Avoidance by Herring of Dissolved Components in Pulp Mill Effluents

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INTRODUCTION

Herring, *Clupea harengus*, avoid whole pulp mill effluent (PME) from a sulfite mill at a concentration threshold equivalent to 2.5-2.9 mg/ℓ as sodium lignosulfonate (WILDISH et al. 1976). This work was designed to identify the soluble chemical components in PME which herring avoid in sea water.

MATERIALS AND METHODS

PME contains a complex mixture of dissolved components, varying with the species of wood used and the chemical treatment it receives. Some major (I, III and IV) and a few minor components present (see MAENPAA et al. 1968; BLOSSER AND GELMAN 1973; KEITH 1976) were tested (see Table 1). Humic acid was included because herring probably encounter this during inshore feeding. Analytical grade compounds were used without further purification. Compounds V, VI, VIII and IX were dissolved in absolute ethanol and VII in polyoxyethylene lauryl ether (final concentration of both 0.1 mℓ/ℓ).

Avoidance tests were made with herring caught and acclimated as previously, in the same experimental apparatus, using the same experimental protocol and analysis (WILDISH et al. 1976). The apparatus consisted of two interconnected arenas (A and B) forming a figure of eight maze into which separate sea water flows of 8-10 ℓ/min were run. The arena walls were 30 cm apart and the interconnection between A and B was limited to 10 x 10 cm to minimize water mixing. Groups of 9-13 fresh herring were used for each test. Control observations were made by noting the number of fish in A or B at 5-min intervals for 13 observations. To test avoidance

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TABLE 1.

Chemical compounds used in avoidance/preference tests.

Number	Name	Formula weight	Supplier
I	Sodium lignosulfonate	-	M.C.B.
II	Humic acid	-	Aldrich
III	D-glucose	180.16	Fisher
IV	Fructose	180.16	"
V	Abietic acid	302.44	K & K Labs.
VI	Dehydroabietic acid	300.44	" "
VII	Tall oil	-	Chem. Serv.
VIII	Furfural	96.09	" "
IX	Catechol	110.11	" "
X	Acetic acid, 99.7%	60.05	Fisher
XI	Formic acid, 88%	46.03	Baker
XII	Acetone	58.08	Fisher

or preference, a toxicant supply was switched on to deliver either to A or B. The supply line was connected to a constant head tank of dissolved test chemical, and 30 min allowed for a steady-state concentration to build up in the arena. Thirteen observations at 5-min intervals were then made in the treatment period.

The proportion, p, is the number of fish observed in A divided by the total number used (9-13) during one observation. The mean proportion ($\bar{p}$) and standard error (S.E.) for the control test are then compared with those for the treatment test. If $\bar{p}$ ± S.E. x t value does not overlap the control, the values are different at the appropriate confidence interval (90%*, 95%**, 99%***) or, if overlapping, are not significantly different (N.D.). Values of p less than the control indicate avoidance, values greater indicate preference.

Concentrations of some compounds in sea water were monitored by U.V. spectrophotometry with control sea water in the reference cell.

RESULTS

Tests of various compounds at a concentration just below the PME avoidance threshold were made as in Table 2 to select compounds for determination of threshold avoidance.

TABLE 2.

Avoidance or preference by herring of PME components and humic acid.

Compound	Nominal concentration mg/ℓ	Control±S.E.		Treatment±S.E.		Significance
I	0.78	0.49	0.04	0.27	0.02	***
II	1.13	0.71	0.02	0.55	0.03	***
III	1.10	0.60	0.02	0.78	0.02	***
IV	1.12	0.62	0.04	0.53	0.04	N.D.
V	0.99	0.53	0.02	0.51	0.02	N.D.
VI	1.05	0.60	0.04	0.62	0.03	*
VII	1.14	0.50	0.03	0.55	0.03	N.D.
VIII	1.16	0.66	0.04	0.70	0.05	N.D.
IX	1.19	0.61	0.05	0.63	0.05	N.D.
X	1.22	0.66	0.04	0.56	0.04	N.D.
XI	1.25	0.52	0.03	0.52	0.03	N.D.
XII	2.43	0.64	0.03	0.43	0.02	***

Most avoidance tests were duplicated or triplicated and confirmed the results shown in Table 2. Ethanol or polyoxyethylene lauryl ether did not cause avoidance (not shown) at the concentrations used to solubilize some of the compounds.

Only sodium lignosulfonate and humic acid were avoided at ~1 mg/ℓ (3 replications) and consequently attempts were made to determine the avoidance threshold (Table 3) of these substances. Fish exposed to D-glucose (III) and dehydroabietic acid (VI) showed a significant preference ($\bar{p}$ treatment > $\bar{p}$ control) but the results could not be replicated (III 1.08 mg/ℓ N.D., 1.05 mg/ℓ N.D., VI 1.0 mg/ℓ N.D.). Acetone (2.43 mg/ℓ*** avoidance, 2.44 mg/ℓ N.D.) gave conflicting results on replication. A possible explanation for these anomalous results is that the compounds are recognized by ectodermal receptors of the herring and used as a conditioning stimulus in learning to use one side of the maze in a way unconnected with avoidance responses.

TABLE 3.

Avoidance responses of herring at various concentrations of sodium lignosulfonate and humic acid.

Concentration mg/ℓ nominal	measured	Control	± S.E.	Treatment	± S.E.	Significance
I. Sodium lignosulfonate						
14.09	7.50	0.55	0.07	0.02	0.01	***
7.58	4.50	0.59	0.02	0.51	0.02	N.D.
0.78	-	0.71	0.02	0.51	0.03	***
0.34	-	0.56	0.02	0.33	0.03	***
0.16	-	0.61	0.04	0.55	0.02	N.D.
0.09	-	0.53	0.03	0.51	0.02	N.D.
II. Humic acid						
9.38	5.75	0.58	0.02	0.29	0.05	***
5.15	4.50	0.53	0.02	0.31	0.03	***
3.12	1.25	0.58	0.02	0.24	0.04	***
1.06	-	0.58	0.02	0.29	0.05	***
0.20	-	0.56	0.02	0.26	0.04	***
0.15	-	0.60	0.05	0.58	0.02	N.D.
0.10	-	0.60	0.01	0.67	0.03	*

Nominal concentration thresholds estimated by interpolation from data in Table 3 were for sodium lignosulfonate 0.1-0.3 mg/ℓ and humic acid 0.1-0.2 mg/ℓ. The measured concentrations differed by up to 50% from the calculated concentrations.

Measurements of sodium lignosulfonate and humic acid by U.V. spectrophotometry proved not to be sufficiently sensitive for this work. Sensitivity limits are controlled by sea water background absorbance (A250 nm = 10 - 57 x 10^{-3}) which varied considerably between tests and between sides A and B of the maze. Because of strong absorbance at 277 nm, compound VIII (furfural) could be measured to at least 1 order of magnitude lower than I and II.

DISCUSSION

Our data suggest that herring avoid long chain complex molecules such as lignosulfonates and perhaps lignin in PME rather than resin acids and breakdown products of low molecular weight which are known to be relatively toxic (LEACH and THAKORE 1973, 1975; ROGERS et al. 1975).

Since spent sulfite liquor contains 50-65% lignosulfonate (BLOSSER and GELLMAN 1973) the actual proportion of lignosulfonate in the PME previously tested (WILDISH et al. 1976) would be 1.2-1.9 mg/ℓ. This concentration is 4-19 times greater than the calculated avoidance threshold of herring with pure sodium lignosulfonate. Possible explanations for the discrepancy are that herring respond to chemical differences of the two lignosulfonate samples tested, or are affected by masking of lignosulfonate in PME by other chemicals present.

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