Overt Avoidance Reaction of Rainbow Trout Fry to Nine Herbicides

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Herbicides are used extensively to control aquatic and ditch-bank vegetation in and along the complex irrigation systems of the western United States. Many of those systems transport relatively cool water, and therefore support trout fisheries in reservoirs and some connecting canals (SCHOETTGER, unpublished). Extensive plant growth in and along canals causes particularly acute problems because it restricts water flow and reduces the capacity of the canals. The application of herbicides is the most feasible and economical approach to controlling such plants. In general, because the chemicals are applied in lateral canals, concentrations effective for plant control are diluted before the treated water is used in irrigation or returned to streams and reservoirs. The effect of many of these herbicidal chemicals on trout is not well known. Presumably, however, if trout can detect these materials in water, they may be able to avoid toxic concentrations, particularly in main and lateral canals, provided there is suitable alternate habitat elsewhere. On the other hand, localized habitats in streams and reservoirs receiving irrigation waters may become essentially unsuitable for trout if the fish detect and avoid even trace amounts of the chemicals.

Numerous investigators have demonstrated that both fresh-water and marine organisms are able to selectively avoid concentrations of certain organic and inorganic pollutants (DANDY 1972 , HANSEN 1966, 1967, 1968, 1969; HANSEN et al. 1972, 1973, 1974; JONES 1947; JONES et al. 1956; KYNARD 1974; LAWRENCE and SCHERER 1974; SCHERER 1975; SHELFORD and POWERS 1915; SPRAGUE 1964; SUMMERFELT and LEWIS 1967). Because fish may avoid herbicides used in irrigation systems, studies were undertaken to determine when concentrations of 2,4-D (DMA), Aquathol K®[1/], copper sulfate, dalapon, diquat, xylene, trichloroacetic acid, acrolein, and glyphosate might be avoided by rainbow trout fry (Salmo gairdneri). The rainbow trout was selected for the tests because it is an important sport and commercial species, is stocked in western reservoirs, and provides a sport fishery in some irrigation canals.

[1/] Reference to trade names does not imply Government endorsement of commercial products

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Methods and Materials

Test fish obtained from the Bellvue State Fish Hatchery, Fort Collins, Colorado, 2 weeks after swim-up, were acclimated in experimental water for 3 weeks before avoidance tests were begun. Charcoal filtered Denver city water was used in all experiments. Water quality was as follows: temperature 11.0 ($\pm$2.0)°C; pH, 8.0; hardness, 89.5 mg/l as $CaCO_3$; conductivity, 340 mohms/cm; chlorine and chloramines, $<$0.01 mg/l; and dissolved oxygen, 6.7 mg/l.

The nine herbicides tested, all of which are in use or may be used in the control of aquatic and ditchbank plants by the U.S. Bureau of Reclamation (BARTLEY, personal communication)[2/], were: 2,4-D (DMA) (dimethylamine salt of 2,4-dichlorophenoxyacetic acid); Aquathol K® (dipotassium salt of 7-oxabicyclo (2.2.1) heptane-2,3-dicarboxylic acid); copper sulfate; dalapon (2,2-dichloropropionic acid); glyphosate (isopropyl-amine salt of n-phosphonomethyl glycine); diquat (6,7-dihydrodipyrido (1,2:2',1'-C) pyrazdiium); emulsified xylene (p 1,4-dimethyl benzene); sodium salt of trichloroacetic acid (TCA); and acrolein (acrylaldehyde). The amount of active ingredient in each herbicide and the expected concentration in water after routine field applications are given in Table 1.

The Y-shaped avoidance maze was constructed in accordance with specifications reported by HANSEN et al. (1972). Flow rates into each arm of the maze were maintained at 400 ml/min for all toxicants except 0.0001 mg/l $CuSO_4$, for which the flow rates were increased to 800 ml/min.

Herbicides were administered from a Meriotte bottle at the inlet of one arm while fresh water was administered at an equivalent rate at the inlet of the other arm. The herbicide concentrations were calculated rather than measured, except for the treatments with dalapon and 0.01 mg/l $CuSO_4$; in these tests the concentrations were measured analytically and found to equal at least 92% of the calculated values.

Fish were placed in the holding area of the maze for 15 min before the toxicant was administered, and then were allowed equal access to the arms with treated and untreated water. The numbers of fish in each arm were recorded 1 hr later. Fish were used only once. Tests at each concentration were repeated five times with 10 fish per trial. Meriotte bottles containing toxicant and water were reversed after each trial to eliminate preference for either section.

Avoidance of herbicides was evaluated statistically by the chi-square goodness of fit test on the assumption that if fish could not discriminate between treated and untreated water, they would leave the holding area and enter each section of the maze with equal frequency. Avoidance was considered statistically significant if the probability that observed distributions would occur by chance was 5% or less.

[2/] Bartley, T.R., Environmental Sciences Section, U.S. Bureau of Reclamation, Denver Federal Center, Denver, Colorado.

Discussion and Results

Rainbow trout fry avoided one or more concentrations of copper sulfate, dalapon, 2,4-D (DMA), xylene and acrolein, but displayed no avoidance of glyphosate, Aquathol K, diquat or TCA at the experimental concentrations (Table 1). The reaction of the fry to xylene was ambivalent: significant attraction to a 0.01 mg/l concentration and avoidance of a 0.1 mg/l concentration were observed. The reason for this dispairity is unclear, however, a similar unexplained biphasic response was also observed in tests of drilling fluids (LAWRENCE and SCHERER 1974).

The lowest concentration avoided by the test fish presumably approximates the lowest level of toxicant perception, at least within one order of magnitude. The lowest concentration of each herbicide avoided was 0.0001 mg/l of $CuSO_4$; 0.1 mg/l of xylene and acrolein; 1.0 mg/l of dalapon and 2,4-D (DMA); 1.0 mg/l of TCA; and 10.0 mg/l of glyphosate, Aquathol K and diquat. Except for $CuCO_4$, acrolein and xylene, no avoidance was observed at concentrations of the herbicide below those expected to be used or recommended for use in the field. Avoidance reactions to each herbicide, except acrolein, occurred well below the respective 96 h LC50 values. Also, in each exposure where a significant avoidance was observed, the avoidance was greater than 69% indicating that the fish had a pronounced capacity to select water free of herbicide.

Copper sulfate induced an avoidance reaction at a much lower concentration than did other herbicides. Previous studies by SPRAGUE (1964) also demonstrated that fish avoided much lower concentrations of metals than of compounds such as herbicides or organochlorine and organophosphate insecticides (HANSEN 1969: HANSEN et al. 1972, 1974; KYNARD 1974; SCHERER 1975). The reason for the ability of fish to detect heavy metals at concentrations lower than at which they detect other pollutants is unclear. However, GARDNER and LaROCHE (1973) have demonstrated histopathological lesions of the olfactory lobe in fish exposed to copper. These lesions may enhance the sensitivity of the avoidance response simply by irritating the olfactory system.

The test fish in the present study demonstrated levels of avoidance that differed widly among the organic herbicides tested. The underlying mechanisms involved in distinguishing between different organic compounds are unclear, but are possibly related to the ability of the olfactory system to discriminate between certain side chains in complex organic molecules (HARA 1972).

In general, the results of these studies suggest that rainbow trout would not be killed by, and would not avoid, relatively low concentrations of dalapon, 2,4-D (DMA), glyphosate, Aquathol K, diquat, or TCA. However, trout present in acrolein-treated water probably would not avoid a lethal exposure. Trout strongly avoided low concentrations of copper sulfate and 0.1 mg/l xylene; although these chemicals are non-toxic at the levels tested, their presence in the water may influence the selection of habitat by fish.

Table 1

Avoidance reactions of rainbow trout fry to three concentrations of nine herbicides

Herbicide	96 hr LC_{50} for 1/ rainbow trout mg/l	Expected water concentration when applied at recommended rates mg/l	Experimental concentration mg/l	Fish in untreated water after 1 hr 2/ %
$CuSO_4 \cdot 5H_2O$	0.14	0.005-0.01 when treated at 0.05-0.1 lb/cfs/day	0.0001 3/	79**
			0.001 3/	86***
			0.01 3/	78*
Dalapon (powder, 74% active ingredient)	340	0.2 when treated 4/ at 10 lb/acre	0.1	60
			1.0	82***
			10.00	96***
2,4-D (DMA) (4 lb/gal acid equivalent)	100	0.1 when treated 4/ at 1.5-2.5 lb/acre	0.1	49
			1.0	70*
			10.0	75**
Glyphosate (3 lb/gal acid equivalent)	50	0.1 when treated 4/ at 2 lb/acre	0.1	51
			1.0	50
			10.0	48
Aquathol K (3 lb/gal acid equivalent)	150	1.0-2.0 lake treatment	0.1	42
			1.0	55
			10.0	64
Diquat (2 lb/gal acid equivalent	12	0.2-0.5 in static treatment	0.1	53
			1.0	51
			10.0	56

Table 1 - continued

Avoidance reactions of rainbow trout fry to three concentrations of nine herbicides

Herbicide	96 hr LC_{50} for rainbow trout mg/1	Expected water concentration when applied at recommended rates mg/1	Experimental concentration mg/1	Fish in untreated water after 1 hr %
Xylene +2% AD-410 (technical grade)	10	740 when treated at 10 gal/cfs/30 min.	0.001	42
			0.01	30*
			0.1	79**
TCA (technical grade)	10	0.2 when treated5/q at 20-40 lb/acre	0.01	53
			0.1	40
			1.0	62
Acrolein (technical grade)	0.14 24 hr LC_{50}	0.1 when treated for 48 hr	0.001	49
			0.01	56
			0.1	88***

1/ Unpublished toxicity data from Fish-Pesticide Lab, Columbia, Mo.; except for TCA, which is 48 h LC_{50} for chinook salmon (*Oncorhynchus tschawytscha*)

2/ * = significant at $P<0.05$
** = significant at $P<0.01$
*** = significant at $P<0.001$

3/ Cu ion

4/ Ditchbank application

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