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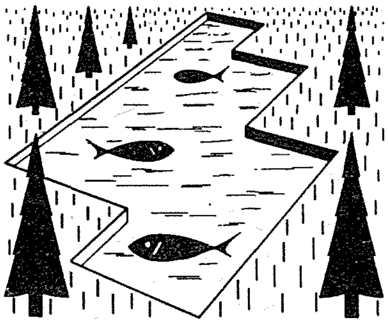

WATER QUALITY CRITERIA FOR EUROPEAN FRESHWATER FISH

Report on zinc and freshwater fish

EUROPEAN INLAND FISHERIES ADVISORY COMMISSION
FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
ROME, 1973

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WATER QUALITY CRITERIA FOR EUROPEAN FRESHWATER FISH

Report on Zinc and Freshwater Fish

prepared by

European Inland Fisheries Advisory Commission
Working Party on Water Quality Criteria
for European Freshwater Fish

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 1973

PREPARATION OF THIS DOCUMENT

The background of this paper is described in the Foreword to the report itself. The paper was prepared by the European Inland Fisheries Advisory Commission (EIFAC) Working Party on Water Quality Criteria for European Freshwater Fish.
The report is being issued in this series where the first eight documents of the Working Party were published: "Report on finely divided solids and inland fisheries", EIFAC Tech. Pap., (1):21 p., 1964; "Report on extreme pH values and inland fisheries", EIFAC.Tech.Pap., (4):24 p., 1968; "Report on water temperature and inland fisheries based mainly on Slavonic literature", EIFAC Tech.Pap., (6):32 p., 1968; "List of literature on the effect of water temperature on fish", EIFAC Tech.Pap., (8):8 p., 1969; "Report on ammonia and inland fisheries", EIFAC Tech.Pap., (11):12 p., 1970; "Report on monohydric phenols and inland fisheries", EIFAC Tech.Pap., (15):18 p., 1972; "Report on dissolved oxygen and inland fisheries", EIFAC Tech.Pap., (19):10 p., 1973; "Report on chlorine and freshwater fish", (20):11 p., 1973.

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FOREWORD

This is the ninth technical paper on water quality criteria for European freshwater fish prepared for the European Inland Fisheries Advisory Commission (EIFAC) - an intergovernmental organization with a membership of 23 countries. The Commission has been active in its efforts to establish water quality criteria for European freshwater fish since its Second Session, Paris, 1962, when it took note of a recommendation of the United Nations Conference on Water Pollution Problems in Europe, 1961, that EIFAC take the initiative in drawing up water quality requirements with respect to fisheries1/.

As was stated in its first eight reports on water quality criteria2/, the Commission "agreed that the proper management of a river system demands that water of suitable quality be provided for each use that is made or intended to be made of it and that the attainment and maintenance of such quality is normally to be sought through the control of pollution. It was necessary, therefore, to know the standards of quality required for each particular use in order to determine the degree of pollution control necessary and to forecast the probable effect of augmented or new discharges of effluents. It was pointed out that water quality standards for drinking water had been well defined by the World Health Organization (WHO) and that standards for certain agricultural and industrial uses are also well defined. However, water quality criteria for fish have not received the attention that they deserve. All too often, water has been considered quite adequate for fish as long as there has been no obvious mortality which can be ascribed to known pollutants. Degradation of the aquatic habitat through pollution and decrease in the annual production and subsequent harvest of fish have often passed unnoted.

With such reasoning in mind, it was agreed that the establishment of water quality criteria for European freshwater fish be undertaken by the Commission. This was to be accomplished by a critical examination of the literature, and very possibly experimentation to clear up contradictions and fill in gaps of knowledge, followed by recommendations as to desirable requirements for various aquatic organisms or groups of aquatic organisms with respect to the various qualities of water. The final criteria were to be published and given wide dissemination."

To accomplish this task, the Second Session of the Commission appointed a Working Party of experts selected on the basis of their knowledge of physical, chemical and biological requirements of European freshwater fish in relation to the topics to be studied.

This Working Party prepared its first report on finely divided solids and inland fisheries (see footnote2/) which was submitted to the Commission at its Third Session, Scharfling am Mondsee, 1964, where it was unanimously approved3/.

1/ See, respectively: EIFAC Report, Second Session, 1962, p. 21-2
UN (1961) Conference on Water Pollution Problems in Europe, held in Geneva from 22 February to 3 March 1961
Documents submitted to the Conference. Vols. I-III, United Nations, Geneva, 600 p.

2/ Report on Finely Divided Solids and Inland Fisheries, EIFAC tech.Pap., (1):21 p., 1964
Report on Extreme pH Values and Inland Fisheries, EIFAC tech.Pap., (4):18 p., 1968
Report on Water Temperature and Inland Fisheries based mainly on Slavonic Literature, EIFAC tech.Pap., (6):32 p., 1968
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Report on Monohydric Phenols and Inland Fisheries, EIFAC tech.Pap., (15):18 p., 1972
Report on Dissolved Oxygen and Inland Fisheries, EIFAC Tech.Pap., (19):10 p., 1973
Report on Chlorine and Freshwater Fish, EIFAC Tech.Pap., (20):11 p., 1973

3/ EIFAC Report, Third Session, 1964, p. 11

The Third Session then suggested that the following studies be considered by the Working Party:

- water temperature (including a review of the effect of heated discharges);
- dissolved oxygen and carbon dioxide; pH; toxic substances including heavy metals, phenols and pesticides and herbicides.

Elevated temperature was given first priority, and a draft on this subject was prepared by the Working Party during the following inter-sessional period. (At the Third Session the work of the Commission was re-organized into three Sub-Commissions, one of which, Sub-Commission III - Fish and Polluted Water - regrouped all the activities of EIFAC in the field of water pollution. The Working Party on Water Quality Criteria for European Freshwater Fish has since functioned under this Sub-Commission.)

The Fourth Session of the Commission, Belgrade, 1966, after having studied this first draft of review of literature on the effects of water temperature on aquatic life concluded that such a review required more effort than the resources of the Commission permitted at the time. Meanwhile, it suggested that a water quality report for extreme pH values be prepared for the next Session of EIFAC, and that a report on dissolved oxygen be prepared when funds become available for a full-time consultant4/.

The report on extreme pH values and inland fisheries (see footnote 2/) was published in 1968, in time for presentation at the Fifth Session of EIFAC, Rome, 1968, where it was unanimously approved5/.

At its Fifth Session the Commission again reviewed priorities for future studies and decided to undertake critical reviews on the effects of ammonia and phenols on freshwater fishes.

It also recommended that guidance as to its future work in the field of water pollution control, including the development of water quality criteria, be taken from the FAO/EIFAC Symposium on the Nature and Extent of Water Pollution Problems affecting Inland Fisheries in Europe which was later held in Jablonna, Poland, 15-16 May 1970, just before the Sixth Session of EIFAC.

The Fifth Session also approved in draft a report on water temperature and inland fisheries based mainly on Slavonic literature. The report was published in November 1968 as the third in the EIFAC water quality criteria series, and was followed in 1969 by the fourth publication in the series, a list of literature on the effect of water temperature on fish. (See footnote 2/ for both papers.)

Following the Jablonna Symposium6/, the Sixth Session of EIFAC, Krakow, 1970, again reviewed the Commission's programme with respect to water quality criteria7/. Noting that a report on ammonia was almost complete, it approved continuance of work on phenols, and the current work begun by the Working Party on copper, zinc and mercury, and recommended the addition of cyanides, detergents, chlorine and hydrocarbons as items for future reviews. It also recommended eventual resumption of work on water temperature and the preparation of a review based on a critical worldwide report on dissolved oxygen prepared for FAO8/.

4/ EIFAC Report, Fourth Session, 1966, p. 12

5/ EIFAC Report, Fifth Session, 1968, pp. 14-5

6/ Holden, A.V. and R. Lloyd (1972), Symposium on the Nature and Extent of Water Pollution Problems affecting Inland Fisheries in Europe. Synthesis of National Reports, EIFAC tech.Pap., (16):20 p.

7/ EIFAC Report, Sixth Session, 1970, p. 13

8/ Doudoroff, Peter and Dean L. Shumway (1970), Dissolved Oxygen Requirements of Freshwater Fishes. FAO Fish.tech.Pap., (86):291 p.

After the Sixth Session of EIFAC, the EIFAC Working Party has published reports on ammonia and monohydric phenols as the fifth and sixth reviews in this EIFAC series of water quality papers 2/ which were presented to the Seventh Session of EIFAC (Amsterdam, 1972 9/) where they were unanimously approved.

Since the Seventh Session, the EIFAC Working Party on Water Quality Criteria has drafted reviews on dissolved oxygen, chlorine and zinc which were studied at its 11th and 12th meetings held in Rome (15-17 January 1973) and Karlsruhe (25 May 1973), respectively. The reports on dissolved oxygen and chlorine have been published as the seventh and eighth reviews of this series 2/ and will be presented to the Eighth Session of EIFAC, which is scheduled to be held in U.K. (Aviemore, Scotland, 6-10 May 1974). The Working Party is continuing an active literature search on mercury and copper.

The ninth review, which follows, is the one on zinc and freshwater fish. For the preparation of this report, the following experts were appointed to the EIFAC Working Party on Water Quality Criteria:

Mr. J.S. Alabaster	(United Kingdom), Convener
Dr. D. Calamari	(Italy)
Mr. M. Grande	(Norway)
Dr. T.B. Hasselrot	(Sweden)
Mr. R. Lloyd	(United Kingdom)
Dr. A.W. Lysak	(Poland)
Dr. W.K. Besch	(Germany, Fed. Rep.)

FAO Secretariat:

Mr. J.-L. Gaudet - Acting Secretary to EIFAC

The preparation of the present review on zinc and freshwater fish was accomplished largely by Dr. M. Grande (Norway) who prepared the basic manuscript to be reviewed by the members of the Working Party.

The Working Party used the same general basis for their work on which they had agreed for the preparation of their first report that:

"Water quality criteria for freshwater fish should ideally permit all stages in the life cycles to be successfully completed and, in addition, should not produce conditions in a river water which would either taint the flesh of the fish or cause them to avoid a stretch of river where they would otherwise be present, or give rise to accumulation of deleterious substances in fish to such a degree that they are potentially harmful when consumed. Indirect factors like those affecting fish-food organisms must also be considered should they prove to be important."

This report will be presented to the Eighth Session of EIFAC (Aviemore, Scotland, United Kingdom).

The Seventh Session of EIFAC (see footnote 9/) suggested as possible future subjects for reviews cadmium and lead.

9/ EIFAC Report, Seventh Session, 1973, p. 18

SUMMARY

There are extensive data on the toxicity of zinc to fish under laboratory conditions, supported to some extent by field data on fish kills, but there are virtually no field observations to indicate the concentrations of zinc that are not inimical to fish populations or fisheries, mainly because the analytical data are too meagre, information on water hardness - perhaps the most important factor affecting the toxicity of zinc to aquatic animals - is missing and details of the status of the fish population are not given. For these reasons only tentative criteria can be suggested which may have to be revised when more adequate field data become available.

The toxicity of solutions containing zinc is mainly attributable to the zinc ion and perhaps also to particulate zinc present as the basic carbonate or the hydroxide held in suspension. It is modified by water quality, being reduced in particular by an increase in hardness and also temperature, salinity and suspended solids and increased by a decrease in dissolved oxygen. The effect of pH, however, is uncertain.

The acute toxicity of zinc in the presence of other heavy metals and other common pollutants seems to be largely simply additive but there is no evidence that the chronic toxicity of different poisons in a mixture is also additive. The effect of zinc is modified, and can be reduced, by acclimation and by the age of the fish.

A low but significant mortality has been found among rainbow trout exposed continuously for 4 months to constant concentrations of 0.2 of the 5-d LC50 and among rudd exposed for $8\frac{1}{2}$ months to 0.3 of the 7-d LC50.

Laboratory studies of avoidance reactions have shown that Atlantic salmon and rainbow trout may avoid concentrations of zinc in soft water which are 0.14-0.01 of the 7-d LC50. Avoidance reactions have also been observed at 0.35-0.43 of the 7-d LC50 by migrating Atlantic salmon in a river polluted with copper and zinc. Carp and goldfish show avoidance of 0.3-0.45 of lethal concentrations under laboratory conditions.

Field observations show that brown trout populations were present when the concentration of zinc was less than 0.06 of the 2-d LC50 to rainbow trout or when the annual 50 and 95 percentiles were up to 0.05 and 0.19 of the LC50 respectively; coarse fish were present when the corresponding percentiles were 0.02 and 0.11 of the 5-d LC50 to roach.

Because concentrations of zinc in freshwaters fluctuate, being distributed approximately lognormally over a period of a year, tentative criteria are expressed as annual 50- and 95-percentile values.

Pending the availability of more information it is tentatively recommended that for the maintenance of thriving populations of fish the annual 95 percentile of concentration of zinc should be no greater than 0.1 of the appropriate 7-d LC50 at 15°C; thus the criteria in terms of concentration of zinc would depend upon water hardness and type of fish as shown in Table I.

The concentration of 0.03 mg Zn/l for salmonids in very soft water may be too severe if brown trout only are present, since this species appears to survive successfully at higher concentrations; in such cases a 95-percentile concentration of 0.2 of the 7-d LC50 (0.06 mg Zn/l) may be more appropriate. For the minnow it might be more appropriate to set a more stringent standard but further data would be desirable to support and explain the existing laboratory findings.

The values of the corresponding annual 50 percentiles would be approximately 0.25 of the proposed 95 percentiles unless the distribution was much wider in range than has hitherto been found or was not lognormal, resulting in a larger ratio between the 95 percentile and

higher percentiles, in which cases more stringent standards would be appropriate. Where other poisons are present and dissolved-oxygen concentrations are below the air-saturation value, allowance should also be made for their contribution to the toxicity.

Table I. Maximum annual 95-percentile concentrations of zinc (mg Zn/l)

Water hardness (mg/l $CaCO_3$)	Coarse fish except minnow	Salmonids
10	0.3	0.03
50	0.7	0.2
100	1.0	0.3
500	2.0	0.5

1. INTRODUCTION

(1) Zinc is an essential trace element in living organisms, being involved in nucleic acid synthesis and occurring in many enzymes. It occurs widely in nature as the sulphide, carbonate and hydrated silicate ores, frequently accompanied by other metals, mainly iron and cadmium. It is used for galvanizing, in brass and other alloys, and some of its compounds, including the oxide, chloride, chromate, and sulphide, are widely used in other industries. Consequently, it can be an important pollutant from both mining and other industrial processes.

(2) Background values of zinc in natural inland surface waters may vary from 0.001 to 0.2 mg Zn/l or even higher, e.g. O'Connor (1968). Wastes containing zinc are often acidic and may also have a high content of copper, iron, cadmium, and other heavy metals. Zinc may occur in water as the free cation, as soluble zinc complexes, or be adsorbed on suspended matter.

(3) Zinc wastes can have a direct toxicity to aquatic life, and fisheries can be affected by either zinc alone, or more often together with copper and other metals.

(4) Effects of zinc on fish have been critically reviewed, for example by Doudoroff and Katz (1953), Skidmore (1964), and Kemp et al. (1971). Rarely will references earlier than 1953 be cited in this review.

2. DIRECT LETHAL ACTION ON FISH

2.1 Symptoms of poisoning and mode of action

(5) Early workers reported that zinc salts precipitated the mucus on the gills of fish, causing them to die from suffocation, although calcium salts inhibited precipitation. However, Lloyd (1960) and Mount (1966) observed little or no precipitation of mucus of rainbow trout (*Salmo gairdneri*) at low, lethal concentrations and histological examinations showed that the epithelial cells of the gill secondary lamellae became swollen, separated from the pillar cells and finally sloughed off (Parry, in Lloyd, 1960). Skidmore (1970) and Skidmore and Tovell (1972) concluded that under such conditions the fish died from asphyxia, rather than osmotic stress, an hypothesis supported by the increased concentrations of lactic acid and reduced concentrations of pyruvic acid in the tissues of trout killed by high concentrations of zinc (Burton, Jones, and Cairns, 1972). Other symptoms observed have been darkening in colour and increased swimming activity (Affleck, 1952) and a curvature of the backbone in the minnow (*Phoxinus phoxinus*) after chronic exposure (Bengtsson, 1972). Vertebral damage was found by B.E. Bengtsson (personal communication) in adult minnow after 30 days exposure to concentrations of zinc nitrate as low as 0.2 mg Zn/l (0.06 of the 4-d LC50, the concentration lethal to 50 percent of the fish at 4 days).

(6) The ratio of zinc in the gill to that in the operculum is diagnostic for death of many American species caused by high concentrations of zinc under experimental conditions, the ratio being close to unity in controls and up to 100:1 in poisoned fish; however, ratios close to unity have not been found in the wild among carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*) (Mount, 1964).

(7) Little is known of sub-lethal physiological effects, except that after six months exposure to 1.6-2.0 mg Zn/l rudd (*Scardinius erythrophthalmus*) had very low levels of fat and glycogen in the liver (Ministry of Technology, 1966); also stickleback (*Gasterosteus aculeatus*) show an increase in the number and activity of the 'chloride cells' of the gills (Matthiessen and Brafield, 1973).

2.2 Factors affecting acutely lethal levels

(8) Under given constant environmental conditions the period of survival of test batches of fish increases with decrease in the concentration of zinc in solution, lines relating survival time to concentration often being such that there is little difference between concentrations lethal at one day and those lethal at several days; a curvilinear relation thus exists between the logarithm of zinc concentration and the logarithm of median period of survival of fish. However, changes in some environmental factors can alter the shape and position of these curves, giving a series of curves which converge toward a common asymptotic ('threshold') concentration, or which diverge to give different threshold concentrations, or in some cases causing the curves to cross each other. However, the inherent variability in response within the population from which test batches are taken leads to uncertainty as to precision and accuracy of the values observed; for example with the 5-d LC50 of zinc measured using batches of 10 fish, the 95 percent confidence limits are often roughly between ± 10 and ± 20 percent, depending upon the species (Ball, 1967); furthermore, the limits are even wider for concentrations corresponding to a percentage response lower than 50, as shown recently for salmonids (Nehring and Goettl, 1973).

(a) Temperature

(9) Lloyd (1960) demonstrated that an increase in temperature from 13.5 to 21.5°C decreased the survival time of rainbow trout in solutions of zinc sulphate in a hard water, the Q_{10} for concentrations between 5 and 10 mg Zn/l being about 2.4; the 5-d LC50 (3.5 mg Zn/l) was not, however, appreciably altered. Similar experiments (Ministry of Technology, 1968) showed that the 2-d LC50 at 2°C could be as high as 4 mg/l compared with 2 mg/l at 12°C. Sprague (1964) found values of Q_{10} of 4 and 10 for Atlantic salmon (*Salmo salar*) tested in concentrations of 0.6 to 3 mg/l in a soft water, but the 8-d LC50 at 5 and 15°C was at least 0.9 and 0.6 mg Zn/l respectively; later work at 5, 11 and 15°C (J.B. Sprague and W.G. Carson, personal communication) indicates that while the 2-d LC50 was about 0.6 mg Zn/l the 8-d was about 0.16, 0.26, and 0.6 mg Zn/l at the three temperatures respectively. On the other hand the effect of temperature in the range 3 to 19°C is negligible in water having a hardness of 320-350 mg/l as $CaCO_3$ (P.V. Hodsen and J.B. Sprague, personal communication).

(10) Thus a reduction in temperature below about 15°C may increase the time of survival of rainbow trout and salmon at acutely lethal concentrations of zinc yet reduce the lethal threshold value such that the 7-d LC50 for salmon at 5°C is reduced to almost a quarter (i.e. toxicity is increased nearly four-fold). There is no information available on the effect of temperature on the toxicity of zinc to coarse fish.

(b) Dissolved oxygen

(11) Lloyd (1960) using rainbow trout unacclimated to low dissolved oxygen prior to testing found that the 1-d LC50 was reduced by about 30 percent by a reduction from 100 to 40 percent of the air-saturation value. However, when the fish were acclimated beforehand to the level of dissolved oxygen used in the tests the 1-d LC50 was similar (about 3 mg Zn/l) at 9.5, 6.3, and 3.7 mg/l dissolved oxygen, though times of survival at 4-12 mg Zn/l were slightly lower at the lowest concentrations of dissolved oxygen. Cairns and Scheier (1957a) studied bluegill (*Lepomis macrochirus*) and found that when the dissolved-oxygen concentration was fluctuating over several hours between 9 and 5 mg/l the 96-h LC50 for zinc chloride was 8.0 mg Zn/l, and when the fluctuations were between 9 and 2 mg/l it was 4.9 mg Zn/l.

(12) Thus a reduction in dissolved oxygen can reduce the LC50 of zinc for rainbow trout unacclimated to the dissolved oxygen beforehand but not for those that are acclimated.

(c) pH value

(13) The solubility of zinc salts in natural waters decreases with an increase in pH value above about 7, though delay in reaching equilibrium can result in supersaturated solutions. However, in hard water, precipitated zinc held in suspension at pH about 8 seems to be about

as toxic to rainbow trout as zinc ions in solution (Lloyd, 1960). On the other hand, Sprague (1964) found an increase in survival time of Atlantic salmon parr in soft water when the pH was increased from 7.1–7.5 to 7.9–9.3, which he considered was accounted for by the reduced amount of zinc calculated to be in solution. Further preliminary results (J.B. Sprague and U. Zitko, personal communication) show that the 5-d LC50 values were about 2 mg Zn/l at pH 4–5.5 and 0.4 to 0.7 mg Zn/l at pH values between 6 and 8. Mount (1966), using fathead minnow (Pimephales promelas) found that at a given hardness (50, 100 or 200 mg as $CaCO_3$) the 96-h LC50 of zinc was always lower at a pH of about 7.6 than at 6.7, and tentatively suggested that zinc in suspension may be more toxic than zinc in solution.

(14) Thus, no definite conclusion can be arrived at for the effect of pH on the toxicity of zinc solutions.

(d) Hardness

(15) Concentrations of zinc acutely lethal to fish are higher in hard than in soft water as shown by Jones (1938) for stickleback, Lloyd (1960) for rainbow trout, and Tabata (1969) for grass carp (Ctenopharyngodon idella). Jones (1938), Affleck (1952), Sreenivasan and Raj (1963), and Tabata (1969) showed that the addition of the calcium ion (as sulphate or chloride) reduced the toxicity of zinc. Tabata (1969) found that the addition of sodium ions also reduced toxicity, whereas additions of potassium ions had no effect when added to a natural water.

(16) Lloyd (1960) observed that the concentration of zinc that was lethal to rainbow trout in 2½ days in a water of total hardness 320 mg/l as $CaCO_3$ was approximately eight-fold higher than in water of total hardness 12 mg/l. For the three levels of hardness tested, 12, 50 and 320 mg/l $CaCO_3$, the 2½-d LC50's were approximately 0.5, 2 and 4 mg Zn/l, and Lloyd and Herbert (1962) suggested that a linear relation existed between the logarithm of LC50 and the logarithm of total hardness. Recent work (J.F. de L.G. Solbé, personal communication) tends to confirm this in that for a hardness of 504 mg/l as $CaCO_3$, the 48-h LC50 for rainbow trout was 4.8 mg Zn/l. However, steelhead trout (Salmo gairdneri) (G.A. Chapman, personal communication) have recently been shown to have a greater sensitivity than expected from other published results with rainbow trout. All these tests were carried out with fish acclimated to water of the appropriate hardness. Lloyd (1965) showed that rainbow trout reared in soft water were more sensitive to zinc poisoning in soft water than those reared in hard water and tested following immediate transfer to soft water. It was also found that the fish that had been kept in hard water had to be maintained in soft water for at least 5 days before they became as sensitive as those reared in soft water (see also para. 25).

(17) The relation between LC50 and hardness is illustrated in Fig. 1 for rainbow trout, rudd and brook trout (Salvelinus fontinalis), the only other species for which comparable data are available.

(e) Salinity

(18) Herbert and Wakeford (1964) showed that the 48-h LC50 of zinc sulphate for yearling rainbow trout and Atlantic salmon smolts was at a maximum in 35 and 40 percent sea water, being about 15 and 13 times greater than in fresh water for the two species respectively, while in 72 percent sea water it was respectively about 6 and about 8 times greater. They suggest that maximum resistance occurs when the water is approximately isotonic (=31 percent sea water) with the blood of Atlantic salmon smolts acclimated to 100 percent sea water.

(f) Organic matter

(19) Experiments have shown that the acute lethal effect of zinc on salmonids is reduced when chelating materials such as NTA (nitrilotriacetic acid) and EDTA (ethylenediamine tetraacetic acid) are added to the water at neutral pH values (Sprague, 1968a, Grande, 1967). Humic substances, amino acid, polypeptides and other soluble organic matter may complex zinc

sufficiently to influence the toxicity of total concentration of the metal to fish, although the toxicity of sewage effluents containing zinc could be predicted accurately from their total zinc content together with those of other poisons present (Lloyd and Jordan, 1964).

(g) Suspended solids

(20) The toxicity of mine water containing heavy metals is reduced when it is mixed with wastes from the flotation process, which contain high concentrations of finely ground silica quartz or other minerals capable of adsorbing heavy metals (Svenska Gruvföreningen, 1960). Also, preliminary studies with rainbow trout (Ministry of Technology, 1971) suggest that the 48-h LC50 for total concentration of zinc is increased from about 3 mg Zn/l to about 4 mg Zn/l with increase in concentration of either organic or inorganic suspended solids from 10 to 400 mg/l.

(h) Age and size

(21) Rainbow trout fry at the beginning of the free feeding stage were somewhat more vulnerable than adult fish, while eyed eggs were the most tolerant, the 5-d LC50 being at least 4 times higher than that of adults (Edwards and Brown, 1966). Goodman (1951) found an increase in resistance in this species between the ages of 2 and 10 weeks, but this may have been partly attributable to their having been hatched and reared in water containing 1 mg Zn/l. J.P. Goettl (personal communication) found that the 4-d LC50 was approximately 1.2 and 4.5 mg Zn/l in hard water (325 mg/l as calcium carbonate) for fish weighing on average 2 g and 28.3 g respectively, and 0.2 and 0.4 mg Zn/l in soft water (30 mg/l as calcium carbonate) for those of 4.9 g and 30.5 g respectively. With Atlantic salmon, Grande (1967) obtained only a 30 percent hatch of eggs in soft water (hardness 14 mg/l as calcium carbonate) containing 0.04 mg Zn/l, which was less than 0.1 of the 21-d LC50 for yolk-sac fry and fingerlings.

(22) B.E. Bengtsson (personal communication) found a significantly increased mortality among newly hatched minnow compared with adults at concentrations of zinc nitrate of 0.08 mg Zn/l (0.025 of the 4-d LC50 of adults); underyearlings and yearlings were of intermediate sensitivity. Bengtsson (1972) also found in long-term studies at 0.13, 0.2, and 0.31 mg Zn/l in soft water (mean hardness of 70 mg/l as $CaCO_3$) that the percentage mortality of juvenile minnow was 10, 22, and 50, but for mature minnow it was only 0, 10, and 30 respectively. On the other hand, Jones (1938) found no difference in resistance to zinc between juvenile (18-20 mm) and mature (45-50 mm) stickleback.

(i) Acclimation to zinc

(23) Goodman (1951) observed that the time of survival of fingerling rainbow trout in 6 and 10 mg Zn/l increased after acclimation for 40 days in water containing 2 mg Zn/l. Similar results were obtained by Affleck (1952) with both rainbow and brown trout, and by Lloyd (1960) and Edwards and Brown (1966) with rainbow trout. Lloyd (1960) found a significant, almost two-fold, increase in survival time among rainbow trout which had survived solutions containing 2.5 and 3.5 mg Zn/l for 14 days and had then been exposed to a concentration of 10 mg Zn/l as compared with fish that had been kept beforehand in clean water. Edwards and Brown (1966) found a 40 percent increase in the 48-h LC50 of fish kept at 0.5 of the 48-h LC50 for 60 days, but not of those kept at 0.6 of the 48-h LC50. Data of J.B. Sprague (personal communication) indicate that with acclimation to sub-lethal concentrations for several months the 7-d LC50 of zinc for Atlantic salmon may be increased two or three-fold.

(24) Pickering and Vigor (1965) found that one-day-old eggs of fathead minnow exposed to 1.1 mg Zn/l hatched and the fry lived for at least 3 days, whereas all the newly-hatched fry not previously exposed to zinc died within 24 hours of being exposed to this concentration; the duration of this tolerance was not, however, investigated. Edwards and Brown (1966) observed that rudd which had survived long exposure to 25 mg Zn/l could live longer at 40 to 60 mg Zn/l than a similar batch previously held in clean water.

(25) These results are supported by field experiments (Schofield, 1965) during the summer in Honnedga Lake, U.S.A. (hardness 1-3 mg/l as $CaCO_3$), when the concentration of zinc was between 0.01 and 0.15 mg Zn/l; caged brook trout transferred from Cortland hatchery (hardness about 80 mg/l as $CaCO_3$), where the maximum concentration of zinc was 0.01 mg Zn/l, died whereas almost all those from Cornell hatchery (hardness about 60 mg/l as $CaCO_3$) in which the concentration was up to 1.52 mg Zn/l, survived the transfer for 9 days. Furthermore the mortality among the Cortland fish was reduced with prior acclimation for 6 days to Cornell hatchery water.

(26) In another experiment rainbow trout were acclimated to a sub-lethal concentration (0.8 mg Zn/l) and then exposed to a detergent (alkyl benzene sulphonate) alone and to mixtures of zinc and detergent. The toxicity of the detergent tested alone was similar for fish from both treatments, but when tested in the presence of 0.8 mg Zn/l the mixture was more toxic to the fish previously exposed to zinc (Brown, Mitrovic, and Stark, 1968).

(27) Thus, some species of salmonids and coarse fish survive longer at lethal concentrations of zinc when previously exposed as eggs or juvenile fish to sub-lethal concentrations; under the most favourable conditions of acclimation to zinc the 7-d LC50 may be increased two or three-fold. However, exposure to sub-lethal concentrations of zinc can reduce the resistance of trout to subsequent additional exposure to a detergent.

(j) Inbreeding

(28) Rachlin and Perlmutter (1968) found a ten-fold increase in tolerance to zinc in an inbred strain of platyfish (*Xiphophorus maculatus*).

(k) Joint action of zinc and other heavy metals

(29) Lloyd (1961) studied the survival of rainbow trout in zinc and copper sulphates and in mixtures of the two in a hard borehole water and in an artificial soft water (total hardness 320 mg/l and 14-20 mg/l respectively as $CaCO_3$). By expressing the concentrations of the metals in the mixtures as proportions of their individual 3-d LC50 in hard water and 7-d LC50 in soft water, median periods of survival were related to concentrations of the two metals when present together. He postulated that copper and zinc exerted their acute toxic action in a similar way and that their toxicity in relatively low lethal concentrations was simply additive. Higher acutely lethal concentrations of the mixture in soft water, however, exhibited synergism, in that survival times were much shorter than with the equivalent amount of either heavy metal alone - an effect also found by Doudoroff (1952) using fathead minnow in soft water.

(30) Sprague and Ramsay (1965) tested juvenile Atlantic salmon in zinc and copper sulphates and in mixtures of the two in a water with a total hardness of 14 mg/l as $CaCO_3$. The 7-d LC50 of the mixture could be accounted for by simple addition of the corresponding LC50's of the separate metals as described above (para. 29).

(31) The 48-h LC50 to rainbow trout of mixtures of copper, zinc and nickel has also been found (Brown and Dalton, 1970) to be adequately predicted by summation of the fractional 48-h LC50 values of the separate poisons.

(l) Joint action of zinc and other poisons

(32) The toxicity to fathead minnow of solutions containing sodium cyanide and zinc sulphate was related mainly to the level of molecular cyanide present (Doudoroff *et al.*, 1966).

(33) Herbert and Shurben (1964) tested rainbow trout in solutions of ammonium chloride and zinc sulphate in both hard and soft water and also in the presence of reduced dissolved oxygen, and found that 50 percent of the fish were killed at 48 hours when the sum of the proportions of the 48-h LC50 of each poison reached approximately 1.0. Similar results have

been found for mixtures of phenol and zinc sulphate (Herbert and Vandyke, 1964), zinc, copper and phenol (Brown and Dalton, 1970), and zinc and a detergent (alkyl benzene sulphonate) (Brown, Mitrovic, and Stark, 1968).

(34) Brown, Jordan, and Tiller (1969) varied the proportion of zinc, ammonia, and phenol in mixtures tested with rainbow trout, and found that when zinc predominated the toxicity of the mixtures was less than expected, assuming simple addition of the fractional toxicities of the three poisons; it is possible that when zinc predominated the amount of ammonia and/or phenol present was too low to exert any effect on the fish.

(35) Several sewage effluents were studied by Lloyd and Jordan (1963 and 1964). The toxicity to rainbow trout of one containing a considerable amount of zinc in addition to other heavy metals, ammonia, detergents, phenol, and cyanides, when tested in hard and soft water, was similar to that predicted from the concentration of zinc and other poisons present. Similar results were found for some fishless rivers in England (Herbert, Jordan and Lloyd, 1965) containing zinc, copper, ammonia, and phenol.

(36) In general the effect of zinc in the presence of other common poisons on the 48-h LC50 of the mixture to rainbow trout can be calculated by simple addition of the fractional 48-h LC50 of the individual poisons.

2.3 Summary of toxicity data

(a) Acutely lethal values

(i) Salmonid eggs

(37) Concentrations reported to be lethal to salmonids vary from about 0.01 mg to more than 10 mg Zn/l, depending mainly on the water hardness and species of fish (Fig. 1) and stage in the life-cycle and also the duration of tests. Some results which give no details of these relevant factors are therefore not considered in this report.

(38) Rainbow trout eggs failed to hatch in a soft water (4.3 mg/l as $CaCO_3$) containing 0.04 mg Zn/l (Affleck, 1952). Yet J.P. Goettl (personal communication) found that the eyed eggs showed no ill effects when kept for 100 and 140 days respectively in a soft water (25 mg/l as $CaCO_3$) containing 0.1 mg Zn/l and in a hard water (340 mg/l as $CaCO_3$) containing 0.4 mg Zn/l.

(39) With brown trout eggs (Ministry of Technology, 1966) there was no significant mortality at 0.06, 0.2 and 0.6 mg Zn/l in water having hardnesses equivalent to 12.5, 50 and 200 mg/l as $CaCO_3$ respectively; the 5-d LC50 was lowest at the early eyed stage, being about 0.5 mg Zn/l and 2.5 mg Zn/l at the extreme values of hardness. Corresponding values for eggs of rainbow trout at the late eyed stage were 3 mg Zn/l and >10 mg Zn/l in the soft and hard water respectively.

(40) Schofield (1965) found between 80 and 85 percent survival of batches of eggs of brook trout in waters having hardnesses of between 1 and 3 mg/l as $CaCO_3$ and containing between 0.02 and 0.04 mg Zn/l.

(41) On the other hand, the hatch of salmon was only 30 percent at a concentration of 0.04 mg Zn/l in water having a temperature of 10°C a hardness of 14 mg/l as $CaCO_3$ and a pH of 6.4 (Grande, 1967), though Brånin and Paulsson (1971) found that in a water of similar quality (about 13 mg/l as $CaCO_3$ and a pH of 6.1-6.4) they developed successfully even at 0.1 mg Zn/l, while there was about 90 percent mortality at 1.0 mg Zn/l.

(ii) Salmonid fry, juveniles and adults

(42) The results of tests with rainbow trout are shown in Fig. 1 (as closed circles) together with those for two other species. The line relating the logarithm of LC50 for rainbow trout to logarithm of water hardness appears to be curvilinear only if the results of Affleck (1952) and G.A. Chapman (personal communication) are given the same weight as the other points.

(43) Affleck (1952) obtained a complete kill of rainbow trout but a complete survival of brown trout at 0.1 mg Zn/l. The greater resistance of brown trout is confirmed by Goodman (1951), Grande (1967) and Nehring and Goettl (1973). Sprague's data (1964) suggest that salmon are slightly more sensitive than rainbow trout, a conclusion also supported by Grande (1967). Brook trout on the other hand appear to be slightly more resistant than rainbow trout (Sprague, 1964; Schofield, 1965, compared with Affleck, 1952 and Nehring and Goettl, 1973).

(iii) Coarse fish

(44) No work has been reported on the young stages of coarse fish, except juvenile minnow (Bengtsson, 1972), the 10-d LC50 values for which in soft water (1.5-2.5 mg Zn/l) appear to be slightly higher than those of salmonid fish. The most resistant species appear to be rudd (Ball, 1967) and goldfish (Carassius auratus) (Pickering and Henderson, 1964), and data for these and other species are shown in Fig. 2.

(b) Long-term lethal values

(45) Edwards and Brown (1966) kept rainbow trout for about 4 months in hard water (320 mg/l as $CaCO_3$) containing 0.6, 1.6, and 2.0 mg Zn/l (equivalent to about 0.2, 0.5 and 0.6 of the 5-d LC50 respectively); the percentage mortality at 6 d was 0, 5, and 7; and at 4 months 16, 18, and 22 respectively.

(46) Somewhat similar experiments have recently been carried out with rudd in hard water at 8-15°C (V.M. Brown and T.L. Shaw, personal communication). The LC50 was 24 mg Zn/l at 5 days and 10.5 mg Zn/l at 8½ months; at the end of this period there was 10 percent mortality at a concentration of 7 mg/l (0.3 of the 5-d LC50) and none at 4 and 2 mg Zn/l.

(47) The minnow was kept for about 3 months in soft water (hardness about 70 mg/l as $CaCO_3$) containing low concentrations of zinc (Bengtsson, 1972); the 90-d LC50 for juvenile fish is estimated at 0.3 mg Zn/l. Stickleback survived 29 days at 6.0 mg Zn/l in water having a hardness of 280 mg/l as $CaCO_3$ (Matthiessen and Brafield, 1973).

3. SUB-LETHAL EFFECTS ON FISH

3.1 Uptake and loss of zinc

(a) Eggs and fry

(48) Experiments by Wedemeyer (1968) on eggs of coho salmon (Oncorhynchus kisutch) indicate that uptake of zinc from the water is mainly by adsorption on the chorion (70 percent), the remainder being by diffusion into the egg (26, 2, and 1 percent being in the perivitelline fluid, the yolk, and the embryo respectively).

(49) Vladimirov (1971) found that the net uptake of zinc within one month by the fry of common carp was higher in those exposed to 5 mg $ZnSO_4$/l than in those exposed to 0.05 and 0.5 mg/l.

(b) Adults

(50) Zinc is taken up by fish directly from the water, especially by the mucus and gills (Skidmore, 1964). Considerable amounts are also found in the eye, kidney, bone and the gastro-intestinal tract, with lower amounts in liver, spleen, and muscle (Saiki and Mori, 1955; Joyner, 1961; Mount, 1964). High concentrations have also been found in the gonads (Nazarenko, 1972). By plugging the oesophagus of brown bullhead (Ictalurus nebulosus) Joyner (1961) showed that swallowed water did not contribute to the zinc in the gastro-intestinal tract.

(51) He found that an initial high rate of uptake was followed by a decline after about 12 hours. On the other hand Slater (1961) found that the rate of accumulation over 48 hours was approximately constant in salmonids and was highest in brown trout, lowest in rainbow trout, and intermediate in cutthroat trout (Salmo clarki). Observations by Nazarenko (1972) suggest that in bream (Abramis brama) the accumulation of zinc is greater than in roach (Rutilus rutilus) and is greater in summer than in winter.

(52) Cairns and Scheier (1957) conclude from their results that accumulation may be faster in soft water than in hard but that temperature has probably no appreciable effect.

(53) Joyner and Eisler (1961) found that most of the zinc taken up within 24 hours by fingerling chinook salmon (Oncorhynchus tshawytscha) exposed to solutions of 0.2 mg Zn/l was retained when the fish were kept in flowing water for 63 days, during which time there was a gradual redistribution of zinc to the vertebral column, head and viscera at the expense of other tissues. On the other hand, Joyner (1961) observed that nearly half of the zinc that accumulated in brown bullhead during prolonged exposure to 6 mg Zn/l was lost during the first day after transfer of the fish to flowing fresh water, and the rate of loss was reduced later.

(54) Mechanisms for redistribution would account for the ratio of zinc in the gill to that in the operculum being higher in fish poisoned by high concentrations of zinc than in those exposed to sub-lethal concentrations, and excretion and differences in exposure would account for the variation in concentrations found by Mount (1964) between different samples of fish from the wild.

3.2 Reproduction

(55) Brungs (1969) found that reproduction in fathead minnow was almost totally inhibited at zinc concentrations that had no effect on survival, growth and maturation. The number of eggs produced per female at 0.18 mg Zn/l was only 17 percent of that produced at 0.03 mg Zn/l. The 4-d LC50 was 8.4 to 10 mg Zn/l, and 15 percent mortality occurred at 2.8 mg Zn/l in the 10-month test period.

(56) No experiments have been carried out on the effect of zinc on the reproduction of European species of fish.

3.3 Growth

(57) In tests lasting about 4 months during which rainbow trout were exposed to concentrations of between 0.6 and 2.0 mg Zn/l (0.2 to 0.6 of the 5-d LC50) in hard water (Edwards and Brown, 1966) there was a small but significant mortality among the fish, and while growth rates, and weights of liver and kidney (expressed as percentage of total body weight) were apparently similar at all concentrations and for the control, consumption of pelleted food (but not that of maggots) per fish was reduced by almost 20 percent at 2.0 mg Zn/l.

(58) Rudd, when tested over a period of 8½ months (V.M. Brown and T.L. Shaw, personal communication), also showed no marked tendency for a lower production of biomass at concentrations up to 4 mg Zn/l (0.17 of 5-d LC50).

(59) Results for other species have been somewhat different. Pickering (1968), using bluegill over a 20-day period, found that growth was enhanced at 1 mg Zn/l and reduced at 4 mg Zn/l (the 20-d LC50 was 7.2 to 12 mg Zn/l). Bengtsson (1972) however did not find such a stimulating effect of low concentrations on the growth of minnow, and Brungs (1969) found that the growth of fathead minnow was reduced when they were exposed to a concentration of 2.8 mg Zn/l for 30 days (approximately 0.3 of the 4-d LC50), but not at lower concentrations.

(60) Thus the growth of rainbow trout and rudd is apparently unaffected at up to 0.6 and 0.17 respectively of the 5-d LC50, while stimulation of growth at lower fractions of the LC50 has been found in bluegill but not in minnow or fathead minnow.

3.4 Behaviour

(61) Sprague (1964a) used Atlantic salmon parr in a soft water (hardness 18 mg/l as $CaCO_3$) in an avoidance trough in the laboratory. The lowest concentrations causing the average fish to show significant avoidance (EC50) were 0.053 mg Zn/l for zinc sulphate alone and 0.006 mg Zn/l for zinc in the presence of 0.0004 mg Cu/l. These were equivalent to 0.14 and 0.07 of the 7-d LC50 respectively when the natural metal contents in the test water (0.002 mg Cu/l and 0.003 mg Zn/l) were included.

(62) Studies of salmon passing through a salmon counting fence in the Northwest Miramichi River, Canada (Sprague, Elson and Saunders, 1965; Saunders and Sprague, 1967) showed that downstream returns during the upstream migration rose from between 1 and 3 percent during 6 years before pollution by mine wastes to between 10 and 22 percent during 4 years of pollution, and runs to the head-waters were delayed and reduced in number. This apparent avoidance seemed to occur at about 0.35-0.43 of the 7-d LC50 of copper and zinc, while a level of 0.8 of the LC50 may have blocked all upstream movement. Of the salmon returning downstream prematurely about 31 percent re-ascended. There was little or no difference between avoidance reactions of fish that had been previously exposed to pollution as smolts and those not.

(63) The EC50 for rainbow trout avoidance of zinc under laboratory conditions at 9.5°C and 17°C was 0.005 mg Zn/l (0.01 of the 7-d LC50) and was not changed when acclimation levels were increased from 0.003 to 0.013 mg Zn/l (Sprague, 1968).

(64) Syazuki (1964) and Ishio (1966) report avoidance at 0.3 and 0.45 of concentrations lethal to common carp (*Cyprinus carpio*) and goldfish; Jones (1947), however, found that stickleback (*Pygosteus pungitius*) showed avoidance only of lethal concentrations.

(65) Thus salmon show avoidance of concentrations of zinc equivalent to 0.14 of the 7-d LC50 under laboratory conditions and 0.35-0.43 of the LC50 in the field. In the laboratory rainbow trout react to 0.01 of the LC50 and coarse fish to 0.3-0.45.

3.5 Resistance to disease

(66) Observations by Pippy and Hare (1969) suggest that in the Miramichi River, Canada, a surge of copper and zinc pollution in late June 1967, accompanied by unusually high river temperatures may have encouraged an infection of disease in Atlantic salmon caused by the bacterium, *Aeromonas liquifaciens* (*A. punctata*). However, peak concentrations of copper and zinc when the mortality started were 1.1 times the 7-d LC50.

(67) Hiller and Perlmutter (1971) found that the amount of virus causing IPN (infectious pancreatic necrosis) in tissue cultures of rainbow trout gonad increased significantly when cultured at 10 mg Zn/l but not at 7.5 mg Zn/l.

4. FIELD OBSERVATIONS ON FISH

(68) There are many reports of fish kills in rivers polluted by water from mining industries, but because of the complexity of the wastes and paucity of analytical data it is usually difficult to identify the effect of zinc alone. However, some data are available from experiments with fish held in cages, from fish farms supplied with zinc-contaminated water and from surveys of the status of fish populations in natural waters containing zinc.

4.1 Caged fish experiments

(69) Hasselrot (1965) found a high mortality among caged yearling salmon and adult minnow in a polluted river in Sweden when mean and maximum concentrations were 0.15-0.28 and 0.25-0.95 mg Zn/l respectively, the water hardness being 21-48 mg/l as $CaCO_3$. His conclusion that the zinc was mainly responsible for killing the fish, with possibly some contribution from low concentrations of copper and other metals accords with laboratory data for salmonids (para. 42 and 43). The adult minnow were slightly more resistant than the yearling salmon.

(70) Similarly, the river Molonglo in New South Wales, Australia, has been polluted by mining activities (Weatherley, Beevers and Lake, 1967). Complete mortalities occurred within 16 days among caged rainbow trout (size range 3.9-5.9 cm), and a partial mortality among brown trout (4.5-9.4 cm), when the total zinc concentration fluctuated between 0.1 and 0.5 mg Zn/l, with a total hardness of 10-13 mg/l as $CaCO_3$. The difference in resistance between the two species is consistent with laboratory data (para. 43); the seemingly higher toxicity of zinc is perhaps attributable to the presence of copper. Crucian carp (Carassius carassius) were more resistant than trout, a 40 percent mortality occurring in 16 days when the total zinc fluctuated between 2 and 7 mg Zn/l, total copper fluctuated between 0.04 and 0.08 mg Cu/l, and the hardness was 57 mg/l as $CaCO_3$.

4.2 Fish farms

(71) Brook trout were reared in Cornell hatchery (hardness 44-123 mg/l as $CaCO_3$) where concentrations reached 1.52 mg Zn/l (Schofield, 1965). These maxima are close to median lethal concentrations found under laboratory conditions (para. 43).

4.3 Fish population surveys

(72) When the river Ystwyth, U.K. contained up to 0.8 mg Zn/l and a trace of lead at normal water level, and up to 1.4 mg Zn/l during droughts with a pH of about 6.4 and a calcium content equivalent to about 11.0 mg/l as $CaCO_3$, brown trout, which were plentiful above the sources of pollution, died soon after being washed into the main river during floods; sea trout (Salmo trutta) which occasionally ascended the lower reaches were also sometimes found dead or dying (Jones, 1940). Later (Jones, 1958) when concentrations were reduced to 0.2-0.7 mg Zn/l (approximately 0.5-1.75 of the 2-d LC50 for rainbow trout) and amounts of lead were negligible, brown trout were present in small numbers, sea lamprey (Petromyzon marinus) occurred occasionally but other species, including minnow, stickleback and bullhead (Cottus gobio), were absent.

(73) Another, preliminary, survey of small streams in Wales, U.K., from which single samples for analysis were taken in December, February and July, showed that in those in which the concentration of zinc expressed as a proportion of the 2-d LC50 to rainbow trout was less than 0.06 brown trout were present at a density of 11-46/100 m^2, where the proportion was between 0.06 and 0.09 the density was 7/100 m^2, and where it was between 0.14 and 0.48 the density was 0.4-4/100 m^2 (from data supplied by Cremer and Warner, personal communication).

(74) Data have also been made available (P. Toner, personal communication) for the river Kilmastualla, Ireland, in which samples have been taken at approximately 2-weekly intervals during 1972; these show that at two stations where there was a self-sustaining population of

brown trout, together with some salmon, annual 50 and 95 percentiles for concentrations of zinc expressed as proportions of the 2-d LC50 to rainbow trout were about 0.054 and 0.19 respectively at one station, and about 0.038 and 0.19 at the other. Zinc accounted for about 81 and 66 percent of the predicted 2-d LC50 at the two stations respectively, there being some contribution from other heavy metals and ammonia.

(75) In Norway, soft water lakes and rivers (hardness 2-20 mg/l as $CaCO_3$) contain self-sustaining populations of salmonids in the presence of concentrations of zinc up to 0.15 mg Zn/l (Grande, 1967; Snekvik, personal communication). Where higher concentrations have been observed brown trout, Atlantic salmon, and Arctic char (Salvelinus alpinus) are absent but in most cases copper and other metals are also present. These observations are consistent with the laboratory findings of Lloyd (1960), Sprague (1964 and 1964a) and J.P. Goettl (personal communication), but not with those of Affleck (1952).

(76) Sprague, Elson and Saunders (1965) found that the concentration of zinc and copper in a tributary of the Miramichi River, Canada, averaged 0.24 of the 7-d LC50 in 1962, the maximum value being 0.6 of the 7-d LC50; salmon parr populations were much lower than in unpolluted parts of the river, perhaps partly because the number of available spawning fish was reduced in previous years. Higher concentrations were present in the other years studied.

(77) Schofield (1965) has reported that brook trout occurred in Honnedga Lake, U.S.A. (hardness up to 3 mg/l as $CaCO_3$) where concentrations of zinc ranged from 0.02 to 0.17 mg Zn/l.

(78) In the Willow Brook, U.K., a stream of unusually high hardness, much present as chloride (504 mg/l as $CaCO_3$) and receiving wastes (from a steel works) containing mainly zinc (J.F. de L.G. Solbé, personal communication), roach, tench (Tinca tinca) and stickleback were present in the upper reaches, where the annual 50 and 95-percentile concentrations were 0.9 and 3.7 mg Zn/l (0.04 and 0.19 of the 5-d LC50 for roach), whereas roach, chub (Leuciscus cephalus), minnow, stickleback, eel (Anguilla anguilla) and stoneloach (Nemacheilus barbatulus) were present in the lower reaches where the corresponding percentiles were 0.4 and 2.2 mg Zn/l (0.02 and 0.1 of the 5-d LC50 for roach).

(79) In the river Aire, U.K., which contains a variety of poisons, roach, chub, minnows and stickleback were present in limited reaches where the water was well oxygenated, when the 6-yearly 50-percentile and 95-percentile concentrations of zinc were equivalent to 0.02 and 0.05 respectively of the 5-d LC50 to roach (from data supplied by D. Woodhead, personal communication).

(80) Thus, concentrations of zinc that are lethal to fish in the field are generally similar to those found lethal in the laboratory. Also, field observations show that salmon parr may be present in polluted rivers when the concentration of zinc is no more than 0.15 of the 7-d LC50, but that numbers are much reduced when annual mean and maximum concentrations of zinc and copper are as high as 0.24 and 0.6 of the 7-d LC50 respectively (para. 76). A few species of coarse fish may occur where the annual median and 95-percentile concentration of zinc is 0.04 and 0.19 of the 5-d LC50 of roach and several species may be present where these values are 0.02 and 0.1 of the LC50 or lower (para. 74, 78 and 79).

5. SUMMARY OF DATA ON AQUATIC VEGETATION AND INVERTEBRATES

5.1 Aquatic vegetation

(81) Whitton (1970) reviewed the little that is known about the toxicity of zinc to freshwater algae. Williams and Mount (1965) found that adding 1 mg Zn/l or more to natural periphytic communities in 4 outdoor channels supplied with running water reduced the number of dominant species. Fungi and myxo-bacteria produced a large standing crop at the highest concentration (6.5 mg Zn/l), apparently by utilizing the killed incoming phytoplankton. Organisms highly tolerant of 6.5 mg Zn/l include the bacteria Sphaerotilus natans, Zoogloea ramigera, and Beggiatoa sp., the fungi Alternaria tenuis, and Leptomitus lacteus, the algae

Anacystis sp., Lyngbya sp., Schizothrix calcicola, Oscillatoria, Oocystis lacustris, Spirogyra sp., and Chlamydomonas snowii, Euglena acus, Trachelomonas volvocina, Chrysococcus major, and the diatoms Cymbella tumida, Nitzchia linearis and Synedra ulna.

(82) Whitton (1970a) studied the toxicity of zinc to 25 species of Chlorophyta from flowing water. Cladophora glomerata was among the most sensitive; Ulothrix, Microspora, Spirogyra and Mougeotia were relatively resistant; while all the Oedogonium spp. taken from the wild were of intermediate sensitivity. However Oedogonium and Mougeotia obtained from a zinc-enriched laboratory tank were highly resistant. Values for zinc causing a slight inhibition of growth ranged from 0.2-6.0 mg Zn/l (or to 9.0 mg/l if the algae from a zinc-enriched tank are included). The hardness was not given but is estimated at approximately 20 mg/l as $CaCO_3$. He also concludes (1970) that Lemanea is relatively resistant.

(83) Extensive observations on benthic diatoms by Besch, Ricard and Cantin (1972) in parts of the Miramichi river system affected by mining operations tend to confirm that Synedra ulna and particularly Achnanthes microcephala and Eunotia exiqua are relatively resistant to zinc; macrophytes most affected were submerged species followed by riparian dicotyledons, monocotyledons and Equisetum arvense (Besch and Roberts-Pichette, 1970).

5.2 Invertebrates

(a) Laboratory tests

(84) There are relatively few data on the effects of zinc on invertebrates, especially those that include information on water quality. The 4-d LC50 for the ramshorn snail (Helisoma campanulatum) was 1.27 mg Zn/l and 0.87 mg Zn/l in hard and soft water respectively, and for young specimens of the pond snail (Physa heterotropha) 0.4 mg Zn/l and 0.3 mg Zn/l in hard and soft water (100 mg/l and 20 mg/l total hardness respectively) (Wurtz, 1962), values that are markedly lower than those found by Cairns and Scheier (1957a). He suggests that snails containing haemoglobin (Helisoma spp.) are more tolerant of zinc than those containing haemocyanin (Physa spp.) and also stresses that molluscs would be the first animals eradicated when a stream became overloaded with metals.

(85) Some crustaceans are relatively sensitive to zinc poisoning. Bringmann and Kühn (1959) have reported a 2-d LC50 for young daphnids of 1.8 mg Zn/l in hard water (215 mg/l as $CaCO_3$), a result close to those of Tabata (1969a) which cover a wide range of hardness and are slightly lower than the corresponding values for trout. Anderson (1948), however, reports an EC50 for immobilization of Daphnia magna as low as 0.07 mg Zn/l in water of hardness 100 mg/l as $CaCO_3$ and Biesinger and Christensen (1972) using the same species report a 2-d LC50 of 0.16 mg Zn/l at 18°C in water having a hardness of 45 mg/l expressed as $CaCO_3$.

(86) Among insects, resistance to zinc is variable. Warnick and Bell (1969) studied the effect of zinc on insects in water having a hardness of 44 mg/l and a pH of 7.25. The 10-d LC50 for the mayfly Ephemerella subvaria was 16 mg Zn/l, whereas the 11 and 14-d LC50 for the caddisfly Hydropsyche betteni and the stonefly (Plecoptera) Acroneuria lycorias respectively was 32 mg Zn/l; thus they are considerably more resistant to concentrations lethal within a few days than the fish that have been studied (para. 37-44).

(b) Field observations

(87) Field investigations from zinc-polluted waters have given some information of the tolerance of invertebrates to zinc although it is often difficult to interpret because of the presence of other pollutants. In the river Ystwyth where concentrations of 0.7-1.2 mg Zn/l and 0.05 mg Pb/l were normally present, mollusca, crustaceans, worms (Oligochaeta) and leeches (Hirudinea) were all absent (Jones, 1940), and in a small tributary containing nearly 57 mg Zn/l there was still a considerable number of insect larvae. When concentrations in the main river fell some years later to 0.2-0.7 mg Zn/l the fauna was still almost entirely made up of lithophilous insects (Jones, 1958), Rhitrogena semicolorata, Heptagena

lateralis, Baetis rhodani, Chloroperla tripunctata and Esolus parallelopipedus being the chief species. Sprague, Elson and Saunders (1965) found that caddisflies (Trichoptera) and midges (Chironomidae) were not affected by copper-zinc pollution in the Northwest Miramichi River where concentrations were at least 1.5 times the 7-d LC50 for salmon. Mayflies were more sensitive and few were present when concentrations were about 60 percent higher. However studies in 1969 (W. Besch, personal communication) showed there was no hatch of caddisflies, including Hydropsyche, and mayflies and that there was a marked reduction in gallery-building midges; furthermore larvae of the caddisfly Glossosoma spp. were absent where the concentrations of zinc were about 0.1 of the 7-d LC50 for salmon. In the Molonglo River, Weatherley, Beevers and Lake (1967) found that the number of animals was markedly reduced and that the fauna consisted mostly of bugs (Hemiptera) beetles (Coleoptera), caddisflies, Diptera, and stoneflies in the zinc-polluted part of the river. However, this may also have been due to indirect effects of the pollution.

6. SUMMARY AND CONCLUSIONS

(88) Zinc is a common pollutant in freshwater and occurs in wastes from a variety of industries and other sources. It is often accompanied by copper and other heavy metals and affects freshwater fisheries, presumably mainly by its direct effects on fish but possibly too on some fish-food organisms such as mayflies and daphnia (para. 84-86).

(89) Its mode of toxic action is not yet fully understood, but its toxicity is mainly attributable to the zinc ion, though particulate zinc as the basic carbonate or the hydroxide held in suspension may also be toxic (para. 13).

(90) In acutely toxic concentrations zinc may kill fish by destroying gill epithelial tissue (para. 4). There may also be chronic effects on various organs and enzyme systems (para. 6).

(91) There are few data on the confidence limits for concentrations reported as having lethal or other effects, but those that are available suggest that they are between ± 10 and 20 percent of the LC50. The limits widen progressively as the percentage response departs from 50. There is thus some uncertainty about the precise levels that are lethal to fish and other organisms.

(92) Nevertheless it is clear that the toxicity of zinc is modified by water quality, in particular being reduced by an increase in hardness (para. 15-17). An increase in temperature may shorten the time of survival of fish at concentrations that are rapidly lethal, yet increase it at lower concentrations such that the 7-d LC50 may be increased (para. 9 and 10). Furthermore, toxicity is increased by a decrease in dissolved-oxygen content (para. 11) and decreased by an increase in salinity (para. 18) and suspended solids (para. 20) but probably not to a large extent by organic matter (para. 20). The effect of pH is uncertain (para. 13 and 14). The acute toxicity of zinc in the presence of other heavy metals and other common pollutants seems to be largely simply additive (para. 29-36). However, there is no evidence that the chronic toxicity of different poisons in a mixture is also simply additive. The effect of zinc is modified, and can be reduced, by acclimation and by the age of the fish (para. 21-27).

(93) The concentrations of zinc that are rapidly lethal to fish under laboratory conditions and their dependence upon the hardness of the water are fairly well defined for rainbow trout in the range 10 to 500 mg/l $CaCO_3$ and for some other salmonids at the lower end of the range of hardness, the LC50 values at several days being about 0.4 and 5 mg/l Zn/l at the two extremes of hardness (para. 16 and Fig. 1). However, the very high toxicity to rainbow trout in water containing less than 30 mg/l $CaCO_3$ (para. 43) needs confirmation and explanation.

(94) Data for coarse fish are fewer, but they too indicate that toxicity is reduced with increase in water hardness and that the fish are generally more resistant than trout, LC50 values at 320 mg/l $CaCO_3$ ranging from 8 to 17 mg Zn/l (para. 15 and Fig. 1). The stoneloach and minnow, however, appear to be more sensitive than trout (para. 44 and Fig. 2).

(95) A low but significant mortality has been found among rainbow trout exposed continuously for 4 months to constant concentrations of 0.2 of the 5-d LC50 and among rudd exposed for $8\frac{1}{2}$ months to 0.3 of the 7-d LC50 (para. 45); although food consumption of the trout is reduced under laboratory conditions at 0.6 or more of the LC50, growth is not apparently affected (para. 57). The 3-month LC50 for the minnow is 0.03-0.06 of the approximate 10-d LC50 (para. 44 and 47).

(96) There is some evidence that resistance to disease (para. 66-67) and reproduction (para.55) may be affected in sub-lethal concentrations of zinc, but little systematic work has been done to illuminate these problems or to study their effects in the field.

(97) Laboratory studies of avoidance reactions have shown that Atlantic salmon and rainbow trout may avoid concentrations of zinc in soft water which are 0.14-0.01 of the 7-d LC50 (para. 61-63). Avoidance reactions have also been observed at 0.35-0.43 of the 7-d LC50 by migrating Atlantic salmon in a river polluted with copper and zinc (para. 62). Carp and goldfish show avoidance of 0.3-0.45 of lethal concentrations under laboratory conditions (para. 64).

(98) The resistance of aquatic plants and invertebrates to zinc varies widely. Algae are adversely affected at concentrations of 0.2-9.0 mg Zn/l (para. 82). Larvae of some caddis-flies and midges are highly resistant to zinc, while those of some mayflies may be about as sensitive as salmon. Mollusca, crustaceans, oligochaetes, and leeches appear to be most sensitive (para. 83-85).

(99) Field observations of fish mortalities generally confirm the laboratory studies of lethal concentrations (para. 69-70). Few, however, adequately describe the conditions in rivers and lakes containing zinc and thriving populations of fish. Good populations of brown trout were present in streams when the concentration of zinc was less than 0.06 of the 2-d LC50 to rainbow trout (para. 73) and the fish were also present when the annual 50 and 95 percentiles of the 2-d LC50 were up to 0.05 and 0.19 respectively (para. 74). Salmon were present when the concentration of zinc was no more than 0.15 of the 7-d LC50 (para. 75) but were much reduced in numbers at annual mean and maximum concentrations of 0.24 and 0.6 of the 7-d LC50 (para. 76); a mixed population of coarse fish species, including the minnow, was found in a river of high hardness where the annual median and 95-percentile concentrations of zinc were 0.02 and 0.11 of the 5-d LC50 for the most resistant coarse fish tested, whereas few species were found when the annual median and 95 percentile were 0.04 and 0.19 of the 5-d LC50 (para. 78 and 79).

7. TENTATIVE WATER QUALITY CRITERIA

(100) There are extensive data on the toxicity of zinc to fish under laboratory conditions, supported to some extent by field data on fish kills, but there are virtually no field observations to indicate the concentrations of zinc that are not inimical to fish populations or fisheries, mainly because the analytical data are too meagre, information on water hardness - perhaps the most important factor affecting the toxicity of zinc to aquatic animals - is missing and details of the status of the fish population are not given. For these reasons only tentative criteria can be suggested which may have to be revised when more adequate field data become available.

(101) Because it is known that concentrations of zinc, and indeed of many other natural and artificial constituents of freshwaters containing fish, fluctuate, it is unrealistic to express criteria as single values that should be exceeded for long periods. Instead they could be expressed as one or more values each relating to a percentile distribution for a defined period, distributions that in practice appear to be approximately lognormal. This idea is inherent in the criteria proposed by EIFAC for monohydric phenols and is embodied in those proposed by EIFAC for dissolved oxygen. In the case of zinc the appropriate period might be taken as one year, since there is no reason to expect marked seasonal differences in the effect of this poison on fish, although for salmon the 7-d LC50 at 5°C is about a quarter (0.26) of that at 15°C.

(102) Pending the availability of more information it is tentatively suggested that for the maintenance of thriving populations of fish the annual 95 percentile of concentration of zinc should be no greater than 0.1 of the appropriate 7-d LC50 at 15°C; thus the criteria in terms of concentration of zinc would depend upon water hardness and type of fish as shown in Table II.

Table II. Maximum annual 95-percentile concentrations of zinc (mg Zn/l)

Water hardness (mg/l $CaCO_3$)	Coarse fish except minnow a/	Salmonids
10	0.3	0.03 b/
50	0.7	0.2
100	1.0	0.3
500	2.0	0.5

a/ See para. 104

b/ But see para. 103

(103) The concentration of 0.03 mg Zn/l for salmonids in very soft water may be too severe if brown trout only are present, since this species appears to survive successfully at higher concentrations; in such cases a 95-percentile concentration of 0.2 of the 7-d LC50 (0.06 mg Zn/l) may be more appropriate.

(104) For the minnow it might be more appropriate to set a more stringent standard but further information from the field would be desirable to support and explain the existing laboratory data.

(105) The values of the corresponding annual 50 percentiles would be approximately 0.25 of the proposed 95 percentiles were the distributions referred to in para. 74, 78 and 79 regarded as typical. However, if the distribution was much wider in range or was not log-normal, so that the ratio between the 95 percentile and higher percentiles was much larger, more stringent standards would be appropriate.

(106) Furthermore, where other poisons are present and dissolved-oxygen concentrations are below the air-saturation value, allowance should be made for their contribution to the toxicity.

●	Rainbow trout	(*Salmo gairdneri*)	1. Affleck (1952) 2. Lloyd (1960) 3. Lloyd (1961) 4. Ball (1967) 5. Goettl (personal communication) 6. Solbé (personal communication) 7. Chapman (personal communication)
×	Salmon	(*Salmo salar*)	8. Sprague (1964a)
⊙	Brook trout	(*Salvelinus fontinalis*)	9. Sprague (1964b) 10. Schofield (1965)

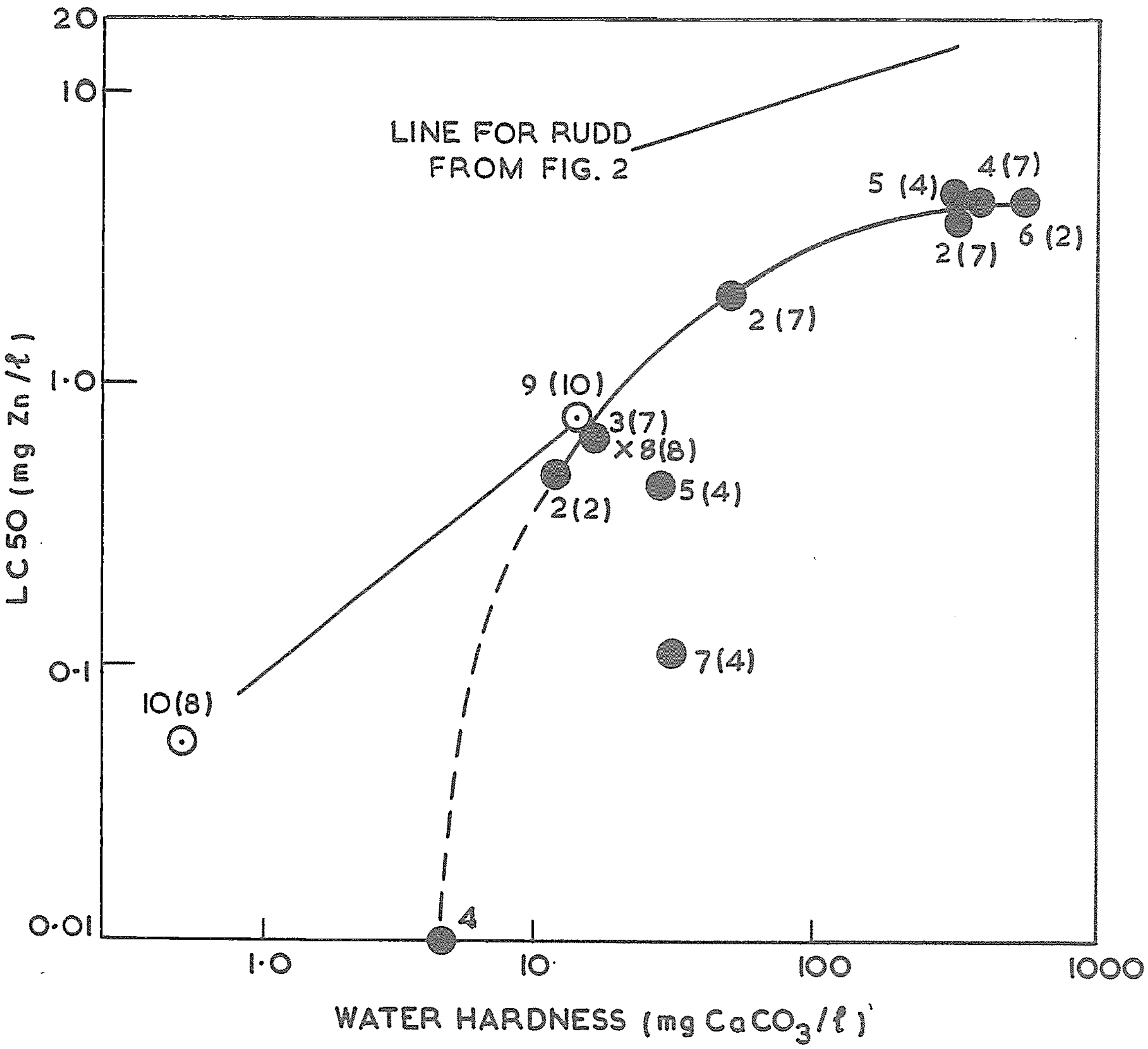

Fig. 1. Median lethal concentration of zinc to salmonid fish (time in days shown in parentheses). Line for rudd from Fig. 2 shown for comparison

●	Rudd	(*Erythrophthalmus scardinius*)	1. Ministry of Technology (1967) 2. Ministry of Technology (1966b)
⊙	Roach	(*Rutilus rutilus*)	3. Ball (1967)
⊡	Perch	(*Perca fluviatilis*)	4. Ball (1967)
△	Bream	(*Abramis brama*)	5. Ball (1967)
▽	Gudgeon	(*Gobio gobio*)	6. Ball (1967)
■	Common carp	(*Cyprinus carpio*)	7. Nehring (1964)
▼	Goldfish	(*Carassius auratus*)	8. Pickering and Henderson (1964)
▲	Crucian carp	(*Carassius carassius*)	9. Weatherley *et al*.(1966)
×	Stickleback	(*Gasterosteus aculeatus*)	10. Jones (1938) 11. Matthiessen & Brafield (in press)
✱	Minnow	(*Phoxinus phoxinus*)	12. Bengtsson (1972)
+	Stoneloach	(*Nemacheilus barbatulus*)	13. Solbé (personal communication)

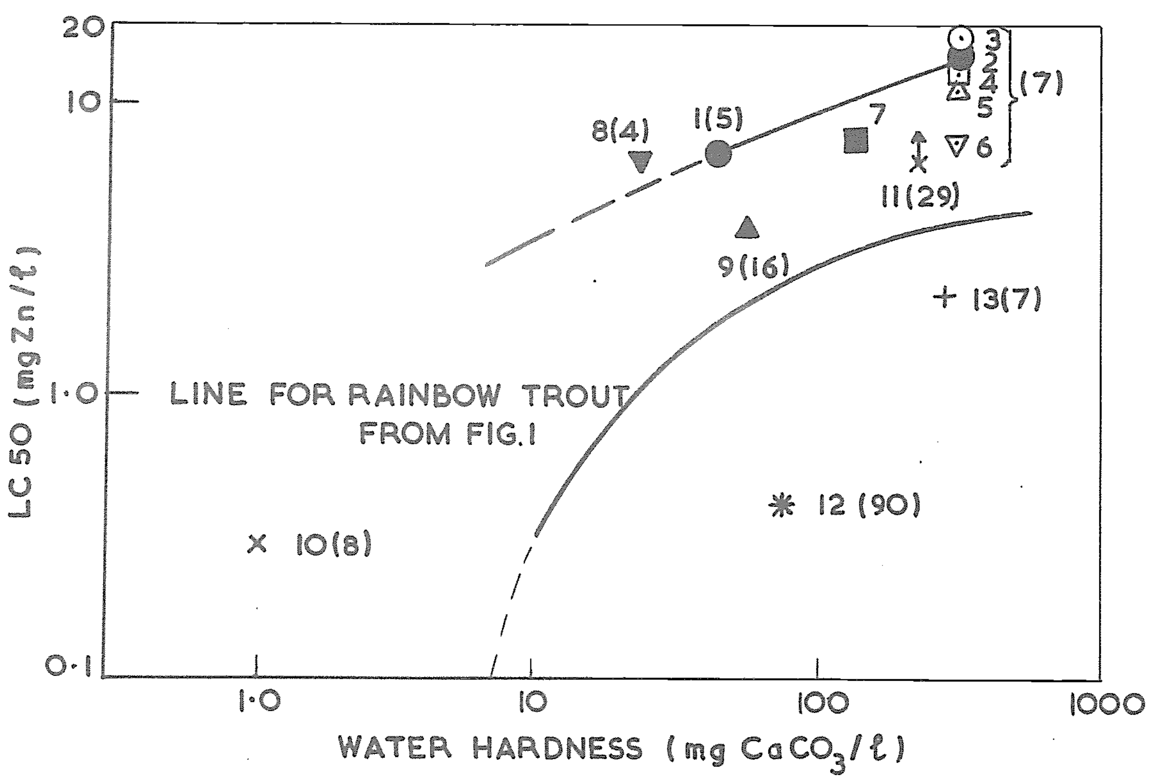

Fig. 2. Median lethal concentration of zinc to coarse fish (time in days shown in parentheses). Line for rainbow trout from Fig.1 shown for comparison

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