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AVOIDANCE REACTIONS OF RAINBOW TROUT TO ZINC SULPHATE SOLUTIONS

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Abstract—Rainbow trout (*Salmo gairdnerii* Richardson) showed strong avoidance reactions to sublethal concentrations of zinc sulphate. The threshold avoidance level was 5.6 μg/l of zinc added to laboratory water. This is only 0.01 of the lethal threshold concentration. There were no significant differences in threshold avoidance levels at 9.5° and 17°C., nor when background of zinc in the water was increased during acclimation and/or testing, from 3 μg/l to 13 μg/l.

THE PURPOSES of this research were to show: firstly whether rainbow trout spontaneously avoid such low concentrations of zinc sulphate as do Atlantic salmon (SPRAGUE, 1964); secondly the effect of water temperature on the avoidance reaction; and thirdly the effect of an increased "background" of zinc sulphate in the water.

MATERIALS AND METHODS

Rainbow trout (*Salmo gairdnerii* Richardson) were obtained in May 1964 from Lindloff hatchery, Cape Breton Island, Nova Scotia, operated by the Canadian Department of Fisheries. Fish were acclimated at St. Andrews, at the test-temperature, for at least 3 weeks, in cylindrical Fiberglas tanks with about 150 l. of aerated and continuously-flowing water. Fish were fed to repletion once a day with shavings of frozen beef liver, and remained healthy in appearance. Size-range of fish at time of testing was 6.5–12.0 cm.

Laboratory water came from a soft water lake, and was essentially as previously described (SPRAGUE, 1964). Its total hardness was between 13 and 15 mg/l as $CaCO_3$ during these experiments. Test temperatures were within 0.5° of 17°C or 9.5°, and acclimation temperatures within 1.0°. Average pH was 7.2 and range was pH 7.0–7.5.

Stock solutions and test-concentrations of zinc sulphate were analyzed by the mixed-color dithizone method and all concentrations are stated as μg/l of zinc.

Test apparatus and procedure

The aim was to document the spontaneous, untrained response made by fish when they were presented with a distinct choice between clean water and a given concentration of pollutant. The boundary between "clean" and "polluted" water was kept as sharp as possible, similar to the approach of SHELFORD (1917), BISHAI (1962), and JONES (1964).

The avoidance apparatus was essentially a horizontal Plexiglas tube or trough, 1.14 m long and 14.6 cm inside diameter. Water flowed into each end and out at the centre. Changes from a previous description (SPRAGUE, 1964) were: (1) improved visual shielding; (2) flow control by constant heads and capillaries to eliminate pump vibration; and (3) water flow increased from 2 to 3 l/min into each end.

Performance of the apparatus was better than previously described because of increased water flow. Tests with dye showed a sharp vertical boundary at the middle, which re-established itself within 1 min after major disturbance. All traces of dye cleared from the trough in 6 min. Chemical analyses of water samples taken at the end of each test-period showed that desired concentrations of pollutants were being attained within limits of accuracy of the chemical method.

The improved test procedure was as follows:

(1) A continuous flow of 3 l/min of water entered each end of the trough.

(2) A single fish was put into the trough and allowed 30 min for accustomization.

(3) The weakest concentration of pollutant was tested first by adding a flow of stock solution to one water inflow. For a given concentration the "polluted" side was alternated with respect to successive test-fish. Extraneous left- or right-hand effects were thus balanced. Pollutant flow continued for 20 min and during the last 10 min the fish's movements along the trough were recorded.

(4) A water sample was taken half-way along the polluted side for measurement of zinc concentration, and temperature and pH were measured *in situ* on each side.

(5) Pollutant was stopped and the apparatus cleared itself during the next 10 min.

(6) The next stronger concentration in the series was tested by adding stock solution on the alternate side to that used in step (3).

(7) Steps (4), (5), and (6) were repeated until the series of concentrations was completed. The fish was discarded.

(8) The procedure was repeated on another fish.

Checks indicated that responses of fish at the last and highest concentrations were little different, whether or not they had previously been exposed to the stepped series of lower concentrations.

Results are expressed in two ways as previously described (SPRAGUE, 1964). In the first, the time which a fish spent in the clean side of the trough was calculated as a percentage of the total test-time of 10 min. A time-response of 50 per cent indicates perfectly neutral response, time-responses from 51 to 100 per cent indicate avoidance, and responses from 0 to 49 per cent indicate preference. This is illustrated in FIG. 1.

The second method analyzed performance of a fish as an all-or-none response. Relative shortness of individual "visits" on each side of the trough was compared for significant difference (5 per cent level) by the Kolmogorov–Smirnov test. FIGURE 2 shows part of this analysis.

RESULTS*

Rainbow trout avoided solutions of zinc sulphate in a regular and predictable pattern (FIG. 1). Test-results for both temperatures and both backgrounds of zinc are included in FIG. 1, since neither parameter affected avoidance reactions. This lack of effect is described at the end of the Results section for simplicity of presentation.

At the lowest concentration, 1 μg/l Zn, the median time-response is nearly neutral; i.e. only a little above 50 per cent. The overall response at this lowest concentration may be considered as a control for the remainder of the test-concentrations. At each higher concentration, fish spent successively more time in clean water, demonstrating

* Data are recorded in original manuscript number 1073 on file at the Biological Station, St. Andrews, N.B., copies on request.

stronger avoidance reactions. The strong response at 100 μg/l Zn is nearly maximal, since results are similar at the next higher concentration, 320 μg/l.

Estimating the threshold response

The threshold may be estimated by using the all-or-none feature of the same data. Standard probit analysis may be applied (LITCHFIELD and WILCOXON, 1949) by plotting the proportion of fish showing net significant avoidance at each concentration.

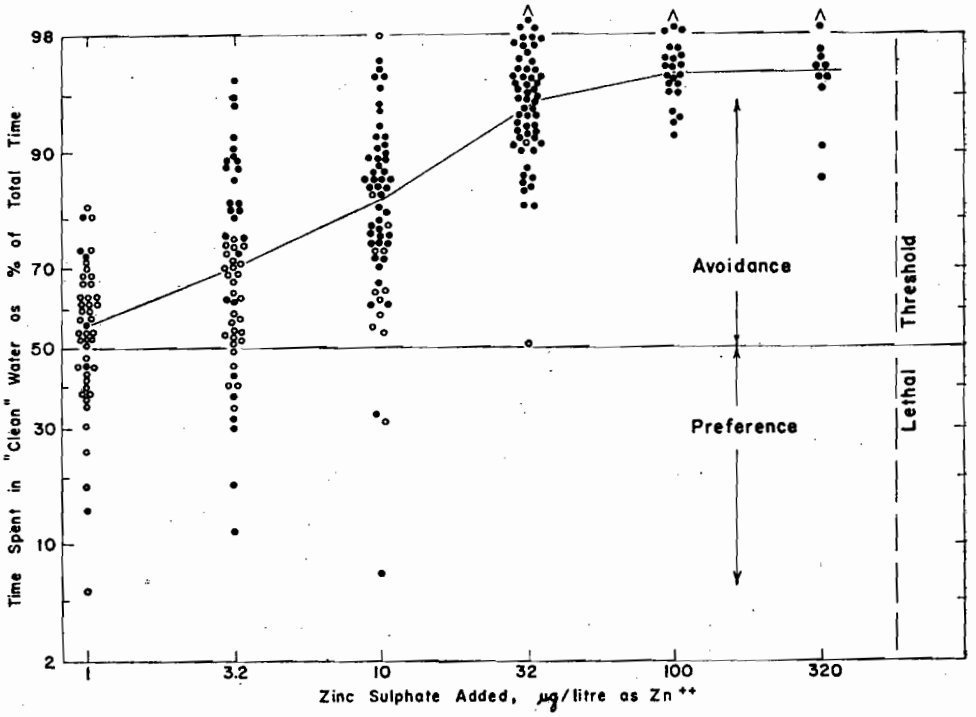

FIG. 1. Avoidance of solutions of zinc sulphate by rainbow trout, with performance of each fish treated as a graded response. A solid point represents statistically significant choice by one fish, an open point a non-significant response. A response of 50 per cent is neutral. The line represents median response. Concentration is on a logarithmic scale, and response on a probability scale.

Net significant avoidance is calculated as the number of fish showing significant avoidance minus the number of fish showing significant preference, divided by the total number of fish at that concentration and multiplied by 100. This has been plotted in FIG. 2 and the straight line is obviously an excellent fit (p value of 0.98).

Most modern investigators would agree that a threshold is "that stimulus intensity at which a positive response can be expected in 50 per cent of the presentations" (BUERKLE, 1967). Accordingly, in this experiment the *threshold avoidance level* is 5.6 μg/l Zn, the concentration at which the fitted line in FIG. 2 cuts the 50 per cent response line. The 95 per cent confidence limits are 4.3 and 7.3 μg/l Zn (LITCHFIELD and WILCOXON, 1949).

There was a natural trace of zinc, approximately 3 μg/l Zn, in our laboratory water. Even this becomes significant in the experiment because of the low threshold avoidance level. The trout actually avoided a threshold of $3+5.6 = 8.6$ μg/l Zn in preference for 3 μg/l.

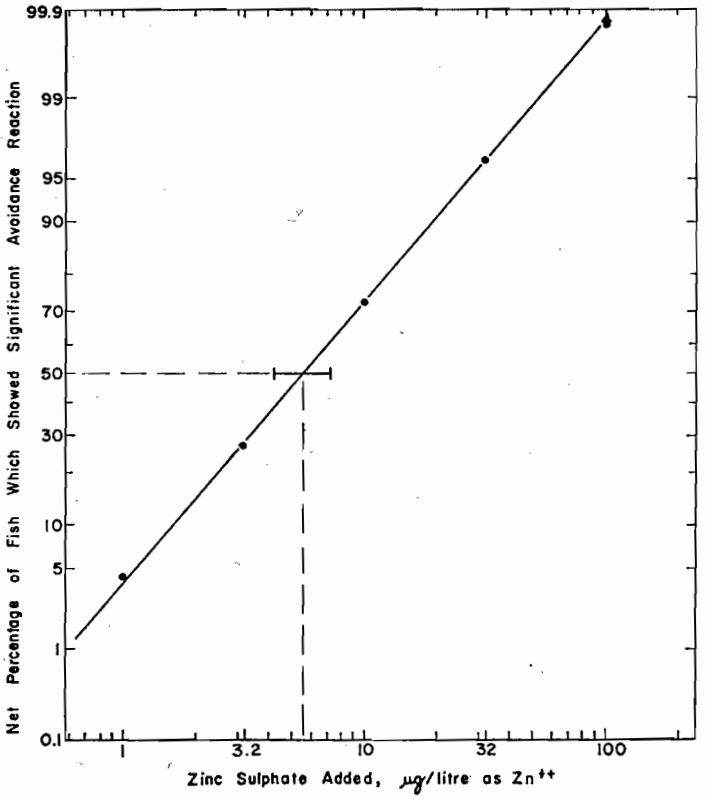

FIG. 2. Avoidance of solutions of zinc sulphate by rainbow trout, with performance of fish treated as a quantal response. Based on data shown in FIG. 1. Threshold avoidance level is determined by 50 per cent significant response. The 95 per cent confidence limits of threshold are shown.

There was also about 2 μg/l of copper in laboratory water. Copper and zinc are additive in causing both lethal and avoidance reactions (LLOYD, 1961; SPRAGUE, 1964; SPRAGUE and RAMSAY, 1965). However it seems reasonable to assume that trout can distinguish between salts of the two metals in solution, and therefore a trace of copper should not affect results of this experiment.

Effect of water temperature

Results described above include a comparison of avoidance reactions at 17°C and 9.5°C, on trout fully acclimated to the test-temperature. Threshold avoidance levels (and 95 per cent confidence limits) for 17°C were 4.3 (3.0 and 6.2) μg/l, and for 9.5°C were 5.4 (3.2 and 9.1) μg/l of zinc added to laboratory water. The slight difference in thresholds is not statistically significant, and there were no apparent differences in

other aspects of the data. Results from the two temperatures were therefore combined and included in the general analysis.

Effect of background zinc concentration

One tank of fish was acclimated for 3 weeks to ordinary laboratory water containing only 3 μg/l Zn, another tank received an additional 10 μg/l. Some fish from each tank were tested in the usual way, others were tested with an added background of 10 μg/l zinc in both sides of the trough.

Results were similar for the four combinations of conditions. Threshold avoidance levels were 4–7 μg/l of added zinc with no significant differences, and no apparent cause–effect relation to background. Results were accordingly combined and included in the general results given above.

DISCUSSION

It is reasonable that fish should show similar avoidance reactions to zinc at 9.5°C and 17°C. There seems to be no *a priori* reason why reactions should be different. It is possible that somewhat different avoidance responses would be obtained in tests near the limits of the temperature range for rainbow trout, about 10° higher and lower than the temperatures tested.

With increased background concentration of zinc, we would expect a reduction in the fish's ability to discriminate and avoid added zinc. ISHIO (1965) concludes that such proportional reduction of discrimination follows the Weber–Fechner relation. Such a change was not found in the present work when the background was increased from 3 to 13 μg/l of zinc. Perhaps a further increase in background would have raised the threshold avoidance level.

The remarkably low avoidance threshold of 5.6 μg/l is just about 0.01 times the lethal threshold concentration of 570 μg/l Zn for rainbow trout at this water hardness (LLOYD and HERBERT, 1962). That is, under these experimental conditions, rainbow trout avoided 0.01 *toxic units* of zinc sulphate (SPRAGUE and RAMSAY, 1965).

The threshold avoidance level for rainbow trout is almost ten times lower than the threshold of 53 μg/l Zn shown by Atlantic salmon under the same experimental conditions (SPRAGUE, 1964). The difference is of course statistically significant (LITCHFIELD and WILCOXON, 1949). The difference between the two species of *Salmo* may not be in sensory perception or in spontaneous dislike of dissolved zinc sulphate, but in behaviour characteristics. Young salmon tend to sit in one place in our test-trough while rainbow trout swim more freely and thus become aware of any available choice between waters. This relative immobility of small salmon has been commented upon by HÖGLUND (1961).

In any case it is evident that the previously-reported avoidance by salmon of sublethal concentrations of heavy metals is in no way peculiar. Rainbow trout avoid even lower levels. Other authors confirm avoidance of sublethal zinc concentrations by fish. ISHIO (1965) reports avoidance of 0.45 of the lethal concentration, and SYAZUKI (1964), 0.3 of the lethal concentration, not as low as our threshold of 0.01 toxic units of zinc for rainbow trout. A puzzling exception is JONES' (1964) summary showing that sticklebacks and some other fish usually failed to avoid mildly-lethal concentrations of metal, including zinc sulphate.

Comparison with avoidance reactions to other types of pollutants will be made in a paper to be published in the proceedings of the fourth international conference on water pollution research, to be held September 1968.

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RESUME

Les truites arc-en-ciel (*Salmo gairdnerii* Richardson) ont évité fortement les concentrations sous-léthales de sulfate de zinc. Les truites ont évité un seuil de 5.6 μg/l de zinc, ajouté à l'eau du laboratoire. Ce seuil n'est que 0.01 du seuil de concentration léthale. Les réactions des truites démontrent aucune différence significative d'éviter le zinc, aux températures de 9.5° et 17°C. Egalement, les seuils qui furent évités n'étaient pas changés si la concentration de zinc dans l'eau du laboratoire était élevée de 3 μg/l à 13 μg/l pendant l'acclimatation ou durant la période d'essai.

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