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AVOIDANCE REACTIONS OF SALMONID FISH TO REPRESENTATIVE POLLUTANTS

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AVOIDANCE of polluted waters by fish is often named as one of four or five probable sublethal effects of pollution. However, there have been relatively few investigations to demonstrate whether avoidance reactions are in fact of great importance.

The purpose of the present research was (1) to determine whether there is a general pattern of spontaneous avoidance reactions by fish, (2) to attempt extrapolation of the laboratory findings to field situations, to predict behaviour of fish if their natural habitat were affected by these pollutants.

MATERIALS AND METHODS

Rainbow trout (*Salmo gairdnerii* Richardson) and Atlantic salmon (*Salmo salar* L.) were obtained from hatcheries of the Canada Department of Fisheries. Size-range during tests was 7.7 to 14.8 cm. Acclimation and feeding followed standard practice (Sprague, in press).

The laboratory water was very soft, 13 to 16 mg/l. hardness as $CaCO_3$; other qualities have been described (Sprague, 1964). Water passed through an activated carbon filter and gave no chlorine reaction. Test temperatures were within 0.2° of 17°C, acclimation within 1.0°. Range of pH was 7.0 to 7.5, except that tests with detergent had pH 7.9 at the highest concentration, and tests with chlorine had pH 8.4 maximum. These pH values apparently would not in themselves cause avoidance (Ishio, 1965; Bishai, 1962; Jones, 1964).

The avoidance apparatus was a horizontal plexiglass trough. Water flowed into each end and out the centre, with pollutant on one or other side (Sprague, in press). In tests with pulp mill waste, 2 l./min total liquid entered each end, instead of the usual 3 l./min. Water samples confirmed theoretical concentrations of the other three pollutants, within accuracy limits of chemical tests.

Pollutants

"New Nylon Dreft" was purchased retail in 1961. It contained no bleach, 20% $NaSO_4$, 32% complex phosphates, and 28% alkyl benzene sulphonate (ABS). Test-concentrations are stated as mg/l. of ABS measured by the methylene blue method. Phenol concentrations were measured by the Gibb's method as mg/l. of phenol. Chlorine solutions were made from calcium hypochlorite, and standardized as mg/l. of available chlorine by the orthotolidine method.

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Neutralized bleached kraft pulp mill effluent (BKME) was standardized and made up exactly as described by Betts and Wilson (1966), and came from the same mills. Mixed and neutralized BKME was used in experiments within 4 days of generation at the mill. Calculated concentrations of BKME are stated as parts per million or percentage by volume. Other experiments used unbleached Kraft effluent. Concentrations are stated so as to be equivalent to BKME in content of Kraft screen room effluent, i.e. as if water were substituted for the other two components of BKME (Betts and Wilson, 1966).

RESULTS WITH DISCUSSION*

Avoidance of ABS detergent

At the lowest concentration tested, 0.001 mg/l. of ABS, overall response was neutral (Fig. 1). That is, for eleven fish tested, the median response was to spend about half of the ten-minute test-period swimming in the "polluted" side, and half in the "clean" side of the trough. Time-responses of individual fish were scattered above and below this median response.

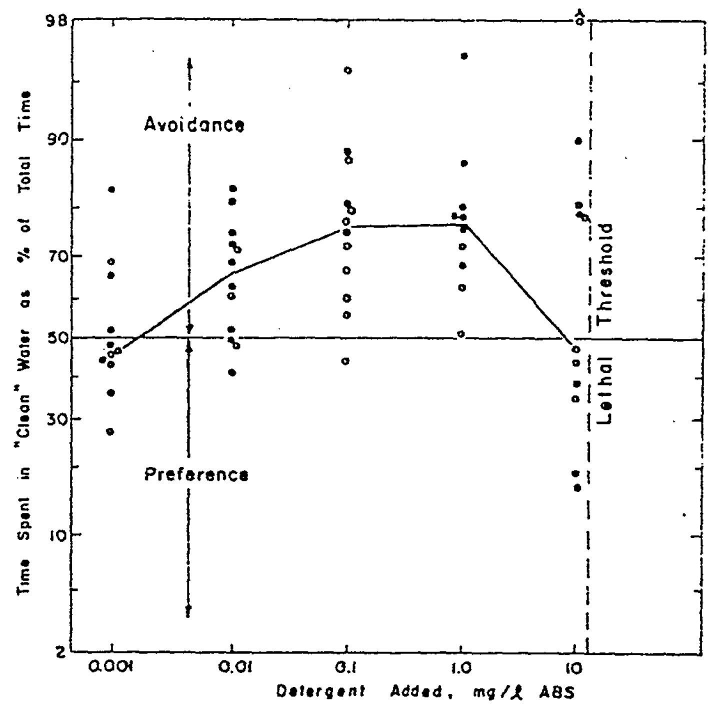

FIG. 1. Avoidance of solutions of ABS detergent by rainbow trout, with performance of each fish treated as a graded response. A solid circle represents statistically significant choice by one fish, an open circle a non-significant response. A response of 50% is neutral. The line represents median response. Concentration is on a logarithmic scale, and response on a probability scale. The unexpected lack of avoidance at 10 mg/l. seems to be because some fish were confused.

* Data are in original manuscript number 1074 on file at the Biological Station, St. Andrews, N.B., copies on request.

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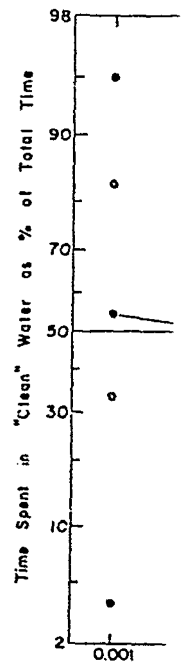

FIG. 2. Lack of av

At intermediate concentrations of 0.01, 0.1, and 1.0 mg/l. ABS, avoidance response increased only gradually.

At 10 mg/l. some trout showed avoidance, but others preferred detergent. Median time-response accordingly dropped to approximately 50%. Some fish were obviously excited, judging from rapid movements. They seemed aware of the detergent, apparently disliked it, but seemed confused and incapable of avoiding it. Permanent functional destruction of chemoreceptors is unlikely, requiring several hours at this concentration (Bardach *et al.*, 1965). Temporary impairment, i.e. sensory adaptation, is a possibility. Or perhaps detergent rinses from the sensory receptors slowly—after being in strong detergent solution, fish would not recognize clean water when they entered it. Inability of trout to avoid detergent would probably persist into lethal concentration (anything higher than 12 mg/l., unpublished results).

An estimate of the threshold avoidance level was made by probit analysis of the proportions of fish showing statistically significant avoidance (Sprague, in press). The results for 10 mg/l. were excluded. The threshold is 0.37 mg/l. of ABS. Its 95% confidence limits are extremely wide, 0.026 mg/l. and 5.3 mg/l., because avoidance changes gradually with concentration. This threshold is somewhat above the concentration of 0.1 to 0.2 mg/l. ABS reported for many rivers, and somewhat below the 0.5 mg/l. present in some rivers, in U.S.A. (Bardach *et al.*, 1965).

Avoidance of Phenol

Rainbow trout did not avoid sublethal solutions of phenol. At each concentration from 0.001 to 10 mg/l., overall time-response was about neutral (Fig. 2). Net numbers

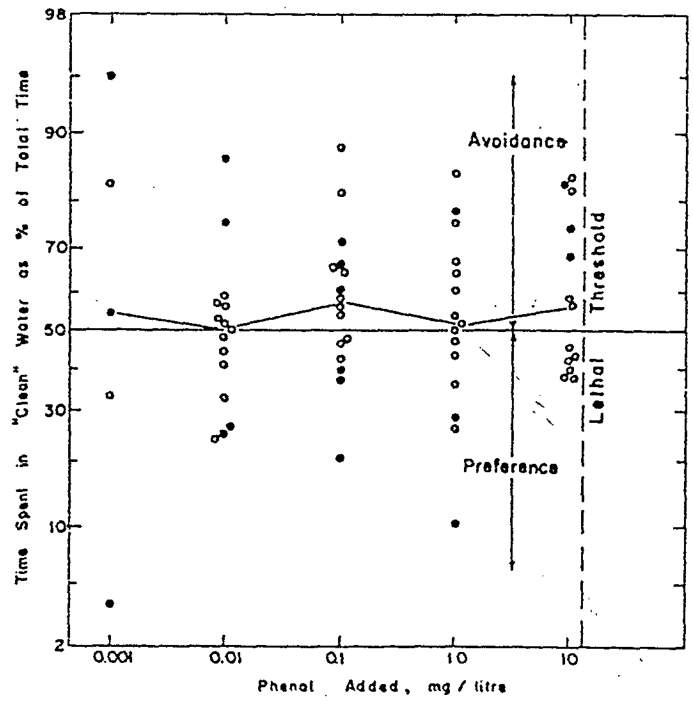

FIG. 2. Lack of avoidance reactions to phenol solutions by rainbow trout.

of statistically significant reactions were also near-neutral. Fish showed no signs of detection such as sudden stops or "coughing" at the midline of the trough, as in experiments with zinc sulphate (Sprague, 1964). Nor were they disturbed at 10 mg/l., judging from swimming speed.

Avoidance reactions were probably inconsistent even at lethal concentrations. Two trout were tested in 30 mg/l. phenol, 2.2 times the lethal threshold. One showed nearly-perfect avoidance, the other showed none, became extremely excited, and finally lost equilibrium.

Avoidance of Chlorine

The response of rainbow trout to solutions of free chlorine was peculiar. Original experiments were therefore repeated, with confirmation.

At the lowest concentration, calculated as 0.001 mg/l. available chlorine, avoidance reaction was slight (Fig. 3). Most fish showed avoidance at a theoretical concentration of 0.01 mg/l. of chlorine, lethal in 12 days according to our laboratory tests.

Surprisingly, most trout preferred 0.1 mg/l. of chlorine which would kill them in about 4 days. There seemed to be an unusual "physiological trap" involving the sense organs. Time and again, trout swam back and forth in the chlorine solution,

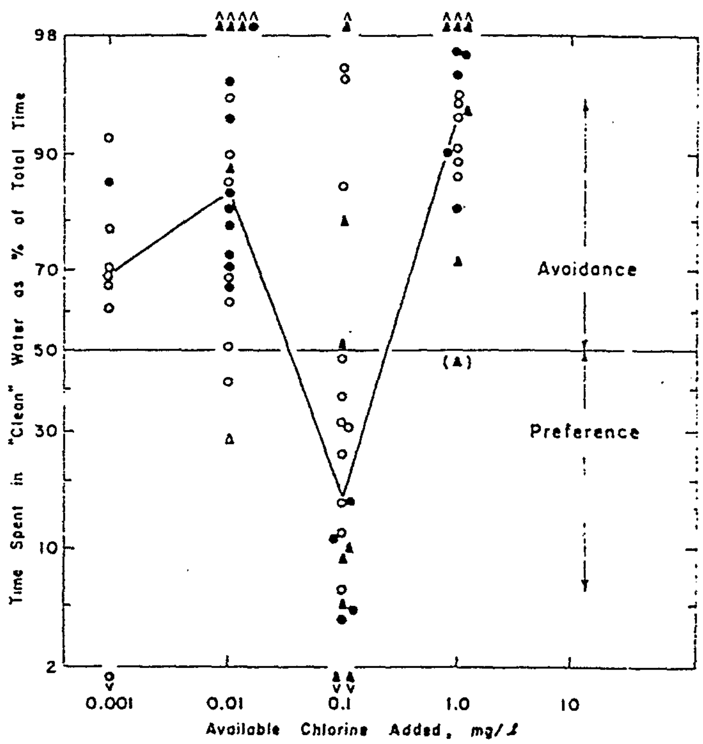

FIG. 3. Avoidance and preference of rainbow trout for solutions of chlorine, added as calcium hypochlorite. A solid circle represents a statistically significant choice by one fish, an open circle represents non-significance, and a triangle represents a response which could not be tested for statistical significance. The bracketed point at 1.0 mg/l. is not comparable with the others since the fish lost equilibrium.

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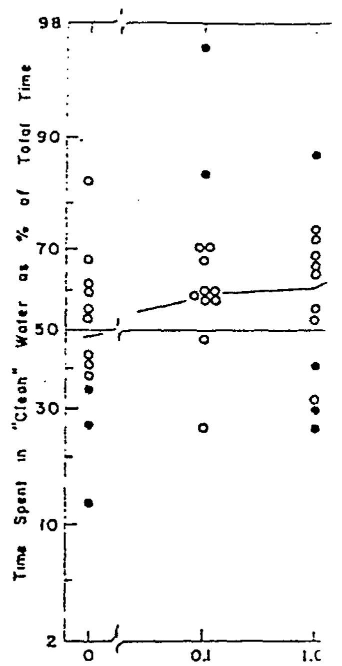

FIG. 4. Avoidance r

reached the boundary with clean water, stopped short, and turned back into the chlorine. Momentary entrance into clean water apparently triggered an unpleasant sensation. Perhaps the sense organs remained deadened if fish stayed in 0·1 mg/l. of chlorine.

Strong avoidance reactions returned at 1·0 mg/l. of chlorine, lethal in 4 hours or less. One test was stopped when a fish lost equilibrium and floated in the polluted side.

Because of the peculiar preference response sandwiched between avoidance responses, estimation of the threshold avoidance level is not attempted. Many apparent examples of nearly-perfect preference or avoidance could not be tested statistically, because fish did not make three visits to the side they disliked, as required by the Kolmogorov-Smirnov test of significance.

Avoidance of Pulp Mill Effluent

Salmon gave a somewhat vague response to BKME (Fig. 4). At 0.1 and 1.0 ppm BKME, responses may be random. Over five higher orders of magnitude, from 10 ppm to 100,000 ppm BKME, almost all fish showed avoidance, but of moderate overall strength. The lethal threshold is not much higher, about 15% BKME as determined in our laboratory and elsewhere (Betts and Wilson, 1966). Avoidance did not increase greatly with concentration, a rather indefinite response. Only at 56% BKME was there strong overall avoidance.

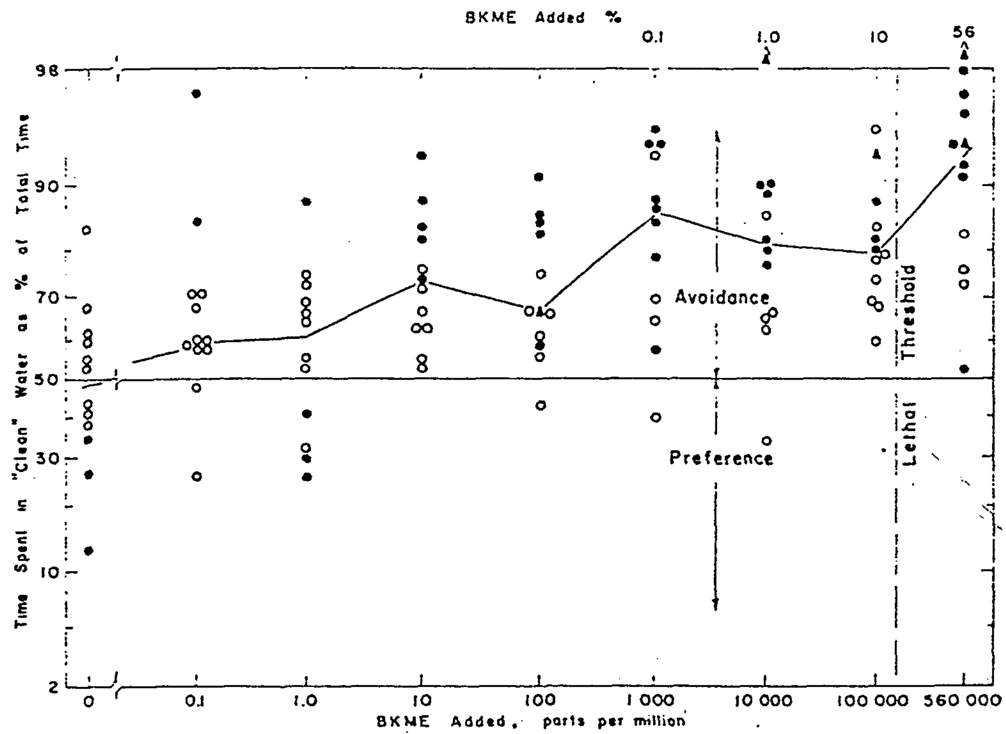

FIG. 4. Avoidance responses of Atlantic salmon parr to neutralized bleached Kraft pulp mill effluent.

Probit analysis would be unrealistic when response is similar over such a wide range of concentration. There seems to be an extremely broad threshold, with about half the fish showing statistically significant avoidance, over the range from 10 ppm to 10%.

A control test with only water in the trough gave the expected random series of time-responses. Three out of 12 fish showed significant "preference"; apparently some performances are significant for reasons unrelated to the pollutant.

Experiments with unbleached KME gave similar results. The only appreciable change was a neutral response at 10 ppm KME instead of mild avoidance. KME at 56% produced somewhat weaker avoidance than did BKME. Similarity between avoidance of KME and BKME suggests that the response depends primarily upon wastes from the Kraft cooking process. This is surprising, since the acidic chlorination effluent contains most of the toxic material (Betts and Wilson, 1966).

GENERAL DISCUSSION

Figure 5 compares reactions to the four pollutants and also includes reactions of rainbow trout to zinc sulphate (Sprague, in press). To make the comparison meaningful

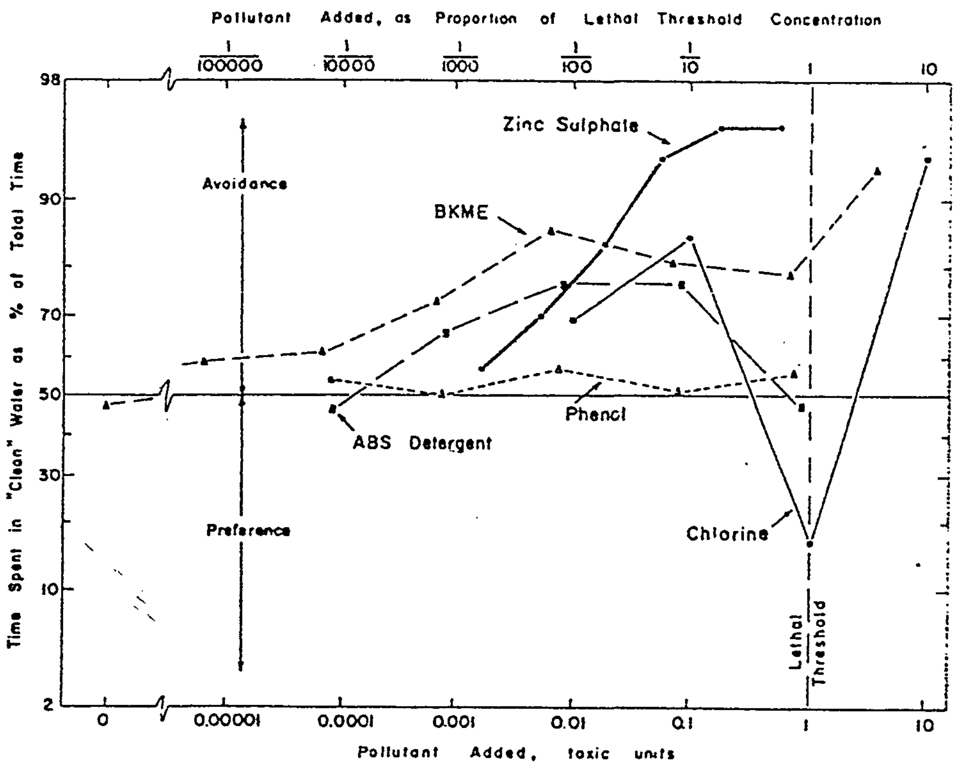

FIG. 5. Median avoidance reactions of salmonid fish to various pollutants. Concentration is expressed in terms of toxicity to fish instead of chemical units. Each point is the median of individual quantitative responses of 5 to 55 fish at that concentration of pollutant.

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in terms of fish survival, concentrations of pollutants are expressed in *toxic units*, or fractions of the lethal threshold concentration (Sprague and Ramsay, 1965). Since no lethal threshold was found for chlorine (unpublished results), the concentration lethal in 4 days was substituted.

Comparison based on toxic units has previously proven effective. Avoidance reactions of salmon to zinc, copper, and mixtures become almost identical on this scale of units (Sprague, Elson and Saunders, 1965, Fig. 4).

However, there is no single pattern in Fig. 5; each pollutant has its own. Nor is there any common relationship between avoidance response and lethal level. Near-lethal concentrations of detergent and phenol are not avoided. A lethal level of chlorine is preferred. Only zinc sulphate elicits sharp and consistent avoidance at sublethal concentrations; rainbow trout show almost complete avoidance at 0.1 toxic units. Atlantic salmon also avoid sublethal concentrations of zinc and copper sulphates, separately or together. If these three lines were in Fig. 5, they would appear similar to the line for zinc sulphate and rainbow trout, but to the right of it, higher by one cycle of concentration, but still clearly sublethal (Sprague, 1964).

Application of Results to Field Situations

The only quantitative comparison of which we are aware, between avoidance reactions of fish in the laboratory and reactions in a polluted river, arises from previous work of the St. Andrews laboratory (Sprague, 1964; Sprague, Elson and Saunders, 1965; Saunders and Sprague, 1967). That comparison may be taken as a basis of prediction, lacking others.

That work shows that the level of metal pollution causing disturbed movements of adult Atlantic salmon in a river is about 18 times higher than the threshold avoidance level for salmon parr in laboratory tests. Unfortunately, this factor of 18 times the laboratory threshold is of limited application. It could be used for detergent, but not for chlorine, phenol, or BKME, since threshold avoidance levels could not be estimated for them by our method. Furthermore, a constant factor applied to the laboratory threshold is unrealistic in view of the diverse relations between threshold response and strong response (Fig. 5).

An alternative approach is to relate the concentration of pollutant which causes avoidance in the river, to the strength of response which this concentration elicits in the lab. Following this approach, we know that movements of adult salmon in a river were disturbed at 0.35 to 0.43 toxic units of copper-zinc pollution (Saunders and Sprague, 1967). Such copper-zinc levels caused small salmon in laboratory tests to spend 88 to 92% of their time in clean water, judging from interpolated median time-responses (Sprague, 1964, Fig. 6).

For the laboratory studies shown in Fig. 5, the concentrations which cause approximately 90% median time-response may be read. For zinc sulphate this is about 0.032 toxic units. Thus we might expect rainbow trout to show avoidance reactions if their natural habitat were polluted to 0.032 toxic units, which is equal to about 0.018 mg/l. Zn in very soft water.

Median time-responses of 90% were also demonstrated for BKME and chlorine. For BKME this occurred at 2.3 toxic units, for chlorine, at 7.8 toxic units. However, lower concentrations of both pollutants elicited a slightly lower median time-response of about 85% in the laboratory. BKME did so at the very low level of 0.0067 toxic units (0.1 % BKME). Chlorine did so at 0.1 toxic units, equivalent to 0.01 mg/l. of chlorine. These concentrations would likely cause avoidance reactions by trout in polluted rivers.

Thus we might expect salmon in nature to stay out of water containing BKME if given an easily available choice of clean water such as one side of a river, and otherwise suitable habitat. However, given no easy alternative of clean water, we could not depend on salmon to avoid almost-lethal levels of BKME since their response is weak at such concentrations. Jones *et al.* (1956) record such a failure of chinook salmon to move out of a polluted section of river.

Trout in nature would probably avoid pollution by chlorine, unless "trapped" by a concentration near 0.1 mg/l.

Detergent pollution would apparently not generate distinct avoidance in field conditions, since median time-response did not reach 90%, or even 80%, in the laboratory. Nor would sublethal phenol cause avoidance since fish did not avoid it even in the laboratory.

Comparison With Other Research

Little work has been done on avoidance of detergents, but Bardach *et al.* (1965) showed that as little as 0.5 mg/l. could damage chemoreceptors in time, so that behaviour was impaired.

For phenol, Jones (1964) concludes that fish have "little chance of avoiding the solution at any concentration" agreeing with our results. Shelford (1917) reports preference of lethal concentrations, although the generalization is suspect. Ishio (1965) and Syazuki (1964) report avoidance thresholds at 1.1 and 2.5 times the lethal concentration, but Skrapek (1963) claims escape reactions at 0.2 to 0.3 of the lethal level. Hiatt *et al.* (1953a) record violent reactions of a marine fish to 20 mg/l. of phenol, and medium reaction to 2 mg/l. which should be sublethal.

The above findings show diversity, but most confirm our finding that trout fail to avoid sublethal concentrations of phenol. This is difficult to reconcile with detection of phenol by trained minnows at maximum concentrations of 0.0005 mg/l.; furthermore, "it was evident that the fish had a natural dislike for the odors" of phenol and *p*-chlorophenol (Hasler and Wisby, 1950). Perhaps some avoidance tests have used phenol concentrations which were too high, dulling sensory perception of fish. However, the lowest concentration of phenol in our tests was 0.001 mg/l., not much higher than the maximum of Hasler and Wisby.

Chlorine at 10 mg/l. violently irritated a marine fish; 1.0 mg/l. caused slight irritation (Hiatt *et al.*, 1953b). We found clear avoidance by trout at the lower concentration, and even at 0.01 mg/l.

Lack of strong avoidance of KME has been documented by others. Chinook salmon avoid 2.5% to 10% KME in sea water (Jones *et al.*, 1956) with about the same forcefulness as our Atlantic salmon. Smelt avoided KME down to 0.5% in a field experiment (Smith and Saalfield, 1955).

Young coho salmon did not avoid any concentrations of KME up to 10% (Jones *et al.*, 1956), nor did silver salmon avoid 3.5% KME (Holland *et al.*, 1960). Our findings of slight avoidance at much lower levels probably result from a more sensitive technique.

Although Höglund (1961) worked with sulphite waste liquor, not BKME, his

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Avoidance of sublethal concentrations of zinc sulphate is also confirmed by Ishio (1965) and Syazuki (1964).

From the above comparisons our procedure seems to be among the more sensitive for detecting avoidance and preference responses. The sharp boundary between clean and polluted water maximizes the opportunity of fish to discriminate, since they orient to chemicals in water by comparisons of intensities in time and space (Bardach *et al.*, 1967; Hemmings, 1966). Analysis of individual response of each fish is also a major factor in sensitivity of our method.

Diversity of avoidance response is evident for different pollutants, different apparatus, and different fish. Hiatt *et al.* (1953b) seem to have introduced some order into this diversity. They suggest that the most effective irritants for fish are inhibitors of sulphydryl groups in enzyme systems of sensory receptors; (1) mercaptide-forming agents such as heavy metals; (2) oxidizing agents; and (3) alkylating agents. Our results seem to fit this hypothesis. Zinc sulphate caused sharp avoidance and obviously fits into the first category. Chlorine also caused distinct avoidance and falls into the second category. Phenol would not ordinarily fit any of the categories, and correspondingly did not stimulate avoidance reactions. The other two pollutants tested contain several or many chemical compounds and relation to the theory is accordingly not clear.

SUMMARY AND CONCLUSIONS

1. Spontaneous avoidance of four common pollutants by small rainbow trout or Atlantic salmon was tested in the laboratory by presenting a sharp choice between clean and polluted water.
2. For a detergent, trout showed a threshold avoidance level of 0.37 mg/l. ABS. However, at 10 mg/l., nearly-lethal, fish were confused and unable to show avoidance. Perhaps this resulted from a lag in rinsing of detergent from sensory receptors.
3. Trout did not avoid phenol at any concentration from 0.001 mg/l. to 10 mg/l. which is nearly lethal.
4. Trout significantly avoided 0.01 mg/l. of available chlorine, lethal in 12 days, and 1.0 mg/l. which is rapidly lethal. Most preferred an intermediate lethal concentration of 0.1 mg/l. Apparently an unusual "sensory trap" kept fish in the chlorine.
5. Salmon showed moderate avoidance of bleached kraft pulp mill effluent (BKME) all the way from 10 ppm to 10% concentration. Lower concentrations were not avoided. A lethal concentration of 56% was strongly avoided. Similar results for unbleached effluent suggest that avoidance is caused by material in the Kraft cooking wastes.
6. These results contrast with sharp avoidance by the same species, of sublethal concentrations of zinc sulphate.
7. There are different patterns of avoidance response for each of these five pollutants. More knowledge is required, to make useful generalizations and predictions about this possible effect of pollution.

8. Based on previous work, avoidance reactions may be expected in polluted natural waters, at concentrations which cause fish in laboratory tests to spend 90% of their time in clean water.
9. Applying this relation to field situations, only zinc sulphate of the five pollutants discussed here, would cause consistent avoidance reactions by salmonid fish at sublethal concentrations. BKME could cause avoidance at low sublethal levels, especially with an easy alternative of clean water. However, salmon might show only weak avoidance of near-lethal levels of BKME. Trout would probably avoid chlorine pollution, unless trapped and killed by certain concentrations. They would probably fail to avoid lethal and mildly-harmful levels of detergent in a river. Trout apparently would not avoid sublethal phenol pollution.

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