Acclimation-Induced Change in Toxicity of Aluminum to Rainbow Trout (*Salmo gairdneri*)

P. L. Orr,[1] R. W. Bradley, J. B. Sprague,[2] and N. J. Hutchinson[3]

Department of Zoology, University of Guelph, Guelph, Ont. N1G 2W1

Orr, P. L., R. W. Bradley, J. B. Sprague, and N. J. Hutchinson. 1986. Acclimation-induced change in toxicity of aluminum to rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 43: 243–246.

When a group of rainbow trout (*Salmo gairdneri*) fingerlings was exposed to 87 μg Al/L for 1 and 2 wk, their threshold lethal concentration (LC50) increased to about 1.8 times the control LC50 of 175 μg Al/L. Raising sublethal exposure to 154 μg/L during the third week resulted in a similar ratio of 1.7 between LC50s of previously exposed and control fish. Thus, prior exposures of 0.5 and 0.9 of the control LC50 resulted in about the same increase in tolerance. The magnitude of the increase was similar to those reported in the literature for other metals. Such acclimation could assist in the survival of fish during spring and autumn surges of aluminum and other metals in surface waters acidified by atmospheric deposition of oxides of sulfur and nitrogen; those environmental changes in aluminum concentration parallel the concentrations used in this research. Experimental water simulated an acid lake with pH of 5.1–5.3 and total hardness of 11 mg/L.

L'exposition, pendant 1 ou 2 sem, d'un groupe d'alevins non vésiculés de truite arc-en-ciel (*Salmo gairdneri*) à une concentration d'aluminium de 87 μg/L s'est traduite par une augmentation du seuil de la concentration létale (CL_{50}) d'environ 1,8 fois par rapport aux témoins (175 μg Al/L). La concentration sublétale a été portée à 154 μg/L pendant une troisième semaine d'exposition et le rapport des CL_{50} des poissons déjà exposés et des témoins est demeuré semblable (1,7). Des expositions antérieures à 0,5 et 0,9 fois la CL_{50} des témoins se traduisaient donc par une augmentation semblable de la tolérance. Cette augmentation est du même ordre que celle signalée dans les publications pour d'autres métaux. Une telle acclimatation pourrait favoriser la survie des poissons pendant les montées soudaines, au printemps et à l'automne, des concentrations d'aluminium et d'autres métaux dans les eaux de surface acidifiées par le dépôt atmosphérique d'oxydes de soufre et d'azote. Les concentrations d'aluminium utilisées pour la présente étude sont du même ordre que celles notées dans le milieu naturel. L'eau utilisée pour l'étude simulait celle d'un lac acide de pH 5,1–5,3 et de dureté totale de 11 mg/L.

Received April 25, 1985
Accepted October 2, 1985
(J8213)

Reçu le 25 avril 1985
Accepté le 2 octobre 1985

Recent work has demonstrated that fish can increase their tolerance of certain metals if they have been previously exposed to a sublethal level of that metal (copper, Dixon and Sprague 1981b; Buckley et al. 1982; McCarter and Roch 1983, 1984; cadmium, Duncan and Klaverkamp 1983; zinc, Bradley and Sprague 1985; and mixtures, Roch and McCarter 1984a). If there were such acclimation to aluminum, it could be significant for survival of fish in waters which are being acidified by atmospheric fallout. Aluminum is one of the metals which increases in concentration in acid lakes and makes a major contribution to toxicity (Schofield and Trojnar 1980; Hutchinson 1984). It is present at sublethal levels during the early stages of acidification of most susceptible lakes (Hutchinson 1984), providing a potential stimulus for acclimation. Increased tolerance might allow fish to survive the dramatic surges in metal concentration which occur during snowmelt and autumn runoff (Driscoll et al. 1980; Schofield and Trojnar 1980).

The present study was designed to show whether fish acclimate to aluminum, as judged by an increase in their tolerance of the metal, and if so, the general magnitude of any change in tolerance. It tested the null hypothesis that prior exposure of trout to a sublethal concentration of aluminum ("pre-exposure") would not alter the lethal threshold of the metal.

The study also intended to show whether a very high level of pre-exposure would elicit a stronger acclimation response than an intermediate pre-exposure. Previous research with copper indicated that the increase in tolerance was proportional to the strength of prior exposure, within limits; pre-exposure to at least 0.18 of the LC50 was required to trigger an increase in tolerance, but there was a "plateau" for maximum increase in tolerance for a pre-exposure range from about 0.4 LC50 up to at least 0.6 LC50 (Dixon and Sprague 1981b). Accordingly, pre-exposures of 0.5 and 0.9 of a control LC50 were used in the present study to test the null hypothesis that there would be no difference between the subsequent tolerances (=incipient lethal levels or threshold LC50s, Fry 1971).

Methods

Procedures were those previously established in our laboratory for work on acclimation, and the descriptions in Dixon and Sprague (1981a, 1981b) are applicable for details of tank sizes, flow rates, fish source, feeding regimes, etc. An outline of the general protocol is repeated here, along with a description of items specific to this experiment.

[1]Present address: 1471 Seagram Avenue, Oakville, Ont. L6L 1W9.
[2]Author to whom reprint requests may be addressed.
[3]Present address: Dorset Research Centre, Ontario Ministry of Environment, Dorset, Ont. P0A 1E0.

Rainbow trout (*Salmo gairdneri*) of 3.5 g initial wet weight were held in flowing experimental water for 4 wk. Trout were then split into two groups of 300 and held in pre-exposure tanks for a further week. Aluminum at 87 μg/L was then started in one pre-exposure tank (day 0) and maintained until day 14; the other pre-exposure tank was the control. On day 14 the aluminum concentration was increased to 154 μg/L for pre-exposed fish. It was necessary to attain the higher pre-exposure level in two stages because preliminary experiments demonstrated that sudden exposure of naive trout to that level caused about 15% mortality.

Lethal tests were initiated on days 0, 7, 14, and 21 using samples of fish collected just before any change in pre-exposure level. There were 10 pre-exposed fish at each of six test concentrations including zero. Control fish that had no prior exposure to Al were tested simultaneously. The flow of test water at a given concentration was split between a test tank of pre-exposed fish and one of control fish to maintain identical conditions and avoid discrepancies of analysis. Randomization of fish and tanks was practised throughout. There was no mortality of fish in zero concentrations of the lethal tests, and mortality during pre-exposure was less than 1%. Each lethal test continued for 6 d and threshold or incipient lethal levels were established in 5 d or less. The 120-h LC50s were estimated by a computerized trimmed Spearman–Karber method (Hamilton et al. 1977), and statistical differences between pairs of LC50s were assessed by standard error of the difference using $P < 0.05$ as a criterion.

The soft water was derived from well water by reverse osmosis and from the university deionized supply, both run through ion-exchange columns. This was followed by the addition of 3.4% of the same hard well water to provide a mix of natural ions. Its quality during holding, pre-exposure, and testing was similar with total hardness 11.2 mg/L as $CaCO_3$ (SD = 4.0, N = 65), temperature 15°C (SD = 0.6, N = 158), and dissolved oxygen 9.0 mg/L (SD = 0.5, N = 55) or greater than 85% saturation. Scans of three samples of control water by inductively coupled plasma emission gave the following average values (milligrams per litre): Na, 9.3; Ca, 2.9; Mg, 1.1; Si, 0.6; K, <1; P, <0.5; B, 0.009; and (micrograms per litre): Pb and Zn, less than the detection limit of 50; Al, Cd, Cr, Fe, and Mn, <10; Cu, <8. The experimental water was acidified by an automatic titrating unit dispersing dilute H_2SO_4. During pre-exposure, pH averaged 5.25 (SD = 0.21, N = 44). The pH values in test tanks for control and pre-exposed fish were nearly identical but they varied slightly among the four successive lethal tests, averaging 5.22 (SD = 0.16), 5.31 (SD = 0.17), 5.09 (SD = 0.15), and 5.18 (SD = 0.09) with $N \geq 80$ in each case. The experimental pH not only simulated an acid lake, but yielded maximal toxicity of aluminum, and minimal changes of aluminum from one chemical form to another.

Stocks solutions of aluminum contained reagent grade $Al_2(SO_4)_3 \cdot 18H_2O$, deionized water, and H_2SO_4 to lower pH to 2.8–3.6 as required to achieve the desired pH in test tanks (the aluminum salt itself had some acidifying effect). Average measured concentrations of total aluminum are used throughout this paper, based on seven samples during pre-exposure and two to four samples for each concentration of each lethal test. Samples were acidified to pH < 2.0. Precision of analysis by flameless AAS was good, judging by the average coefficient of variation of 5.4% on five triplicate samples submitted without the knowledge of the analyst. Measured test concentrations averaged 120% of nominal values but results for each test concentration were relatively constant, with an average coefficient of variation of 6.6% through the four lethal tests, scarcely greater than the analytical variation.

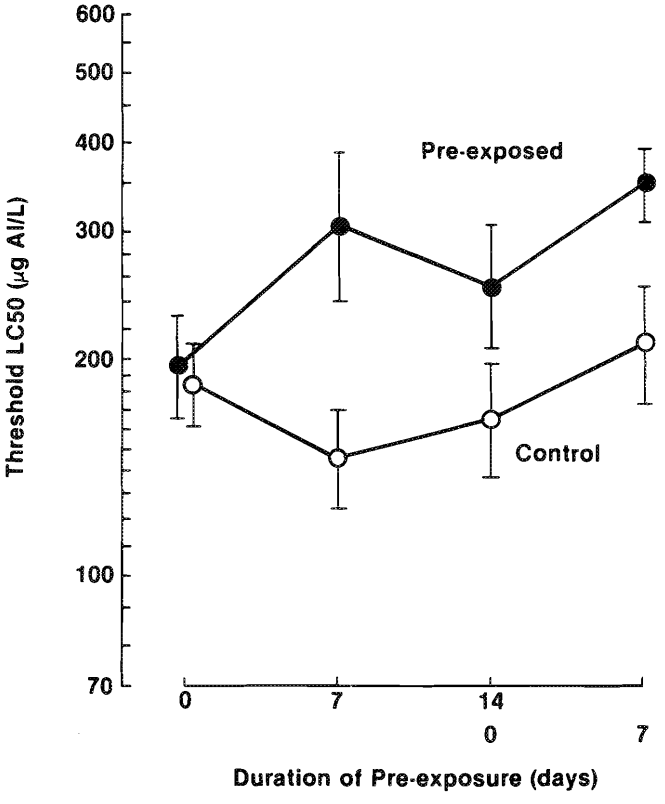

FIG. 1. Changed tolerance of aluminum in rainbow trout pre-exposed to sublethal levels of the metal. The pre-exposed fish were held in 87 μg Al/L for 14 d and then in 154 μg/L for a further 7 d. Control fish were held in <6 μg/L. Total hardness of experimental water averaged 11 mg/L as $CaCO_3$, and pH averages were 5.1–5.3. The 95% confidence limits of the LC50s are shown.

Results

Control LC50s showed some variation (Fig. 1) but the geometric average of the four LC50s determined for unexposed fish at different times was 175 μg/L, very close to the day 0 mean value of 189 μg/L. Thus, the initial 2-wk pre-exposure to 87 μg/L may be considered to represent 0.50 of the control LC50, and the final week of exposure to 154 μg/L represented 0.88 of the control LC50.

After 7 d of pre-exposure, fish had increased their LC50 to 2.1 times that of control fish tested at the same time, and after 14 d the ratio was 1.5 times (Fig. 1). Differences between control and pre-exposure LC50s were statistically significant on each day. An average ratio of 1.8 might be taken to represent the increase in tolerance of aluminum following pre-exposure to 0.50 LC50 (see Discussion). On day 21, after increasing the acclimation concentration, pre-exposed fish were 1.7 times more tolerant than controls, again a significant difference.

Pre-exposed fish did not differ in size from control fish. The trout grew during holding and experimental periods to about 5.5 g on day 0 and 7.9 g on day 21. No statistical difference was demonstrated in wet weights or lengths of pre-exposed fish compared with control fish (three-way ANOVA). Mean wet weights (grams) of pre-exposed trout (control trout in parenthe-

ses) used in tests were as follows: day 0, 5.2 (5.8); day 7, 5.9 (6.0); day 14, 6.3 (6.6); day 21, 7.8 (8.0). Coefficients of variation averaged 29%.

Discussion

The tolerances of aluminum shown by pre-exposed fish were significantly higher than those of control fish and the first null hypothesis is therefore rejected. We conclude that a 1- or 2-wk exposure of rainbow trout to half the lethal level of aluminum results in increased tolerance by a factor in the vicinity of 1.5−2.1, or approximately 1.8-fold.

At 21 d, after increasing the aluminum acclimation concentration, the tolerance ratio of pre-exposed to control fish was similar, at 1.7. We do not consider that our results demonstrate any change in degree of acclimation from 7 to 21 d, either with duration of pre-exposure or with level of pre-exposure. There is within-test variability associated with each LC50 determination as indicated by the confidence limits in Fig. 1, and slight changes of LC50s in opposite directions could greatly alter the ratios. The small drift in control LC50s might be related to change in aluminum toxicity at slightly different test pHs, but in fact such differences, or greater ones, are usual in repeated tests within a laboratory (Sprague 1984). There are no grounds for rejecting the second null hypothesis, and we conclude that pre-exposure to 0.9 LC50 or 0.5 LC50 results in the same degree of increased tolerance. It appears that the "plateau" of maximum increase in tolerance, found for pre-exposures to copper of 0.4−0.6 LC50 by Dixon and Sprague (1981b), may apply to other metals such as aluminum and extend upwards to 0.9 LC50.

Most other values in the literature for lethal levels of aluminum for fish tend to be higher than our control values. Near-threshold LC50s for brook trout (*Salvelinus fontinalis*) fry would be about 420 μg Al/L at pH 5.2 and hardness 5 mg/L (Driscoll et al. 1980), or in the vicinity of 350 μg Al/L judging from data of Schofield and Trojnar (1980), also at pH 5.2. Brook trout fingerlings were reported to show 4-d LC50s of 380 μg Al/L at pH 5.6 and hardness 2 mg/L and 3000 μg Al/L at the same pH and hardness 18 mg/L (Tandjung et al. 1982). At alkaline pH, which would involve major changes in speciation of aluminum, LC50s for rainbow trout are in the thousands of micrograms of total aluminum per litre (Freeman and Everhart 1971).

The pre-exposure to aluminum apparently did not affect growth of trout. Any metabolic cost of activating defence mechanisms must have been small. In similar experiments showing acclimation to copper, growth was reduced for pre-exposure of 0.29 LC50 and higher (Dixon and Sprague 1981b; Buckley et al. 1982). Growth differences could, of course, be the result of changes in appetite and food consumption (e.g. Freeman and Everhart 1971), food conversion efficiency, or activity.

These findings could have relevance for fish in acidified surface waters, in which total aluminum levels of 20−320 μg/L have been reported from the U.S. Adirondacks, 20−600 μg/L from southern Norway, and 190−200 μg/L in Sudbury lakes of Canada (Schofield and Trojnar 1980; Wright et al. 1980; Gunn and Keller 1980). Some of this aluminum forms complexes with inorganic ions, and much of it may be bound by organic ligands and be practically nontoxic (Driscoll et al. 1980; Baker and Schofield 1982). Nevertheless, labile inorganic aluminum in acidified creeks may increase from 50−150 μg/L during much of the year to 200−300 μg/L during spring and autumn runoff (Driscoll et al. 1980) (i.e. the same ranges of "pre-exposures" and LC50s that are reported in the present experiment). The modest degree of acclimation shown by the trout might therefore be of significance in surviving spring and autumn surges of aluminum, along with other metals. Zinc and copper are the two other toxic metals which are mobilized and are therefore important in waters undergoing acidification (Hutchinson 1984) and to which fish can also acclimate (Chapman 1978; Dixon and Sprague 1981b; Bradley and Sprague 1985).

The magnitude of the increase in aluminum tolerance is similar to those reported in the literature for other metals, following pre-exposure to "medium" levels of 0.35−0.6 LC50 (1.9 times for copper and rainbow trout, Dixon and Sprague 1981b; 1.6−1.9 times for copper and coho salmon (*Oncorhynchus kisutch*), McCarter and Roch 1983, 1984; 2.5 times for zinc and rainbow trout, Bradley and Sprague 1985; 1.8 times for cadmium and white sucker (*Catostomus commersoni*), Duncan and Klaverkamp 1983; 1.7 times for cadmium and fathead minnows (*Pimephales promelas*), Benson and Birge 1985; 1.7 times for mixtures of the above metals and chinook salmon (*Oncorhynchus tshawytscha*), Roch and McCarter 1984b; and up to 1.5 times for these mixtures and rainbow trout, Roch and McCarter 1984a). Previous work on acclimation of fish to aluminum was not carried to the stage of estimating tolerance or LC50s. Guthrie and Schofield (1982) found less mortality among brook trout fry and yearlings gradually acclimated during 4−5 d to final levels of pH 5 and 200 or 400 μg Al/L than among fish suddenly exposed. These concentrations of aluminum bracket the LC50s we found for acclimated rainbow trout. Research that assessed acclimation in terms of resistance times instead of tolerance levels has been omitted from discussion.

The rapid development of increased tolerance during 7 d is similar to that found for copper and zinc in our laboratory (Dixon and Sprague 1981b; Bradley and Sprague 1985) and to speed of increase in copper tolerance of coho salmon (McCarter and Roch 1983) and cadmium tolerance of white sucker (Duncan and Klaverkamp 1983). The similarity in degree and rate of acclimation between aluminum and the other metals suggests a similar defence mechanism. The mechanism for acute lethal action of aluminum is also similar to that of other metals, with general necrosis of gills and, in some cases, mucous accumulation with likely results of anoxia and impaired ion exchange (Muniz and Leivestad 1980; Schofield and Trojnar 1980; Baker and Schofield 1982; Tandjung et al. 1982).

We found no clear evidence of a change in degree of acclimation as the pre-exposure continued, and results in the literature for other metals are in general agreement. There appeared to be a trend for copper tolerance to increase from day 7 to day 21 of pre-exposure, but changes were too slight to draw definite conclusions (Dixon and Sprague 1981b). Duncan and Klaverkamp (1983) concluded that there was no difference in cadmium tolerance between 7 and 14 d of pre-exposure. For zinc, Bradley and Sprague (1985) found pre-exposed to control ratios of 2.5 after 1 wk, 2.7 at 2 wk, and 2.3 at 3 wk, again probably chance variation. McCarter and Roch (1983) concluded that there was a steady loss of acquired tolerance during a long pre-exposure to copper, but calculations from their pairs of pre-exposed and control LC50s indicate that the loss was only about 17% from the first to the eighth week.

Other researchers have compared the effects of medium and

high pre-exposure levels, with various findings. As mentioned above, copper tolerance of trout was much the same for pre-exposures of 0.4 and 0.6 LC50 (Dixon and Sprague 1981b). However, coho salmon showed apparently greater increase of copper tolerance at higher pre-exposures (about 1.6, 1.7, 1.9, and 2.2 times control values for pre-exposures of 0.38, 0.50, 0.55, and 0.75 LC50, McCarter and Roch 1983, 1984). White sucker also showed greater gain in tolerance of cadmium (2.45 times) for a high pre-exposure of 0.66 LC50 than they did (1.75 times) for a medium pre-exposure of 0.37 LC50 (Duncan and Klaverkamp 1983). It is difficult to reconcile these findings but we repeat that the ratios derived from individual LC50s could vary by chance, aside from possible species differences.

Acknowledgements

This research was supported by an operating grant from the Natural Sciences and Engineering Research Council of Canada to J. B. Sprague. We thank Prof. E. D. Stevens of the Department of Zoology for his refreshing approach to administrative matters during the research.

References

BAKER, J. P., AND C. L. SCHOFIELD. 1982. Aluminum toxicity to fish in acidic waters. Water Air Soil Pollut. 18: 289–309.

BENSON, W. H., AND W. J. BIRGE. 1985. Heavy metal tolerance and metallothionein induction in fathead minnows: results from field and laboratory investigations. Environ. Toxicol. Chem. 4: 209–217.

BRADLEY, R. W., AND J. B. SPRAGUE. 1985. Acclimation of rainbow trout to zinc: kinetics and mechanism of enhanced tolerance induction. J. Fish. Biol. 22. (In press)

BUCKLEY, J. T., M. ROCH, J.A. MCCARTER, C. A. RENDELL, AND A.T. MATHESON. 1982. Chronic exposure of coho salmon to sublethal concentrations of copper — I. Effect of growth, on accumulation and distribution of copper, and on copper tolerance. Comp. Biochem. Physiol. 72C: 15–19.

CHAPMAN, G. A. 1978. Effects of continuous zinc exposure on sockeye salmon during adult-to-smolt freshwater residency. Trans. Am. Fish. Soc. 107: 828–836.

DIXON, D. G., AND J. B. SPRAGUE. 1981a. Acclimation-induced changes in toxicity of arsenic and cyanide to rainbow trout, *Salmo gairdneri* Richardson. J. Fish Biol. 18: 579–589.

1981b. Acclimation to copper by rainbow trout (*Salmo gairdneri*) — a modifying factor in toxicity. Can. J. Fish. Aquat. Sci. 38: 880–888.

DRISCOLL, C. T. JR., J. P. BAKER, J. J. BISOGNI JR., AND C. L. SCHOFIELD. 1980. Effect of aluminum speciation on fish in dilute acidified waters. Nature (Lond.) 284: 161–164.

DUNCAN, D. A., AND J. F. KLAVERKAMP. 1983. Tolerance and resistance to cadmium in white suckers (*Catostomus commersoni*) previously exposed to cadmium, mercury, zinc, or selenium. Can. J. Fish. Aquat. Sci. 40: 128–138.

FREEMAN, R. A., AND W. H. EVERHART. 1971. Toxicity of aluminum hydroxide complexes in neutral and basic media to rainbow trout. Trans. Am. Fish. Soc. 100: 644–658.

FRY, F. E. J. 1971. The effect of environmental factors on the physiology of fish, p. 1–98. *In* W. S. Hoar and D. J. Randall [ed.] Fish physiology. Vol. VI. Academic Press Inc., New York, NY. 559 p.

GUNN, J. M., AND W. KELLER. 1980. Enhancement of the survival of rainbow trout (*Salmo gairdneri*) eggs and fry in an acid lake through incubation in limestone. Can. J. Fish. Aquat. Sci. 37: 1522–1530.

GUTHRIE, C. A., AND C. L. SCHOFIELD. 1982. Acclimation of brook trout (*Salvelinus fontinalis*) to increased acidity and aluminum concentration, p. 351. *In* R.E. Johnson [ed.] Acid Rain/Fisheries: Proceedings of an International Symposium on Acid Precipitation and Fishery Impacts in Northeastern North America. American Fisheries Society, Bethesda, MD. 357 p.

HAMILTON, M. A., R. C. RUSSO, AND R. V. THURSTON. 1977. Trimmed Spearman–Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 11: 714–719.

HUTCHINSON, N. J. 1984. Studies of trace metal mixtures and factors modifying their lethality to American flagfish in soft, acid water. Ph.D. thesis, University of Guelph, Guelph, Ont. 189 p.

MCCARTER, J. A., AND M. ROCH. 1983. Hepatic methallothionein and resistance to copper in juvenile coho salmon. Comp. Biochem. Physiol. 74C: 133–137.

1984. Chronic exposure of coho salmon to sublethal concentrations of copper. III. Kinetics of metabolism of methallothionein. Comp. Biochem. Physiol. 77C: 83–87.

MUNIZ, I. P., AND H. LEIVESTAD. 1980. Toxic effects of aluminum on the brown trout, *Salmo trutta*, L., p. 320–321. *In* D. Drablos and A. Tollan [ed.] Proceedings of the International Conference on the Ecological Impact of Acid Precipitation. SNSF Project, Oslo, Norway.

ROCH, M., AND J. A. MCCARTER. 1984a. Hepatic methallothionein production and resistance to heavy metals by rainbow trout (*Salmo gairdneri*) — I. Exposed to an artificial mixture of zinc, copper and cadmium. Comp. Biochem. Physiol. 77C: 71–75.

1984b. Metallothionein induction, growth, and survival of chinook salmon exposed to zinc, copper, and cadmium. Bull. Environ. Contam. Toxicol. 32: 478–485.

SCHOFIELD, C. L., AND J. R. TROJNAR. 1980. Aluminum toxicity to brook trout (*Salvelinus fontinalis*) in acidified waters, p. 341–362. *In* T. Y. Toribara, M. W. Miller, and P. E. Morrow [ed.] Polluted rain. Plenum Press, New York, NY. 500 p.

SPRAGUE, J. B. 1984. Factors modifying toxicity, p. 124–163. *In* G. M. Rand and S. R. Petrocelli [ed.] Fundamentals of aquatic toxicology: methods and applications. Hemisphere Publishing Corporation, Washington, DC.

TANDJUNG, S. D., D. V. ROTTIERS, AND R. L. HERMAN. 1982. Histopathological studies on the effects of acid stress to brook trout *Salvelinus fontinalis* (Mitchell) in the presence of sublethal concentrations of aluminum, p. 357. *In* R. E. Johnson [ed.] Acid Rain/Fisheries: Proceedings of an International Symposium on Acidic Precipitation and Fishery Impacts in Northeastern North America. American Fisheries Society, Bethesda, MD. 357 p.

WRIGHT, R. F., N. CONROY, W. T. DICKSON, R. HARRIMAN, A. HENRIKSEN, AND C. L. SCHOFIELD. 1980. Acidified lake districts of the world: a comparison of water chemistry in southern Norway, southern Sweden, southern Scotland, the Adirondack Mountains of New York, and southeastern Ontario, p. 377–379. *In* D. Drablos and A. Tollan [ed.] Proceedings of the International Conference on the Ecological Impact of Acid Precipitation. SNSF Project, Oslo, Norway.