# Toxicity of environmental acid to the rainbow trout: interactions of water hardness, acid type, and exercise

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Classical 7-day lethality tests were used to establish the influence of water hardness (≃ 140 versus ≃ 14 mg/L $CaCO_3$), acid type (HCl versus $H_2SO_4$) and activity level (rest versus exhaustive exercise) on acid toxicity to fingerling rainbow trout (*Salmo gairdneri*) at 15°C. Seven-day mean lethal concentration (LC50) pH's ranged from 4.1 to 4.5. Hardness reduced $H_2SO_4$ toxicity at all pH levels during both rest and exericise, but reduced HCl toxicity only at very low pH levels. Hardness increased HCl toxicity at pH's >3.8. $H_2SO_4$ was generally less toxic than HCl, except at pH's >3.8 in soft water. Exhaustive exercise markedly potentiated $H_2SO_4$ toxicity in both hard and soft water except at very low pH levels. Below pH = 4.4–4.6, critical swimming speed declined linearly by about 4% per 0.1 pH unit. Possible physiological mechanisms responsible for these modifying influences and their ecological significance are discussed.

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Des expériences classiques de létalité d'une durée de 7 jours ont permis d'établir l'influence de la dureté de l'eau (=140 versus 14 mg/L $CaCO_3$), du type d'acidité de l'eau (HCl versus $H_2SO_4$) et de l'activité (repos versus exercice d'épuisement) sur la toxicité des acides chez des alevins de truite arc-en-ciel (*Salmo gairdneri*) gardés à 15°C. Les pH qui entraînent une mortalité de 50% en 7 jours se situent entre 4,1 et 4,5. La dureté de l'eau diminue la toxicité d'$H_2SO_4$ à tous les pH chez les poissons au repos et les poissons actifs, mais elle ne réduit la toxicité d'HCl qu'à des pH très bas. La dureté de l'eau augmente la toxicité d'HCl à des pH supérieurs à 3,8. $H_2SO_4$ est généralement moins toxique qu'HCl, sauf aux pH supérieurs à 3,8 en eau douce. Les exercices d'épuisement augmentent de façon appréciable la puissance toxique d'$H_2SO_4$, en eau dure comme en eau douce, sauf à des pH très bas. A des pH de 4,4–4,6, la vitesse de nage critique diminue de façon linéaire d'environ 4% par 0,1 unité de pH. La discussion porte sur les mécanismes physiologiques qui pourraient être responsables de ces modifications et sur l'importance écologique de ces modifications.

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## Introduction

Acid precipitation is recognized as a serious problem in Canada and abroad (Cogbill and Likens 1974; Oden 1976; Braekke 1976; Dillon *et al.* 1978). It is now common in some areas of south-central Ontario to find lakes acidified to pH's below 4.8 (Beamish and Harvey 1972; Beamish 1974, 1976; Dillon *et al.* 1978; Harvey 1979; Jeffries *et al.* 1979). At such low pH's, the development and survival of rainbow trout (*Salmo gairdneri*) (Lloyd and Jordon 1964; Kwain 1975; McDonald *et al.* 1980) and many other freshwater teleosts (Beamish 1972, 1974; Daye and Garside 1975, 1976; Lievestad *et al.* 1976; Trojnar 1977*a*, 1977*b*) are affected in the laboratory. These results have been broadly supported by field observations showing the disappearance of fish populations from acidified lakes (Beamish and Harvey 1972; Beamish *et al.* 1975; Schofield 1976; Harvey 1979). Nevertheless, our knowledge of the influence of modifying factors on acid toxicity remains scanty. The present study employs classical toxicological techniques (Sprague 1969, 1973) to assess the effects of three possible modifiers of acid toxicity in the rainbow trout: water hardness, acid type, and exercise level. There are reasons to suspect that all three may significantly interact with the toxic mechanism(s) of acid action.

Firstly, the nature of the toxic syndrome in trout varies greatly between hard and soft water, a difference specifically due to the concentration of calcium and not other ions (McDonald *et al.* 1980). There already exist some indications that hardnesss may affect absolute acid toxicity (Lloyd and Jordon 1964; Lievestad *et al.* 1976; Trojnar 1977*b*). In the wild, acidification is almost exclusively a soft water phenomenon. Secondly, there is indirect evidence that the $SO_4^{2-}$ anion itself may be toxic (De Renzis and Maetz 1973), whereas $Cl^-$ anion probably is not; HCl has been the acid most commonly used in laboratory studies, but $H_2SO_4$ is the major pollutant in the wild. Thirdly, loss of major electrolytes and depression of blood pH have both been implicated as key toxic mechanisms of acid action (Packer and Dunson 1970, 1972; Lievestad *et al.* 1976; McWilliams

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and Potts 1978; Neville 1979*a*, 1979*b*; Packer 1979; McDonald *et al.* 1980; McDonald and Wood 1981). Both of these changes also occur during exercise in fish (Wood and Randall 1973; Kiceniuk and Jones 1977; Wood *et al.* 1977) and are due respectively to increases in effective gill permeability and to the endogenous generation of respiratory and metabolic acids. Synergistic effects seem likely.

A final aim was to assess the influence of acid stress on critical swimming speed (CSS; Brett 1964). If acid exposure interferes with $O_2$ transport, a dependence of CSS on environmental pH may well occur (Jones 1971). Furthermore, fish in the wild must swim actively in a variety of normal behaviours (feeding, avoiding predation, vertical migration, spawning etc.). Thus swimming ability, and the alteration in it caused by toxicants, is an important consideration when assessing species survival capability (Sprague 1971).

## Materials and methods

### *Experimental animals*

Fingerling rainbow trout, *Salmo gairdneri* Richardson (weight 3.50 ± 0.09 g, length = 7.59 ± 0.30 cm; $\bar{X}$ ± 1 SE, $n$ = 740), were purchased from a local hatchery (Spring Valley Trout Farm). Fish were kept in a 500-L polyethylene flow-through holding tank prior to acclimation to experimental conditions. Aerated, dechlorinated tap water (hard water; see below) was supplied to the tank (15 ± 3°C: seasonal fluctuations). Fish were fed granular feed, size No. 3 (Martin Feeds, Elmira, Ontario).

### *Toxicity testing*

#### *Apparatus*

Polyethylene tubs were used to test 10 fish at one time. Each tank was an independent 60-L system recirculated at 10 L/min through a coil immersed in an ethylene glycol – water mixture cooled by a box freezer (Woods, Guelph, Ontario). Apparatus in contact with the test water was either glass, plastic, or Tygon; no metal components were employed.

#### *Test water*

Tests were conducted in either hard (hardness ≃ 140 mg/L $CaCO_3$) or soft water (hardness ≃ 14 mg/L $CaCO_3$) at 15 ± 2°C. Major ion concentrations of the hard water (dechlorinated Hamilton tap water) were 2.0–4.0 meq/L $Ca^{2+}$, 0.8–2.0 meq/L $Na^+$, 0.8–2.0 meq/L $Cl^-$, and < 0.1 meq/L $K^+$. Soft water was a 1:10 dilution of hard water with distilled water and had an ion concentration of 0.2–0.4 meq/L $Ca^{2+}$, 0.2–0.4 meq/L $Na^+$, 0.2–1.2 meq/L $Cl^-$, and < 0.1 meq/L $K^+$. Variation in ion levels resulted both from the modifying effects of fish on the water in the recirculating systems and from the additions of acid and base necessary to control pH. $Na^+$, $Cl^-$, and $K^+$ concentrations were monitored at the termination of each test. $Ca^{2+}$ was assayed at the start and end of both acclimation and testing and additional checks were made with any replacement water during testing. Physiological evidence indicates that $Ca^{2+}$ (and not $Cl^-$, $Na^+$, and $K^+$) is the ion solely responsible for the different response of fish to acidified hard and soft water (McDonald *et al.* 1980).

All experimental and acclimation water was acidified to below pH 4.0 with HCl or $H_2SO_4$, as appropriate, and bubbled vigorously with air for 24 h before use to eliminate any complicating $CO_2$ effects (Lloyd and Jordan 1964; Neville 1979*b*). Final adjustments to the desired pH (test or control) were then performed with NaOH and either HCl or $H_2SO_4$ as appropriate. Final concentrations of the added acid anion were 2–3 meq/L in hard water and 0.5–1.0 meq/L in soft water. Subsequently pH's were monitored and adjusted when necessary (at least once a day) with a Radiometer or Corning pH meter and low ionic strength electrode. pH variation was virtually nil at the lower part of the test range (pH = 3.0–3.6) but increased to a maximum of ± 0.1 unit at the upper end (4.6–4.8).

#### *Test procedure*

Prior to testing, fish were acclimated to the experimental temperature and water type for at least 7 days; blood ion status returns to normal within 4 days after a hard water to soft water transfer (McDonald *et al.* 1980). No food was administered to the fish. Ten fish were transferred directly into each test tank from either the acclimation tank (resting tests) or the swimming respirometer (exercise tests; see below). A pH range of 3.0–4.8 in 0.2-unit increments was used and each test was accompanied by a control having the same number of trout pretreated and held under identical conditions at normal pH (i.e. 6.5–8.0). Deaths were monitored as they occurred during the first day of exposure and then two or three times daily until day 7. At death, each fish was measured for total length and wet weight. Trout surviving the 7 days were sacrificed and similarly processed.

#### *Experimental treatments*

Six treatments were tested in total: (1) hard water, $H_2SO_4$, rest; (2) soft water, $H_2SO_4$, rest; (3) hard water, $H_2SO_4$, exercise; (4) soft water, $H_2SO_4$, exercise; (5) hard water, HCl, rest; and (6) soft water, HCl, rest.

### *Exercise procedure*

#### *Apparatus*

Fish were exercised in a 150-L Plexiglas swimming respirometer (Research Instruments Mfg. Co., Guelph, Ontario) similar in design to that of Farmer and Beamish (1969). Water speed in the swimming chamber (40 cm long × 20 cm diameter) was continuously variable to a maximum of 60 cm/s; calibration was via a precision rodometer ("Ott Miniature Current Meter"). Current was generated by a 3 hp (1 hp = 746 W) motor and a heresite laminated impellor (to prevent any heavy metal release during acidification). A cooling jacket of anodized aluminum kept water temperature at 15 ± 2°C; air saturation was maintained during testing.

#### *Test procedure*

Fish were exercised in the respirometer in groups of 10 at each pH level. Critical swimming speed (CSS) for each fish was determined by the method outlined by Brett (1964); speeds were increased in 6.0-cm/s increments and each increment lasted 60 min. The CSS was recorded for each fish when it was overcome by the water speed, forced onto the downstream screen, and became nonresponsive to a mechanical stimulus (prodding). At CSS, the fatigued fish was removed from the swimming chamber by suction into a 19 mm (internal

diameter) Tygon tube. Removal did not damage the fatigued fish and allowed those remaining to continue swimming.

Exhausted fish were immediately placed into a 60-L recirculating system (described above) at the same pH (± 0.05 pH units), water hardness, and temperature (± 1°C). Deaths were monitored over the following 7-day period, with the time of exercise commencement (i.e. first exposure to acid) taken as time zero.

*Statistical procedures*

The experimental design allowed construction of toxicity curves in the form of probit mortality versus log time at each pH, and also in the form of probit mortality versus pH at different times (cf. Sprague 1969, 1973). The former were used to estimate median lethal times (LT50's) at each pH and the latter to estimate median lethal concentrations (LC50's) at selected times. Ninety-five percent confidence limits and statistical comparisons of LT50's and LC50's were assessed by means of the nomographic methods of Litchfield (1949); a significance level of $p \leq 0.05$ was employed. The slopes and intercepts of linear regression relationships (Fig. 4) were considered significantly different when 95% confidence limits did not overlap (Ostle and Mensing 1975).

## Results

*Water hardness effects*

With $H_2SO_4$ as the means of acidification there were consistent hard water versus soft water differences during both rest and exercise (Figs. 1A and 1C). The hard water condition was significantly less toxic than soft water at every point in both trials except at pH = 3.8 during nonexercise tests (Fig. 1A). These effects were clearly reflected in the 4- and 7-day LC50 values which were always greater in soft water though the difference was only significant for the 7-day rest values (Table 1).

When HCl was used to acidify the water, the hard water versus soft water differences were more complex (Fig. 1B). These HCl results have also been reported elsewhere (McDonald *et al.* 1980). As with $H_2SO_4$, soft water was significantly more toxic than hard water at the acutely toxic pH values of 3.0 and 3.2. However, at higher levels, the situation became reversed so that soft water was significantly less toxic at pH 3.8 and above. As a consequence, the LC50 values after the reversal were greater in hard water, the difference becoming significant by 7 days (Table 1).

*Acid type effects*

Acid types were compared only under nonexercise conditions. Below pH = 3.8, $H_2SO_4$ was less toxic than HCl in both soft water and hard water though the differences were consistently significant only in the latter (Figs. 2A, 2B). Above pH = 3.8, the same difference persisted in hard water, whereas in soft water, a crossover of the toxicity curves occurred, so that $H_2SO_4$ became significantly more toxic than HCl. These effects were reflected in the LC50 values (Table 1). By 7 days, the LC50 value for HCl was significantly greater than the corresponding $H_2SO_4$ value in hard water, whereas the reverse was true in soft water.

*Exercise effects*

Exercise exerted a significant effect on acid toxicity under both hard water and soft water conditions, the exercise groups surviving for significantly less time at test pH's above 3.4 (Figs. 3A, 3B). The separation of the toxicity curves was greatest at the intermediate pH of 3.8 and declined at higher values. There were no significant differences between the 4- and 7-day LC50 values (Table 1) in rest and exercise treatments. The exercise condition was significantly less toxic below pH = 3.4 in soft water, whereas no significant difference occurred over the same pH range in hard water (Figs. 3A, 3B).

*Critical swimming speeds*

Under control conditions, the CSS's were about 5.5 L/s and not significantly different between hard water and soft water (Fig. 4). Acute acid exposure had a marked detrimental effect on CSS, which was significantly depressed at all pH's below 4.4 in hard water and below 4.6 in soft water. Over these ranges, CSS varied in an approximately linear fashion with pH, declining by about 4% per 0.1 pH unit. The regression lines differed significantly in slope and intercept but converged at pH = 4.0–4.2. At three acidic pH levels (3.2, 3.4, and 4.4), CSS's were significantly greater in hard water than in soft water.

## Discussion

The present data indicate the long-term nature of acid toxicity. Seven-day LC50's were always higher (i.e. less concentrated) than 4-day values (Table 1) and clear thresholds, at which acute toxicity would cease, were not seen in the toxicity curves over the 7-day experimental period. Such thresholds would be manifested as changes in slope to the point where the toxicity curve became asymptotic to the time axis (Sprague 1969, 1973). (Apparent asymptotes in the exercise trials (Figs. 1C, 3A, 3B) were probably not indicative of thresholds; see below.) This conclusion agrees with Lloyd and Jordan (1964), who observed continuing acid toxicity to trout for at least 15 days, and with Beamish (1972), who found toxicity extending to 42 days in the white sucker. Thus, the 7-day LC50's reported here should not be considered incipient LC50's (Sprague 1969, 1973).

In a toxicity study such as the present, responses in individual animals are essentially quantal, so speculation on the physiological significance of the results is dangerous. However, the opposite process, explanation of toxicological data from physiological results, is much

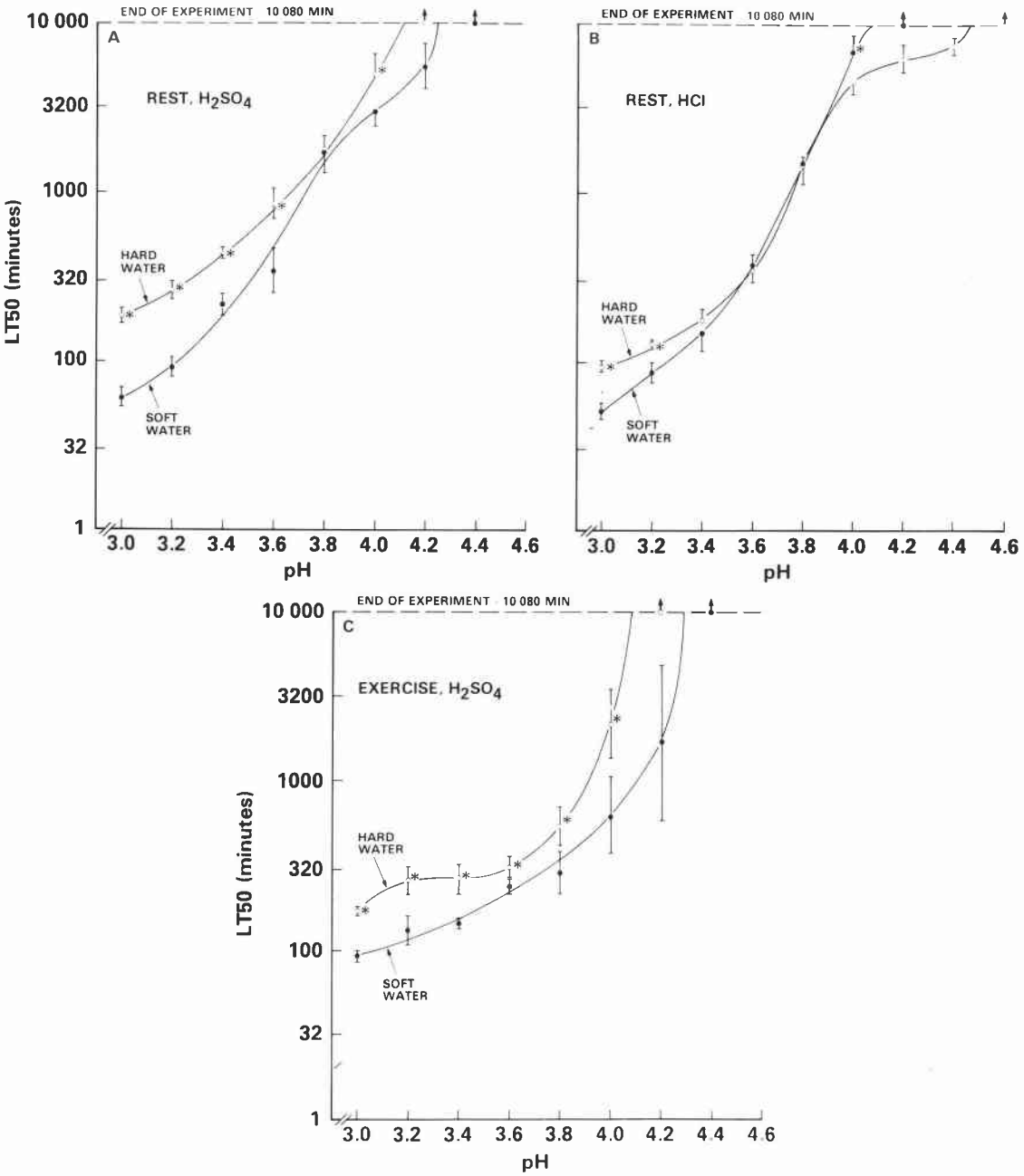


FIG. 1. The influence of water hardness on the relationship between environmental pH and median survival time (LT50, log scale) in fingerling rainbow trout under (A) rest, $H_2SO_4$; (B) rest, HCl; and (C) exercise, $H_2SO_4$ conditions. Each point is based on 10–30 animals. Bars represent 95% confidence intervals. Arrows indicate points where mortalities were less than 50% over the 7-day (10 080 min) experimental period. Curves intersect 10 080 min at the estimated 7-day LC50 values. Asterisks indicate significant differences ($p \leq 0.05$) between LT50's at the same pH.

more meaningful. The influence of modifying factors on acid toxicity must relate to the key toxic mechanism(s) of acid action (Sprague 1971). The explanations are necessarily complex because acid probably kills fish by more than one mechanism. For example, it is currently thought that acute disturbances in $O_2$ uptake may be the toxic event at very low pH's, whereas more gradual disturbances in ionoregulation and acid–base regulation

TABLE 1. LC50 values for 4 and 7 days (pH ± 95% confidence limits) for acid lethality to fingerling rainbow trout under different conditions

| Treatment | Hard water LC50 | Soft water LC50 |
|---|---|---|
| $H_2SO_4$, rest | | |
| 4 days | 3.98±0.11 | 4.12±0.11 |
| 7 days | 4.12±0.08 | 4.25±0.05* |
| $H_2SO_4$, exercise | | |
| 4 days | 3.98±0.11 | 4.20±0.21 |
| 7 days | 4.09±0.15 | 4.30±0.14 |
| HC1, rest | | |
| 4 days | 4.12±0.08 | 3.98±0.16 |
| 7 days | 4.46±0.04† | 4.06±0.08*† |

NOTE: *, significantly different ($p \leq 0.05$) from corresponding hard water value; †, significantly different ($p \leq 0.05$) from corresponding $H_2SO_4$, rest value. There were no significant differences between corresponding $H_2SO_4$, rest, and $H_2SO_4$, exercise, values.

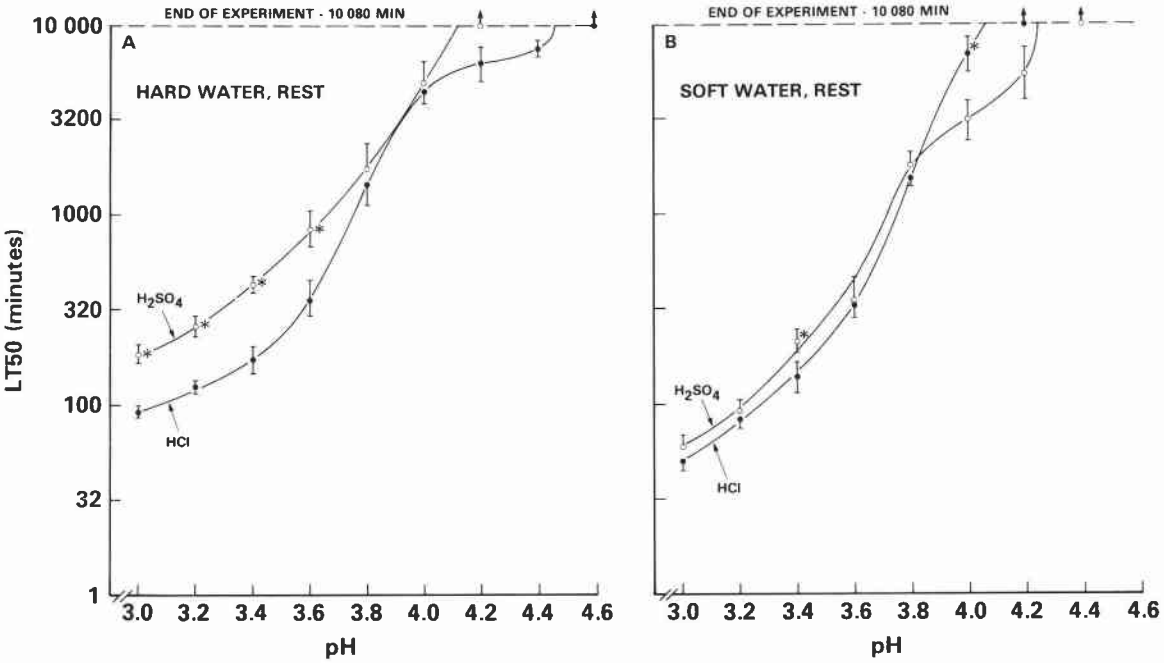


FIG. 2. The influence of acid type (HCl versus $H_2SO_4$) on the relationship between environmental pH and median survival time (LT50, log scale) in the fingerling rainbow trout under (A) hard water, rest, and (B) soft water, rest conditions. Other details as in legend of Fig. 1.

become more important at higher acidic pH's (Fromm 1980; McDonald *et al.* 1980). The relative influence of these may in turn depend upon the environmental calcium level and the $O_2$ demand of the animal.

Hard water conditions significantly reduced acid toxicity relative to soft water conditions at virtually every pH level with $H_2SO_4$ in both rest (Fig. 1A) and exercise (Fig. 1C) treatments. This finding agrees with others showing a general protective effect of higher calcium levels or ionic strength against $H_2SO_4$ toxicity (Lievestad *et al.* 1976; Trojnar 1977*b*; Carrick 1979). The situation with HC1 was more complicated (Fig. 1B); hard water reduced toxicity at low pH (3.0, 3.2), had no effect at intermediate pH, and actually potentiated it at higher pH's (> 3.8). The crossover phenomenon was replicated several times to ensure its validity. These results are very similar to those of Lloyd and Jordan (1964) on trout, who reported that hardness

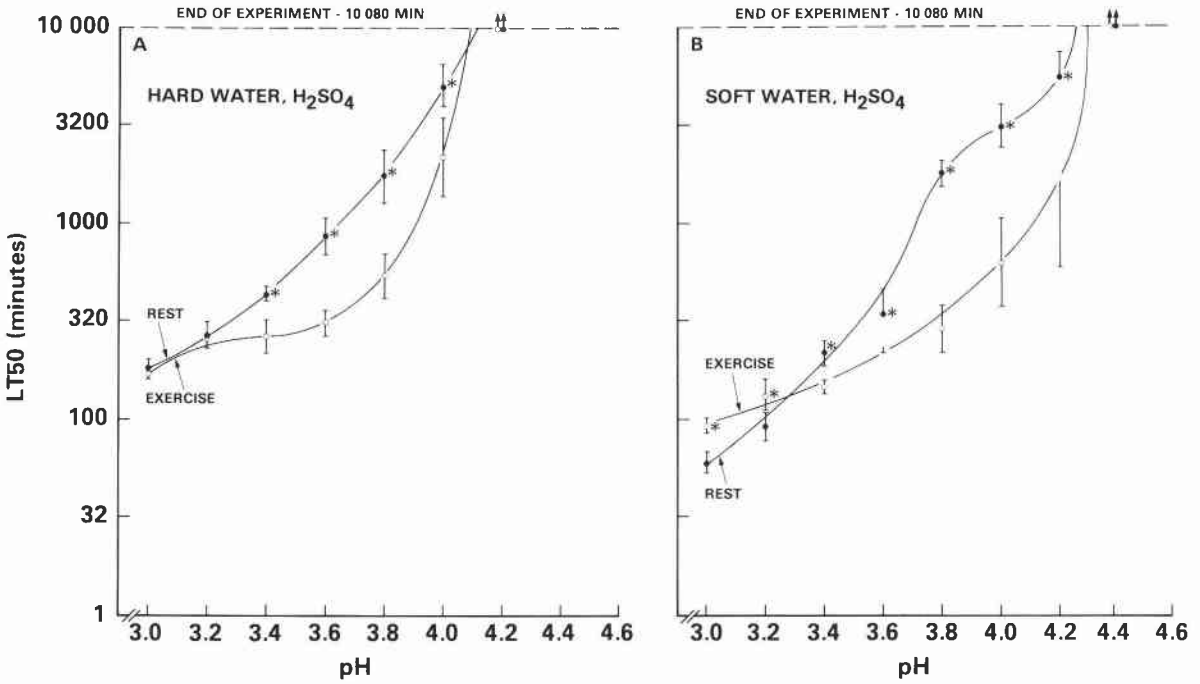


FIG. 3. The influence of exhaustive exercise on the relationship between environmental pH and median survival time (LT50, log scale) in fingerling rainbow trout under (A) hard water, $H_2SO_4$ and (B) soft water, $H_2SO_4$ conditions. Other details as in legend of Fig. 1.

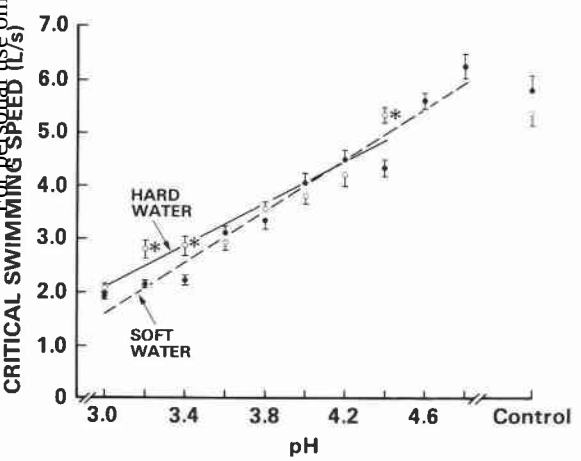


FIG. 4. The relationship in fingerling rainbow trout between environmental pH and critical swimming speed (CSS) in hard water and soft water under $H_2SO_4$ acidification. Points are means ± 1 SE for 10 or 20 fish. Asterisks indicate significant differences ($p \leq 0.05$) between critical swimming speeds in hard and soft water at the same pH. The regression equations are the following:

Hard water CSS = 1.99 (± 0.16) pH − 3.90 (± 0.61) ($r = 0.98$, $p = < 0.001$).

Soft water CSS = 2.40 (± 0.10) pH − 5.59 (± 0.40) ($r = 0.98$, $p = < 0.001$).

extended survival at acutely toxic pH levels, but the effect diminished at higher pH's so that 4-day LC50's were little different between hard and soft water. Indeed an apparent crossover occurred after the termination of their 4-day experiment. Protective effects of water hardness against acid toxicity are probably attributable to the lower electrolyte permeabilities of the gills in high calcium (Cuthbert and Maetz 1972; Eddy 1975; McDonald *et al.* 1980). The anomalous potentiating effect of hard water on HC1 toxicity only at higher pH levels cannot be explained at present, but must reflect some influence of the anionic component as it did not happen with $H_2SO_4$ (see below).

There were clear differences in the toxicities of $H_2SO_4$ and HC1, the former being less toxic at all pH's in hard water (Fig. 2A); the difference was significant from pH = 3.0 to 3.6. The same was true at pH's ≤ 3.8 in soft water, though significance was only attained at pH = 3.4 (Fig. 2B). However the reverse occurred at pH's ≥ 4.0 in soft water (Fig. 2B). As the acids used were analytical grade, the differences can only be attributed to the anions. The greater difference between the $Cl^-$ and $SO_4^{2-}$ effects in hard water than in soft water (Fig. 2B) would therefore reflect the greater anion concentration in the former (2–3 meq/L versus 0.5–1.0 meq/L). These results agree with the only two previous, very

limited, comparisons (Packer and Dunson 1972; Beamish 1972) of the acids using modern technology. Doudoroff and Katz (1950) decided that no definite conclusions could be drawn from earlier comparisons of HCl and $H_2SO_4$ because of experimental or technological deficiencies. Packer and Dunson (1972) found that brook trout survived three times longer at pH = 3.25 in $H_2SO_4$ than in HCl; calcium levels were not given, but the water was probably quite soft, based on the reported sodium level (0.1 meq/L). Beamish (1972) found that white suckers survived slightly longer in $H_2SO_4$ than in HCl at pH's = 3.4–3.8 in hard water of very similar composition to that used here. It seems improbable that the $Cl^-$ anion would be toxic at these levels, so the difference more likely reflects an ameliorative effect of the $SO_4^{2-}$ anion. Perhaps the impermeability of $SO_4^{2-}$ at the gills (Garćia-Romeu and Maetz 1964) retards $H^+$ entry.

The reversal of HCl and $H_2SO_4$ toxicities only in soft water at pH's $\geq$ 4.0 (Fig. 2B) may reflect the intrinsic slow toxicity of the $SO_4^{2-}$ anion itself under low calcium, high permeability conditions. De Renzis and Maetz (1973) reported that goldfish died over a 5-day period when kept at neutral pH in 1.0 meq/L $Na_2SO_4$ in deionized water, but survived when kept in deionized water alone. This toxicity of $SO_4^{2-}$ at acidic pH's $\geq$ 4.0 in soft water can only be viewed as a potentiator of other stresses, for our control fish at neutral pH survived well in similar $SO_4^{2-}$ levels, as did those of Daye and Garside (1976) in various $SO_4^{2-}$ salt solutions.

At acutely toxic pH's (3.0, 3.2), exhaustive exercise either had no effect on acid toxicity (hard water, Fig. 3A) or even extended survival (soft water, Fig. 3B). A failure of $O_2$ uptake or transport is probably the cause of death at these extreme acid levels (Packer and Dunson 1972); Fromm 1980). The flushing action of the water current at the gills may have prevented mucus accumulation or else physiological adaptations to improve $O_2$ transport during exercise may have helped short-term survival here. In any event, the phenomenon probably has little ecological significance. Much more important was the clear potentiation of acid toxicity by exhaustive exercise at pH's $\geq$ 3.4 (Figs. 3A, 3B). The shift in the LC50 at intermediate survival times (e.g. 1000 min) was as much as 0.4 pH units. By 7 days, the exercise toxicity curves rejoined the resting relationships, producing apparent asymptotes to the time axis. These should not be viewed as thresholds (Sprague 1969, 1973) but rather as simple returns to the resting condition *because the exhaustive exercise occurred so long before death that its potentiating influence had worn off*. Such synergism between exercise and acid toxicity is to be expected from the elevated branchial ion losses (Wood and Randall 1973) and depressed blood pH levels (Kiceniuk and Jones 1977; Wood *et al.* 1977) which result respectively from elevated branchial permeability and the endogenous generation of metabolic and respiratory acids during activity. Indeed, exhaustive exercise alone, produced by chasing, is occasionally fatal to adult trout (J. D. Turner, M. S. Graham, and C. M. Wood, unpublished observations), though this did not occur with the control fingerling trout exercised to exhaustion in the respirometer.

Critical swimming speeds were not significantly different between hard and soft water at the same pH at neutrality (control) or at pH = 3.6–4.2, thereby extending the observations of Waiwood and Beamish (1978) at pH = 6.0–8.0. However, below pH = 4.6 (soft water) or 4.4 (hard water), CSS fell about 4% per 0.1 pH unit. The threshold for this effect in hard water may in fact be higher because the pH = 4.4 results did not seem to follow the general trend (Fig. 4). As mortality over the 7-day period did not commence until somewhat lower pH's in the exercised fish (4.1–4.3; Table 1), the reduction of CSS appears to be an indicator of sublethal acid stress. Natural waters with pH's below 4.8 are becoming increasingly common in Ontario and Scandinavia (see Introduction); it is likely that the exercise capabilities of fish surviving in these lakes are similarly reduced. Interference with $O_2$ uptake (branchial mucus production) or blood $O_2$ transport (Bohr and Root effects due to blood acidosis) seem the most likely explanations (cf. Jones 1971), although elevated ionoregulatory costs during activity (Rao 1968; Farmer and Beamish 1969; Wood and Randall 1973) may also be involved. The protective effect of water hardness was again seen, CSS being significantly higher in hard water versus soft water for three acidic test pH's (Fig. 4).

In summary, the major point illustrated by the presented data is the complexity of acid toxicity and its sensitivity to various modifying factors. Acid toxicity is not an absolute for a particular pH value, but a variable dependent upon the water hardness, the nature of the acid, the activity level of the fish, and probably several other variables (e.g. temperature; Kwain 1975). Furthermore, the influence of any one of these modifying factors is not fixed but may change with the pH level and the presence or absence of other modifiers. Any extrapolation from laboratory toxicity data to field survival criteria should attempt to take these interactions into account.

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