FIELD AND LABORATORY STUDIES OF EXPOSURES OF BROWN TROUT TO ACID WATERS

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ABSTRACT. Some recent work on the effects of acid waters on brown trout are presented. Laboratory bioassay experiments have demonstrated that yearling trout are relatively insensitive to pH >4.3. Aluminium is demonstrated to be extremely toxic with suppression of growth occurring at concentrations above 20 μg L^{-1} at pH 4.4 to 5.2. Aluminium toxicity is reduced at high pH (5.9 and 6.3). Field studies carried out on 61 acidic and circumneutral streams in upland areas of England and Wales showed a strong relationship between water quality and standing crop of 1+ brown trout. Measured pH levels *per se* were too high to be directly toxic. On the other hand, heavy metal and Al concentrations could account for low or zero brown trout biomass in the more acidic streams. A mobile bioassay laboratory has been developed to allow controlled bioassay experiments to be carried out in the field. Natural and synthesised waters can be tested concurrently in multi-factorial experiments with *in situ* determinations of pH, Ca, Al (total and monomeric) and other water quality characteristics.

1. INTRODUCTION

There is much evidence which indicates that the survival and growth of fish at low pH are dependent upon other water quality factors, principally Ca and Al (Muniz and Leivestad, 1980; Schofield, 1980; Schofield and Trojnar, 1980; Baker and Schofield, 1982; Driscoll *et al.*, 1980; Brown, 1983; Fivelstad and Leivestad, 1984).
In order to understand the complex interactions between different aspects of water quality it is necessary to conduct carefully controlled bioassay experiments in which the exact composition of the water is known and it is possible to test independently variations in each constituent. There are, however, limitations

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to this bioassay approach when trying to predict responses in natural waters. It needs to be demonstrated that all of the toxic substances in the water or any detoxifying complexing agents have been considered. Also the effect of variable conditions in natural waters needs to be investigated. There is therefore a requirement for a number of approaches ranging from pure bioassay studies through experiments designed to simulate natural conditions, to field studies. This paper reports some of the findings from recent work carried out at the Central Electricity Research Laboratories which have adopted these different approaches.

2. MATERIALS AND METHODS

2.1. Field Studies

A survey of the water chemistry, and fish populations of 61 upland streams in Central and North Wales and the Peak District of England was conducted in 1982-3. These three areas are all characterized by hard, unreactive geologies (principally shales, mudstones or millstone grit) which give rise to oligotrophic waters, but differ in acid deposition rates (25 to 30 and $>$50 kg S ha^{-1} yr^{-1} for Welsh and Peak District sites, respectively; Martin, 1980).
Quantitative sampling was carried out by repeated electrofishing of 50 to 200 m stream reaches, and populations estimated by the 2-catch method of Seber and Le Cren (1967) or Zippin's (1958) multiple capture method. Water quality was sampled on five occasions over the 12-mo period preceding fish sampling. Variables measured were pH and conductivity (in the field), Ca, Mg,Al, Cu, Fe, Pb, Zn, S (by inductively coupled plasma emission spectrometry), NO_3 (UV spectrometry following removal of organic C by filtration through activated C), Cl (chloride ion selective electrode by titration with Ag NO_3) and K by flame emission spectrometry. Additional water sampling was carried out on two occasions in 1985 to measure the concentrations of labile monomeric Al, the fraction considered to be most toxic to fish (Driscoll et al., 1980). This was done using the catechol violet method and cation ion exchange resin by the method of Seip, et al. (1984).

2.2. Laboratory Bioassay

Yearling brown trout (Salmo trutta) from a hatchery in Derbyshire were exposed to various conditions in a flow-through system in which synthetic salt solutions can be maintained at constant pH levels. This experimental system consists of 12 tanks (capacity 100 L) supplied with water which has been recirculated through sand and activated C filters and ion exchange resins to which is added concentrated salt solutions of known composition. All experiments have been conducted at 12^{+} 1.5°K, with a nominal water composition of 1 mg L^{-1} Na,1 mg L^{-1} Ca, 0.5 mg L^{-1} Mg, and 0.3 mg L^{-1}K, all salts added as chlorides. pH is controlled continuously by titration with H_2SO_4 or KOH as appropriate (pH of unregulated tank water is approximately 5.5). The fish were acclimatised to the system for 7 days and then marked individually by subcutaneous injection of latex dye. Growth rates were then measured over 6 weeks when fed on commercial trout pellets at a rate of 2% of biomass per day.

2.3. Field Experiments

To help to overcome the problem of distinguishing toxicological from ecological and other effects, a mobile bioassay laboratory was developed. This enables controlled laboratory toxicity studies to be carried out in the field, on the banks of rivers and lakes. This mobile laboratory is provided with a temperature-controlled section containing the experimental aquaria and dosing pumps and a section containing computerized control and analytical equipment including a continuous-flow autoanalyser for in situ determination of Al and Ca levels. The aquaria are arranged in four columns, each capable of holding a variety of tank combinations, from 8 x 10 L capacity to a maximum of 48 x 0.1 L tanks. The water supplied to each of the four columns can be treated in a variety of ways including carbon filtration, ion exchange and pH control. Computer-controlled dosing pumps supply individual tanks with any required ions. With this system, synthesized waters comparable to those used in conventional laboratory bioassays can be tested alongside natural waters.

This apparatus has been used in an experiment carried out at Loch Fleet, an acid lake in Galloway, SW Scotland. The lake has been devoid of brown trout for the past three decades and a study is now in progress to assess various treatment options that would restore trout fisheries to this and similarly affected waters. The main water quality problems preventing a trout fishery appeared to be low pH (4.0 to 4.5) and associated elevated Al levels (approximately 200 μg L^{-1} total) and low Ca levels (0.7 mg L^{-1}).
Brown trout swim-up fry of two different softwater strains were exposed for 9 days to various experimental treatments, including (a) raw lake water (pH 4.4, 210 μg total Al L^{-1}, 0.7 mg Ca L^{-1}), (b) the same lake water adjusted to the seasonal minimum pH of 4.0 by H_2SO_4 addition, (c) lake water adjusted to pH 5.4 by NaOH addition and (d) control: lake water, deionized and C filtered, Ca and Na added (pH 5.2, 0.7 mg Ca L^{-1}, 1 mg Na L^{-1}, 13 μg Al L^{-1}). The experiment was conducted at a temperature of 10 to 12°K.

3. RESULTS

3.1. Field Studies

A wide range of water chemistry conditions were found during the survey with pH 4.45 to 7.30, 1 to 27 mg L^{-1} Ca, 0.5 to 7 mg L^{-1} Mg, 3 to 12 mg L^{-1} Na, 0.3 to 5 mg L^{-1} K, 0 to 0.02 mg L^{-1} Cu, 0 to 1 mg L^{-1} Fe, 0 to 0.1 mg L^{-1}Zn, 2.5 to 44 mg L^{-1} SO_4, 6 to 22 mg L^{-1} Cl and 0.3 to 8 mg $L^{-1}NO_3$ (based on averages of 5 samples).
The fish populations were dominated by brown trout which was found at all of the fish containing sites. The catches tended to be mostly of the 1+ age group as smaller fish were not sampled very efficiently by the method used. The results are therefore presented for this age group to prevent any bias due to the occasional capture of large older fish on the biomass values.
pH, labile monomeric Al and heavy metal concentrations were the most highly correlated factors with the biomass of the 1+ age group of trout (Figure 1). The toxicity of heavy metals (Cu, Zn, Pb) was found to be well represented by the index MeTox calculated using the method of Brown (1968) based on the toxicity of these metals to rainbow trout (Salmo gairdneri Richardson).

3.2. Laboratory Bioassays

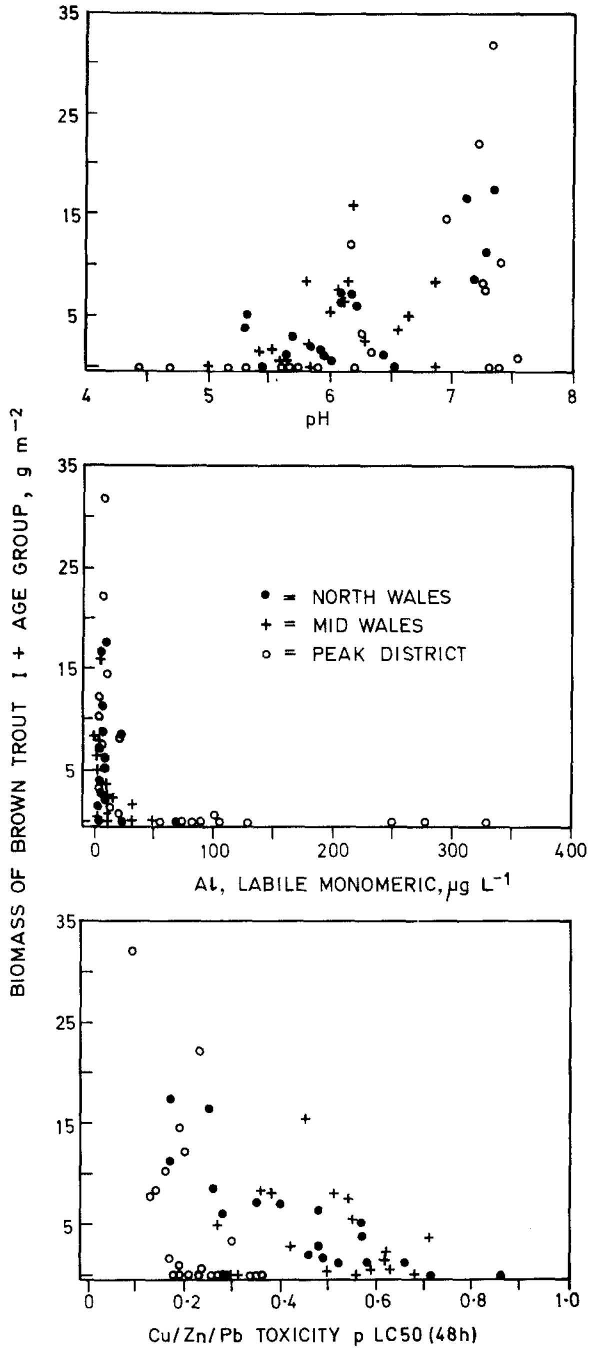

FIG. 1 BROWN TROUT BIOMASS IN RELATION TO WATER QUALITY

The results of an experiment in which brown trout were exposed to pH 4.4, 4.8 or 5.2, at varying additions of aqueous Al (added as Al chloride) are shown in Figure 2. In the absence of Al the fish all showed good growth and survival independent of pH but a threshold concentration of approximately 20 µg L^{-1} Al was found above which growth rates are suppressed. The importance of pH to Al toxicity has been demonstrated by exposing fish to 50 µg L^{-1} of Al at pH 4.3 to 6.2 (Figure 3). Growth rates of the fish at pH 4.3 in the absence of Al were clearly reduced and mortalities were high indicating that for the tested strain of brown trout there is a relatively sharp limit to their long term survival and growth at pH 4.3 to 4.4. The greatest toxicity attributable to Al *per se* occurred at pH 5.1 with relatively little suppression of growth at pH 5.9 and 6.3. This clearly indicates the differing toxicity of the various Al hydroxide complexes which predominate at different pH's. Preliminary analysis using thermodynamic equilibrium calculations to estimate the Al species present in the test conditions (Driscoll *et al.*, 1984) would suggest that the $Al(OH)^{++}$ species is the most toxic form, as was also suggested by Fivelsted and Leivestad (1984), but this needs to be confirmed in further experiments.

3.3. Field Experiments

Figure 4 shows the results of the trout survival experiment conducted in Loch Fleet water (raw or adjusted to pH 4.0 or 5.4) and in optimal control conditions. The results for both stocks of fish indicate that the toxicity of the waters was decreased, by increasing pH from 4.4 to 5.4, to that found in control water lacking Al. Reducing the pH to 4.0 substantially increased toxicity, particularly to the Loch Dee stock.

4. DISCUSSION

The difficulties of interpreting field study results are illustrated by Figure 1 in which relationships with several environmental variables may be found, but there remains a large amount of variability which cannot be accounted for. Also, the environmental variables themselves may be intercorrelated.
The pH values exhibit an association with biomass, and 80% of streams (n = 10) with mean pH <5.5 were fishless compared with 19% of 51 streams with pH > 5.5. Population density and condition factor were also lower in the more acidic streams.
It is generally accepted that pH values above 5.0 *per se* are unlikely to be harmful to fish, and the bioassay studies indicate that good growth rates can be maintained down to pH 4.4 (Figure 2). Only two streams in the study had mean pH values below 5.0 but it is clear that streams of the type studied are susceptible to short-term (<24 hr) episodes of low pH which can only be adequately represen-ted by near-continuous chemical monitoring. Therefore, the relationship observed in Figure 1 may represent an underlying relationship with episodic pH minima.
However, Al and heavy metal concentrations associated with the more acid streams would account for much of the observed variation in biomass. Field studies of toxicity of heavy metals derived from natural outcrops, mine-tailings and also ammunition dumps on the River Mawddach system in West Wales, (Howells *et al.*, 1983) showed that brown trout biomass was negatively correlated with

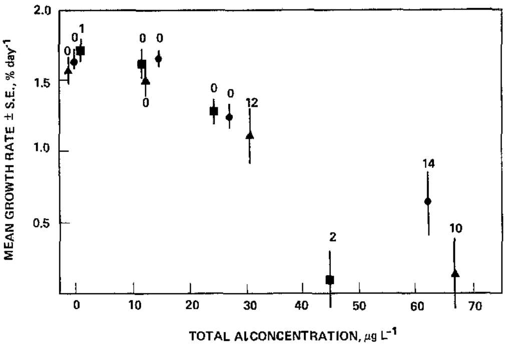

FIG. 2 MEAN GROWTH RATES OVER 6 WEEKS OF BROWN TROUT AT DIFFERENT AL CONCENTRATIONS. ▲ = pH 4.4 ● = pH 4.8 ■ = pH 5.2. NUMBERS REFER TO THE NUMBER OF MORTALITIES (ex 20) OBSERVED IN EACH TANK

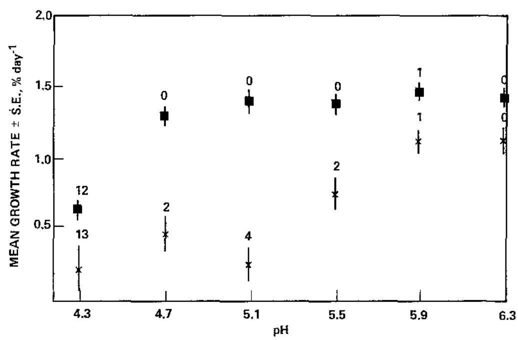

FIG. 3 MEAN GROWTH RATES OVER 6 WEEKS OF BROWN TROUT AT DIFFERENT pH LEVELS. ■ = 0 AL × = NOMINAL 50 µg l-1 AL. NUMBERS REFER TO THE NUMBER OF MORTALITIES (ex 20) OBSERVED IN EACH TANK

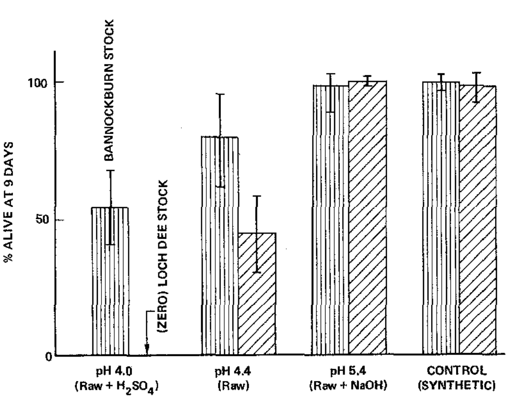

FIG. 4 LOCH FLEET BIOASSAY TEST: SURVIVAL OF TWO SOFT WATER STRAINS OF BROWN TROUT IN RAW AND pH–REGULATED LOCH WATER. CALCIUM LEVEL WAS 0.7 mg L^{-1}, TOTAL AL 210 µg L^{-1}. (BARS REPRESENT 95% CONFIDENCE INTERVALS)

MeTox, zero biomass occurring at around 0.56 of the 48h LC_{50} to rainbow trout. On inspection of Figure 1, it appears that trout biomass was probably controlled primarily by heavy metal toxicity in the Welsh streams, and by Al toxicity in the Peak District streams. Moreover, there is a threshold of approximately 40 $\mu g\ L^{-1}$ labile monomeric Al above which streams were fishless which corresponds well with the threshold found in the bioassay experiments of reduced growth above 20 $\mu g\ L^{-1}$ (Figure 2).

Experimental field studies in which natural conditions can be manipulated help to eliminate the ambiguities which arise in the interpretation of population surveys. Laboratory studies on the toxicity of Al to trout have shown that Al is most toxic at pH values in the range 5.0 to 5.5 (Figure 3; Schofield and Trojnar, 1980; Baker and Schofield,1982; Brown, 1983), hence suggesting that lime applications to Loch Fleet, by raising pH, might increase Al toxicity rather than having the desired ameliorative effect. In fact the toxicity was much reduced at pH 5.4 which suggests that most or all of the Al was not present in toxic form and that pH *per se* is the most important factor in this lake. In a single sample of the Loch Fleet water from the experiment, only about half of the Al (100 $\mu g\ L^{-1}$) at pH 4.5 was in labile monomeric form as estimated by fractionation through a cation exchange column (Driscoll *et al.*, 1984). This alone would not account for the low toxicity attributable to Al and it could be that Al species of low toxicity were present within the labile monomeric fraction due, for example, to fluoride complexation (Driscoll *et al.*,1984; La Zerte, 1984). In future work the role of Al will be considered further by a combination of Al speciation studies and *post mortem* histochemical studies of exposed fish to establish whether or not the fish absorb Al and whether it is in a potentially toxic form.

5. REFERENCES

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