
	14	0	0	0	1	2	3
	20R	0	0	0	0	0	0
	25R	0	7	0	0	0	0
C	0	0	0	0	0	0	0
	14	0	0	0	0	3	3
	20R	2	1	1	3	0	2
	25R	0	0	0	0	0	0

Appendix D.6 Iron in particulate matter levels from 25-day duration iron tests described in chapter five. Values are given in mg Fe g^{-1} dry weight of particulate matter. Multiplication by the atomic weight of iron (56 g) was used to convert mM Fe readings from atomic absorption spectrophotometry to mg Fe $litre^{-1}$. This figure was then corrected for dry weight using the calculation described in section 3.12. The symbol 'R' denotes samples taken from replacement tanks.

	Sample	Target concentration (mg Fe $litre^{-1}$)					
Test	day	0	15	30	40	50	75
A	0	0.299	7.387	8.213	28.778	21.457	11.408
	6	0.025	3.224	4.565	9.219	13.455	24.864
	14	0.076	7.550	7.622	10.298	12.488	63.464
	20R	0.053	5.849	11.128	10.837	17.801	22.664
	25R	0.048	6.749	8.165	10.886	25.348	24.370
B	0	0.056	8.991	24.366	24.811	25.211	17.345
	6	0.000	7.406	15.021	26.954	25.974	29.042
	14	0.000	7.151	10.195	18.288	17.445	18.854
	20R	0.000	3.878	11.751	11.951	15.534	15.303
	25R	0.021	7.648	10.683	12.793	13.387	15.909
C	0	0.104	7.707	15.262	29.494	30.916	30.164
	6	0.064	10.288	15.343	21.438	29.067	29.675
	14	0.083	9.069	12.651	26.951	12.577	23.576
	20R	0.015	5.295	16.810	15.641	13.560	19.219
	25R	0.037	6.754	14.846	11.936	15.592	18.834

Appendix D.7 pH measurements from iron tests described in chapter five. Tank numbers refer to initial (Tank 1) and replacement (Tank 2) tanks. Replicate 1. pH conditions prevalent at addition of animals are underlined.

	Treatment (mg Fe litre^{-1})											
	0		15		30		40		50		75	
Day Tank	1	2	1	2	1	2	1	2	1	2	1	2
-14	8.26		7.90		7.16		7.09		4.04		2.75	
-13	8.06		7.76		7.43		7.36		4.77		2.87	
-12	8.33		8.04		7.72		7.54		5.12		2.69	
-11												
-10	8.36		8.04		7.80		7.57		5.91		3.87	
-9	8.34		8.04		7.80		7.58		6.12		4.01	
-8												
-7												
-6												
-5												
-4												
-3	8.33		8.08		7.90		7.66		6.91		5.10	
-2	8.34		8.07		7.88		7.65		6.95		5.26	
-1	8.38		8.09		7.94		7.73		7.03		5.40	
0	8.38		8.19		8.07		7.86		7.04		5.67	
1	8.04		8.14		7.92		7.87		7.00		6.26	
2												
3												
4	8.08		8.10		7.88		7.82		7.00		6.31	
5												
6	8.20		8.08		7.84		7.64		7.08		7.01	
7		8.21		6.76		4.71		3.08		2.71		2.37
8		8.37		8.10		7.55		7.05		3.93		2.69
9		8.60		8.34		7.98		7.49		4.51		2.86
10		8.62		8.39		8.07		7.59		4.84		2.93
11		8.69		8.43		8.13		7.61		5.09		2.89
12		8.56		8.31		8.04		7.59		5.54		3.26
13		8.53		8.27		7.96		7.58		5.64		3.25
14	8.44	8.45	8.35	8.23	8.17	7.97	7.97	7.58	7.38	6.04	7.29	3.67
15												
16												
17												
18												
19												
20		8.28		8.28		8.04		7.89		7.01		5.43
21												
22												
23												
24												
25		8.37		8.29		8.04		7.93		7.25		6.29

Appendix D.7 continued... Replicate 2. pH conditions prevalent at addition of animals are underlined.

		Treatment (mg Fe litre^{-1})											
		0		15		30		40		50		75	
Day	Tank	1	2	1	2	1	2	1	2	1	2	1	2
-14		8.36		7.66		3.31		2.95		2.83		2.65	
-13													
-12													
-11													
-10		8.64		8.16		6.20		3.73		4.14		3.11	
-9													
-8		8.64		8.19		7.43		3.96		4.43		3.30	
-7		8.56		8.14		7.44		4.10		4.68		3.39	
-6		8.40		8.01		7.36		4.52		5.23		3.48	
-5													
-4													
-3		8.51		8.13		7.56		4.91		6.19		4.13	
-2		8.67		8.27		7.65		5.08		6.73		4.05	
-1		8.39		8.05		7.48		5.03		6.54		4.05	
0		8.44	8.29	8.13	7.99	7.61	6.35	5.25	7.09	6.73	3.26	4.15	3.08
1													
2													
3													
4		7.78	8.42	7.61	8.09	7.21	7.54	6.87	7.25	7.13	6.56	4.23	3.86
5			8.19		7.98		7.91		7.51		7.00		4.08
6		8.02	8.48	7.70	8.24	7.42	7.81	7.00	7.56	7.19	7.05	4.09	4.15
7													
8													
9													
10													
11			8.44		8.18		7.49		7.52		7.15		4.88
12			8.41		8.21		8.04		7.65		7.20		4.96
13			8.43		8.16		8.13		7.71		7.24		4.99
14		8.44	8.57	7.93	8.35	7.67	8.19	7.27	7.79	7.39	7.38	4.53	5.15
15			8.26		7.78		7.90		7.64		7.17		6.31
16													
17													
18													
19													
20			8.45		8.01		8.16		7.96		7.49		7.20
21													
22													
23													
24													
25			7.91		7.34		7.60		7.39		6.91		7.00

Appendix D.7 continued... Replicate 3. pH conditions prevalent at addition of animals are underlined.

		Treatment (mg Fe litre^{-1})											
		0		15		30		40		50		75	
Day	Tank	1	2	1	2	1	2	1	2	1	2	1	2
-13		8.32		8.01		6.63		5.04		3.19		2.60	
-12													
-11													
-10		8.33		8.19		7.75		6.68		3.97		3.11	
-9													
-8		8.52		8.21		7.79		6.96		4.22		3.28	
-7		8.59		8.28		8.01		7.14		4.36		3.40	
-6		8.74		8.41		8.09		7.24		4.61		3.61	
-5													
-4													
-3		8.21		8.01		7.65		6.93		4.99		4.16	
-2		8.31		8.05		7.74		7.05		5.15		4.22	
-1		8.27		8.04		7.76		7.13		5.43		4.41	
0		7.96		7.75		7.29		6.79		5.39		4.67	
1													
2													
3													
4		7.93	8.49	7.78	8.23	7.42	7.88	7.36	7.59	6.78	6.29	5.85	3.15
5			8.21		8.01		7.79		7.52		6.70		3.18
6		8.07		7.94		7.54		7.40		6.85		6.02	
7			8.34		8.31		7.96		7.55		6.78		3.46
8													
9													
10													
11													
12			8.31		8.17		7.85		7.59		6.92		3.88
13													
14		8.38	8.45	8.23	8.31	7.82	8.07	7.53	7.83	7.14	7.30	6.42	4.74
15													
16													
17													
18													
19													
20		Measurements not taken due to failure of pH probe.											
21													
22													
23													
24													
25			8.7*		8.8*		8.7*		8.6*		8.5*		7.7*

* measurements taken using a Philips PW 9418 pH meter which was rather unreliable and therefore figures are approximate.

Appendix D.8 Raw data from 25-day iron tests described in Chapter Five. Census refers to counts of total larval numbers.

Experiment	A						B					
Treatments (mg Fe litre^{-1})												
Counts	0	15	30	40	50	75	0	15	30	40	50	75
Total eggs	313	266	324	328	375	273	343	302	347	269	255	293
Non-viable eggs	115	0	29	58	36	4	6	27	118	4	3	15
Larvae dead in eggmass	0	0	0	0	0	0	0	0	0	0	0	0
Sampled before census I	14	15	9	12	6	10	29	6	7	10	9	8
Census I	186	248	273	163	254	143	296	231	224	243	190	276
Sampled between census I & II	17	25	16	19	18	14	30	15	19	21	12	28
Emerged adults	63	5	0	9	0	0	44	38	19	9	5	0
Census II	92	215	250	113	235	97	217	152	152	207	140	242

Experiment	C					
Treatments (mg Fe litre^{-1})						
Counts	0	15	30	40	50	75
Total eggs	250	245	360	395	288	340
Non-viable eggs	28	0	1	2	5	28
Larvae dead in eggmass	0	0	0	0	0	0
Sampled before census I	21	8	7	19	26	9
Census I	159	104	262	320	259	303
Sampled between census I & II	18	9	23	18	20	32
Emerged adults	12	0	0	0	0	0
Census II	96	57	185	263	239	268

Appendix D.9 Individual dry weight of larvae sampled during the iron tests described in chapter five; (i) Day 14, (ii) Day 20 and (iii) Day 25. Values are given in mg.

(i) Day 14

	Target iron concentration (mg litre^{-1})					
No.	0	15	30	40	50	75
1	0.20	0.03	0.17	0.12	0.18	0.11
2	0.14	0.29	0.08	0.13	0.08	0.10
3	0.26	0.19	0.03	0.18	0.15	0.09
4	0.19	0.12	0.13	0.13	0.17	0.06
5	0.22	0.20	0.25	0.09	0.10	0.05
6	0.12	0.26	0.16	0.10	0.11	0.04
7	0.19	0.22	0.15	0.29	0.11	0.07
8	0.11	0.21	0.13	0.33	0.12	0.06
9	0.20	0.29	0.13	0.18	0.08	0.05
10	0.23	0.20	0.08	0.21	0.09	0.06
11	0.25	0.25	0.12	0.24	0.07	0.05
12	0.22	0.34	0.12	0.03	0.09	0.04
13	0.31	0.18	0.11	0.06	0.10	0.06
14	0.58	0.21	0.08	0.04	0.12	0.06
15	0.40	0.21	0.10	0.06	0.09	0.05
16	0.46	0.28	0.07	0.06	0.09	0.03
17	0.41	0.03	0.04	0.05	0.12	
18	0.60	0.03	0.05	0.04	0.10	
19	0.35	0.02	0.05	0.04	0.09	
20	0.27	0.03	0.08	0.03	0.06	
21	0.48	0.01	0.04	0.05	0.07	
22	0.29		0.06	0.05	0.07	
23	0.22		0.08	0.04	0.12	
24	0.43			0.06	0.07	
25	0.30			0.11	0.12	
26	0.34			0.04	0.09	
27	0.20				0.12	
28	0.33					
29	0.28					
30	0.34					
31	0.26					
32	0.31					

Appendix D.9 continued...

(i) Day 20

	Target iron concentration (mg litre^{-1})					
No.	0	15	30	40	50	75
1	0.58	0.65	0.23	0.18	0.32	0.03
2	1.05	0.58	0.26	0.63	0.29	0.02
3	0.99	0.59	0.33	0.43	0.26	0.03
4	0.80	0.43	0.19	0.61	0.37	0.22
5	0.50	0.53	0.25	0.36	0.35	0.07
6	0.26	0.52	0.24	0.58	0.35	0.10
7	0.29	0.55	0.25	0.63	0.18	0.48
8	0.15	0.54	0.31	0.37	0.31	0.19
9	0.80	0.45	0.41	0.61	1.01	0.13
10	0.24	0.92	0.50	0.12	0.34	0.17
11	0.53	0.88	0.17	0.16	0.52	0.32
12	0.61	0.66	0.28	0.69	0.45	0.14
13	0.60	0.77	0.81	0.67	0.35	0.12
14	0.47	0.65	0.26	0.50	0.55	0.13
15	0.34	0.56	0.56	0.68	0.36	0.13
16	0.58	0.02	0.48	0.44	0.24	0.23
17	0.55	0.16	0.88	0.16	0.24	0.09
18	0.50	0.10	0.06	0.18	0.42	0.12
19	0.49	0.30	0.21	0.53		0.15
20	0.40	0.14	0.19	0.24		0.13
21	0.68	0.18	0.15	0.14		0.13
22	0.43		0.18	0.15		0.16
23	0.26		0.15	0.16		0.10
24	0.51		0.18	0.29		0.12
25	0.76		0.15	0.14		0.12
26	0.16					0.11
27	0.90					0.09
28	0.16					0.19
29	0.97					0.13
30	0.30					0.17
31	0.71					
32	0.82					
33	0.84					
34	1.05					
35	0.51					
36	0.83					

Appendix D.9 continued...

(i) Day 25

	Target iron concentration (mg litre^{-1})					
No.	0	15	30	40	50	75
1	0.98	0.58	0.24	0.67	0.32	0.02
2	1.08	0.64	0.27	0.52	0.27	0.13
3	1.02	0.52	0.24	0.58	0.18	0.15
4	0.44	0.56	0.31	0.58	0.29	0.14
5	0.50	0.56	0.31	0.35	0.27	0.12
6	0.45	0.53	0.25	0.26	0.28	0.10
7	0.73	0.39	0.26	0.14	0.33	0.12
8	0.72	0.65	1.23	0.58	0.16	0.11
9	0.68	0.51	0.83	0.35	0.29	0.17
10	0.59	0.54	0.56	0.34	0.16	0.10
11	0.81	0.66	0.49	0.56	0.32	0.11
12	0.83	0.48	0.63	0.64	0.29	0.19
13	0.55	0.54	0.58	0.49	0.31	0.09
14	0.54	0.46	0.15	0.48	0.44	0.14
15	0.42	0.46	0.11	0.52	0.41	0.10
16	0.47	0.48	0.27	0.55	0.22	0.10
17	0.88	0.86	0.10	0.66	0.39	0.06
18	0.78	0.70	0.09	0.47	0.42	0.11
19	0.48	0.89	0.20	0.14	0.22	0.13
20	0.51	1.06	0.14	0.34	0.28	0.12
21	0.70	0.54	0.18	0.19	0.26	0.17
22	0.71	1.08	0.11	0.08	0.25	0.11
23	0.42	0.33	0.33	0.11	0.43	0.10
24	0.81	0.61	0.14	0.16	0.39	0.21
25	0.90	0.72	0.16	0.11	0.15	0.15
26	0.65	0.55	0.09	0.11	0.13	0.24
27		0.64	0.28	0.08	0.34	0.43
28			0.16	0.21	0.15	0.16
29			0.15	0.31	0.49	0.20
30			0.11	0.23	0.20	0.12
31				0.24	0.22	0.11
32				0.15		0.14
33						0.19
34						0.11
35						0.11
36						0.12
37						0.12
38						0.12
39						0.19
40						0.12
41						0.19
42						0.26
43						0.19

Appendix D.10 Instar stages of larvae sampled during the iron experiments described in chapter five; (i) Day 6, (ii) Day 14, (iii) Day 20 and (iv) Day 25.

(i) Day 6

Test	Target iron concentration (mg litre^{-1})	Instar stage	
		First	Second
A	0	–	1
	15	–	3
	30	–	1
	40	–	2
	50	–	–
	75	–	4
B	0	–	17
	15	–	1
	30	–	–
	40	–	4
	50	–	3
	75	2	2
C	0	–	9
	15	–	–
	30	–	–
	40	–	4
	50	–	10
	75	–	–

(ii) Day 14

Test	Target iron concentration (mg litre^{-1})	Instar stage		
		Second	Third	Fourth
A	0	–	4	9
	15	–		12
	30	–	2	6
	40	–	2	6
	50	–	3	3
	75	2	4	–
B	0	–	1	11
	15	–	1	4
	30	–	3	4
	40	–	4	2
	50	–	2	4
	75	–	4	–
C	0	–	12	–
	15	6	1	–
	30	–	6	1
	40	–	15	–
	50	–	13	3
	75	–	8	–

Appendix D.10 continued...

(iii) Day 20

Test	Target iron concentration (mg litre^{-1})	Instar stage			
		Second	Third	Fourth	Pupae
A	0	–	–	10	–
	15	–	–	9	–
	30	–	–	9	–
	40	–	3	9	–
	50	–	–	8	–
	75	–	5	1	–
B	0	–	–	14	–
	15	–	–	6	–
	30	–	–	8	1
	40	–	1	8	–
	50	–	–	7	–
	75	–	8	3	–
C	0	–	1	11	–
	15	1	4	1	–
	30	–	5	3	–
	40	–	3	2	–
	50	–	–	4	–
	75	–	13	1	–

(iv) Day 25

Test	Target iron concentration (mg litre^{-1})	Instar stage		
		Third	Fourth	Pupae
A	0	–	6	1
	15	–	16	–
	30	–	7	–
	40	–	7	–
	50	–	10	–
	75	2	6	–
B	0	–	15	1
	15	–	8	1
	30	2	6	2
	40	–	11	1
	50	–	5	–
	75	7	10	–
C	0	–	5	1
	15	–	3	–
	30	4	11	–
	40	–	13	–
	50	–	16	–
	75	2	16	–

Appendix D.11 Total iron content of chironomid larvae from 25-day iron tests described in chapter five. The procedure for larval digestion is described in section 3.11. Values are given in mg Fe g^{-1} dry weight of larvae. Multiplication by the atomic weight of iron (56 g) was used to convert mM Fe readings from atomic absorption spectrophotometry to mg Fe $litre^{-1}$. This figure was then corrected for dry weight using the calculation described in section 3.12.

	Sample	Target iron concentration (mg $litre^{-1}$)					
Test	Day	0	15	30	40	50	75
A	14	0.831	3.170	11.915	6.667	5.957	12.727
	20	0.495	2.887	7.935	7.761	9.218	23.830
	25	1.253	3.275	4.468	10.839	9.882	12.584
B	14			No data			
	20	0.806	1.892	3.553	5.477	6.957	10.516
	25	0.000	2.306	1.834	4.468	3.164	7.778
C	14	0.000	0.000	0.000	3.684	7.368	0.000
	20	0.000	0.000	0.000	0.000	2.222	4.884
	25	0.000	0.000	0.000	2.276	9.872	4.317

Appendix D.12 Adult emergence experiment, chapter five; (i) pH, (ii) dissolved/suspended iron (mg Fe litre^{-1}), (iii) sedimented particulate iron (mg Fe g^{-1} dry weight of sediment).

(i)

	Target concentration (mg Fe litre^{-1})											
Tank	0a	0b	0c	15a	15b	15c	30a	30b	30c	50a	50b	50c
Initial	8.73	8.68	8.64	8.46	8.34	8.36	8.19	8.19	8.08	7.64	7.48	7.05
1st replacement	8.62	8.66	8.60	8.20	8.29	8.34	7.00	7.30	7.14	7.12	7.19	6.78
2nd replacement	8.44	8.49	8.48	8.18	8.09	8.12	7.46	7.71	7.70	4.03	6.22	5.46

(ii)

	Target concentration (mg Fe litre^{-1})											
Tank	0a	0b	0c	15a	15b	15c	30a	30b	30c	50a	50b	50c
Initial	<0.056	<0.056	<0.056	<0.056	<0.056	<0.056	<0.056	<0.056	<0.056	<0.056	<0.056	<0.056
1st replacement	<0.056	<0.056	<0.056	<0.056	<0.056	<0.056	<0.056	<0.056	<0.056	0.560	0.280	0.056
2nd replacement	<0.056	<0.056	<0.056	<0.056	<0.056	<0.056	<0.056	<0.056	0.056	0.056	<0.056	0.112

(iii)

	Target concentration (mg Fe litre^{-1})											
Tank	0a	0b	0c	15a	15b	15c	30a	30b	30c	50a	50b	50c
Initial	<0.056	<0.056	<0.056	4.312	3.981	5.591	7.347	3.081	8.785	10.377	10.505	12.463
1st replacement	<0.056	<0.056	<0.056	4.591	2.566	5.890	8.796	6.066	7.614	7.119	9.191	6.983
2nd replacement	<0.056	<0.056	<0.056	2.717	4.424	3.441	4.222	3.421	7.554	11.621	8.191	11.668

Appendix D.13 Adult emergence experiment, chapter five; mortality and emergence of chironomids. The numbers of individuals remaining alive in each culture is given along with the cummulative numbers of emerged and dead individuals at each of four ages. These correspond to the start of the experiment (age 4 days), the first replacement (age 17 days), the second replacement (age 29 days) and the end of the test (age 50 days).

Treatment (mg Fe litre^{-1})		Age (days) 4	17	29	50
0a	no. remaining	50	39	9	0
	no. emerged	–	2	18	18
	no. died	–	9	14	32
0b	no. remaining	50	49	3	0
	no. emerged	–	0	44	45
	no. died	–	1	3	5
0c	no. remaining	50	49	11	1
	no. emerged	–	0	13	15
	no. died	–	1	27	34
15a	no. remaining	50	46	2	0
	no. emerged	–	0	9	9
	no. died	–	4	39	41
15b	no. remaining	50	42	2	0
	no. emerged	–	6	35	35
	no. died	–	2	13	15
15c	no. remaining	50	45	27	4
	no. emerged	–	0	1	5
	no. died	–	5	22	41

Treatment (mg Fe litre^{-1})		Age (days) 4	17	29	50
30a	no. remaining	50	40	13	0
	no. emerged	–	0	11	13
	no. died	–	10	26	37
30b	no. remaining	50	46	8	0
	no. emerged	–	1	9	12
	no. died	–	3	33	38
30c	no. remaining	50	45	28	3
	no. emerged	–	0	4	10
	no. died	–	5	18	37
50a	no. remaining	50	37	19	6
	no. emerged	–	0	0	3
	no. died	–	13	31	41
50b	no. remaining	50	44	22	7
	no. emerged	–	0	0	3
	no. died	–	6	28	40
50c	no. remaining	50	42	18	4
	no. emerged	–	0	0	6
	no. died	–	8	32	40

Appendix D.14 Adult emergence experiment, chapter five; numbers of male and female flies emerged each day. (m = male, f = female)

	Target iton concentration (mg litre^{-1})																							
Age	0 a		0 b		0 c		15 a		15 b		15 c		30 a		30 b		30 c		50 a		50b		50 c	
(days)	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f
17	2								6						1									
18	5		1		2		1		4															
19	4		7	1	6	1	2		1															
20	3		3	1	3	1	4		2	2			1											
21	1		3							1														
22	1		1	3			2		3	2														
23			2	2					2	3			1											
24		1	2	5					1	2					3									
25				4						2														
26			1	5						2	1		1		1									
27				2										2	3	1	2							
28	1			1						2			2	2			2							
29													1	1										
30														1										
31																								
32																								
33																								
34																								
35				1									1		2				1			1		
36																1	2						2	
37																								
38											1													
39						1								1				2					1	
40																		1						
41																								
42																								
43																								
44																								
45						1													2			1	2	1
46												2												
47																								
48																								
49																								
50											1							1				1		

Appendix E

Raw data fron Chapter Six

Appendix E.1 Survival and emergence of *C. riparius* over the 10-day exposure to target iron treaments (mg Fe litre-1) and natural reservoir sediment (NS), chapter six.

	Treatment					
	0	15	30	50	75	NS
Initial numbers	50	50	50	50	50	50
No. larvae recovered	25	32	37	42	42	39
No. pupae recovered	18	10	9	5	7	4
No. emerged adults	6	8	4	2	0	4
Total no. recovered	49	48	50	49	49	47

Appendix E.2 Larval dry weight data for larvae recovered from target iron treatments (mg Fe litre-1) and natural reservoir sediment (NS), chapter six. Values are given in mg.

Treatment					
0	15	30	50	75	NS
1.65	0.86	0.70	0.77	1.20	1.54
1.30	1.43	1.12	1.20	1.03	0.59
1.37	1.33	0.59	0.54	0.76	0.95
0.86	0.71	1.29	1.03	0.85	0.85
1.71	0.47	0.84	0.94	0.20	1.20
1.05	0.18	0.93	0.51	0.68	0.82
0.92	1.22	0.72	1.00	0.61	1.25
0.58	1.24	0.93	0.75	0.81	1.22
1.04	2.08	0.67	0.40	0.98	0.37
0.82	1.08	0.88	1.05	0.26	0.54
	1.71	1.07	0.92	1.00	1.63
	1.03	0.82	0.93	0.79	0.83
	0.90	0.59	0.47	0.58	0.82
	1.29	0.92	0.65	0.39	1.51
	1.45	0.67	0.24	0.89	1.73
	0.82	0.92	0.78	0.71	0.77
	0.82	0.77	0.71	0.81	1.07
	1.87	0.90	0.78	0.72	1.32
	1.00	1.02	0.55	1.32	1.81
		1.25	0.62	0.42	1.18
		0.76	0.56	0.66	1.26
		1.36	0.29	0.40	0.69
		0.69	0.74	0.74	1.37
		0.72	0.69	0.69	1.32
		0.97	0.52	0.49	1.06
		0.68	1.35	1.06	0.70
		0.93	0.60	0.81	0.92
			1.15	0.93	0.83
			1.87	0.84	1.08
			1.16	0.95	0.49
				0.64	1.12
					1.12
					1.11

Appendix E.3 Total iron concentrations of (i)larvae, (ii) pupae and (iii) adults recovered from target iron treatments (mg Fe litre-1) and natural reservoir sediment (NS). Values are given in mg Fe g^{-1} dry weight.

(i) Total iron content of larvae

Treatment					
0	15	30	50	75	NS
0.017	1.758	2.760	2.982	10.663	10.127
0.000	1.978	1.925	3.523	8.237	4.888
0.020	0.484	3.607	3.007	7.589	3.331
0.000	1.499	2.062	4.757	5.172	4.315
0.016	1.132	0.667	5.064	2.800	4.387
0.000	0.156	3.131	1.373	2.459	6.112
0.000	1.538	5.133	2.632	3.580	6.384
0.000	1.400	0.331	4.443	1.763	3.030
0.000	1.427	2.716	2.380	2.057	3.027
0.000	1.063	4.105	2.507	2.585	5.963
	0.884	1.846	3.652	1.456	4.415
	0.652	0.550	3.312	4.147	4.993
	0.964	2.322	2.145	2.020	3.107
	1.411	0.854	1.680	4.779	2.707
	0.599	1.400	2.683	5.241	3.803
	0.034	3.678	1.436	3.712	2.764
	0.649	0.487	2.090	2.603	2.591
	1.213	2.218	2.405	2.109	3.755
	0.952	2.333	1.578	1.517	2.166
		3.075	0.452	1.973	3.631
		2.330	2.700	0.867	2.822
		2.763	1.352	1.145	1.826
		1.935	1.324	0.350	3.250
		2.029	1.055	1.173	2.609
		3.189	0.808	3.449	1.215
		0.924	0.850	1.143	2.600
		3.624	0.700	1.400	2.252
		1.626	2.897	1.002	2.260
			1.033	0.723	3.215
			2.172	6.800	4.457
				1.238	2.900
				0.788	4.050
					1.514

Appendix E.3 continued...

(ii) Total iron content of pupae

Treatment					
0	15	30	50	75	NS
0.000	0.385	0.233	0.000	0.456	0.227
0.000	0.000	0.056	0.142	1.491	0.000
0.000	0.376	0.062	0.251	0.516	0.084
0.000	0.076	0.191	0.678	1.015	0.334
0.000	0.089	0.546	0.600	0.686	
1.090	3.118	0.400		0.195	
0.144	0.754	0.321		0.132	
0.311	0.680	1.115			
0.039	0.902	0.700			
0.471	0.596				
0.824					
0.486					
0.027					
0.673					
0.753					
0.400					
0.031					
0.284					

(iii) Total iron content of adults

Treatment					
0	15	30	50	75	NS
0.243	0.000	0.000	0.000	–	0.000
0.000	0.000	0.000	0.000		
0.000	0.000	0.000			
0.067	0.158				
0.035	0.192				
0.000	0.034				