THRESHOLD OF PERCEPTION OF EUGENOL IN JUVENILE SOCKEYE SALMON

by

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INTRODUCTION

In order to evaluate the effect of environmental changes caused by high dams on the homing capabilities of salmon, a better understanding of the mechanisms of homing behavior is needed. Hasler and Wisby (1951) theorized that homing behavior of salmon during the later stages of migration is based on a response to the odor of the home stream. Field studies with both coho salmon (Oncorhynchus kisutch) (Wisby and Hasler, 1954) and chinook salmon (O. tshawytscha)[1/] tend to support this theory. Hasler and Wisby suggested that it might be possible to condition young salmon to a synthetic odor substance and later decoy them to this odor when they return from the ocean to spawn. Such a procedure might have useful management applications in identifying and separating stocks of migrating adults. Prior to any investigation of this hypothesis, information is necessary concerning levels of sensitivity to odor compounds which might be used.

The chemical, eugenol, was selected for study since the minnow (Phoximus laevis) was found to detect its presence by means of the olfactory sense alone (Neurath, 1949). The threshold of perception of eugenol was determined for juvenile sockeye (O. nerka) in studies conducted at facilities of the Fish-Passage Research Program, Seattle, Washington. Results of this study are reported here.

MATERIALS AND METHODS

One hundred sockeye salmon obtained from the National Hatchery at Leavenworth, Washington, were held for 3 days before the beginning of the study in circular metal tanks, which were flushed with dechlorinated city water. During this period they were fed a diet of dry food pellets. The fish were 1 year old at the beginning of the study.

A modified form of the experimental setup used by Tarrant (1964) was employed (fig. 1). The 77-gallon plywood aquarium was flushed with dechlorinated city water at the rate of 117 gallons per hour. Water was introduced from the bottom

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1/ Groves, A. B., G. B. Collins, and P. S. Trefethen. Sensory factors in the homing of adult chinook salmon (Oncorhynchus tshawytscha). Manuscript in preparation.

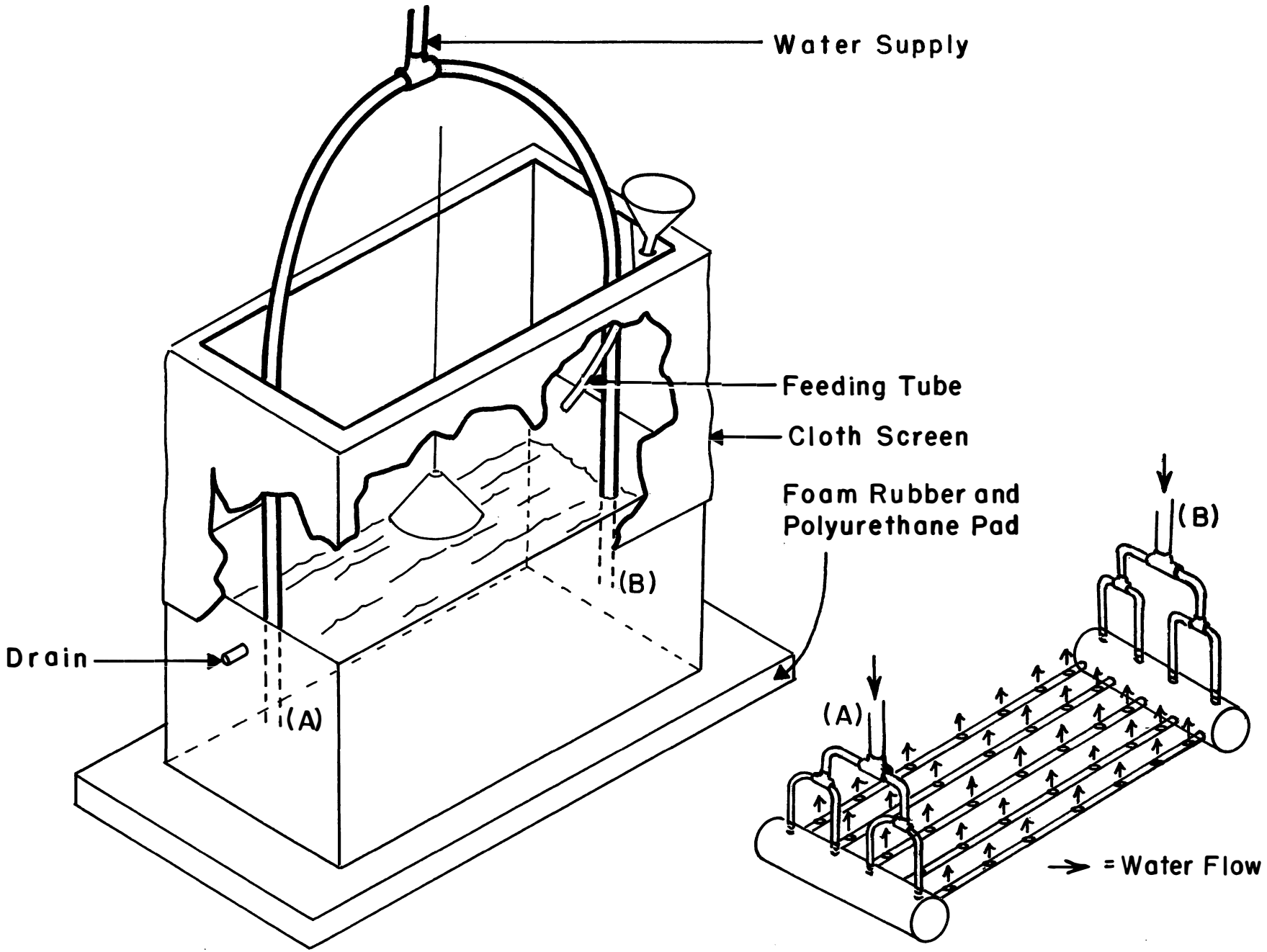

Figure 1.--Diagram of aquarium used for conditioning and testing juvenile sockeye salmon and plastic water diffuser for uniform dispersal of inflowing water. The water diffuser was located inside the aquarium.

of the aquarium through a plastic water diffuser (fig. 1) designed to disperse the water uniformly. Continuous illumination was provided by a 75-watt incandescent bulb placed 8 inches above the water surface, and undetected observation of the fish was possible from a darkened area above the light source.

Eugenol (only slightly soluble in water) was first dissolved in ethanol to facilitate uniform dispersal throughout the aquarium since ethanol mixes freely with both eugenol and water. Tests indicated that ethanol was not detected at the concentrations used in this study. Units of approximately 5 cc. of eugenol solution were injected through a serum stopper in the water supply line with a hypodermic syringe. A solution of methylene blue was injected periodically to determine the rate of mixing within the aquarium. Complete mixing occurred in an average time of 34 seconds after injection.

Forty seconds after the eugenol solution was injected, dry food pellets were dropped into the water through a tube above the aquarium (fig. 1). The fish fed actively at the water surface in the area where the food was introduced. After several associations the fish became conditioned to the chemical stimulus, which would then evoke a response prior to the introduction of food. The conditioned response consisted of increased activity followed by surface feeding behavior. Although the fish responded as a group, some individuals may have been cued by the movements of other fish.

The concentration of eugenol used in each test was calculated on the basis of the total volume of water in the aquarium. In order to insure complete flushing of the chemical from the aquarium, associations were limited to one a day. The fish were given 26 associations at a concentration of 2.50 p.p.m. followed by 34 associations at a concentration of 5.00 p.p.m. (The concentration was strengthened in order to hasten conditioning.) Concentrations were then attenuated in successive associations until the chemical stimulus no longer evoked a conditioned response. In this manner the threshold of perception of eugenol was determined. Ethanol, equal in volume to the test solution, was injected as a control before several of the associations. The sequence of associations and controls was varied.

RESULTS

Within 36 associations the conditioned response was well established, and after the 60th association attenuation of

the concentration was begun. The fish responded to all concentrations of eugenol greater than 2.20 p.p.m. in less than 40 seconds after injection. The time required to respond increased at lower concentrations (table 1), and reinforcement of the response was delayed proportionately. If no surface feeding behavior occurred within 180 seconds after injection of the chemical, food was introduced, and the response was considered negative. At concentrations below 0.20 p.p.m., 240 seconds were allowed before the introduction of the food.

All concentrations of 0.32 p.p.m. or greater evoked a positive response. The percentage of positive responses decreased rapidly at lower concentrations (table 2). At 0.20 p.p.m. conditioned responses were generally weaker and more delayed than at stronger concentrations. Responses at 0.18 p.p.m. were negative with the exception of one weak response occurring 200 seconds after injection of the chemical. In tests at 0.16 p.p.m. there were no positive responses or any indication that the chemical was detected.

The fish were again tested with a more concentrated solution of eugenol, which evoked a strong conditioned response. The concentration was attenuated to 0.16 p.p.m., and the fish failed to respond at this level. This procedure was repeated twice with identical results.

In 32 control tests with ethanol there were 30 negative responses (table 2). The two positive responses in the control tests occurred after a period in which tests were run at approximately the same time each day for several weeks. Greater care was then taken in varying the time of day at which the tests were run, and no further positive control responses occurred.

DISCUSSION

Concentrations of eugenol used in the study were calculated on the basis of the total volume of water in the aquarium. Since concentrated solutions were introduced with the water supply and were dispersed throughout the aquarium, there is some question whether the fish may have responded to a stronger solution than the calculated concentration. However, the time from injection of the chemical until the fish responded (table 1) always exceeded the average time required for complete mixing within the aquarium. At 0.18 p.p.m. the response time exceeded the time required for mixing by a factor of six.

Below 2.30 p.p.m. the response time increased in geometric progression as the concentration of the chemical was

Table 1.--Average response times of a group of 100 juvenile sockeye salmon[1/] at different concentrations of eugenol.

Concentration[2/] (p.p.m.)	Number of tests	Average time from injection of eugenol until conditioned response occurred (seconds)
4.50 - 5.00	2	35
3.50 - 4.00	2	35
2.50 - 3.00	5	35
2.00 - 2.40	9	44
1.50 - 1.90	6	48
1.00 - 1.40	5	60
0.50 - 0.90	9	81
0.36 - 0.40	13	113
0.30 - 0.34	11	115
0.24 - 0.28	18	114
0.18 - 0.22	16	128

1/ Prior to the tests the fish had received 60 associations of eugenol with food.

2/ Between 2.50 and 5.00 p.p.m. the concentration was reduced by 0.50 p.p.m. at each attenuation step. Between 0.50 and 2.40 p.p.m. the concentration was reduced by 0.10 p.p.m. at each attenuation step. Between 0.18 and 0.40 p.p.m. the concentration was reduced by 0.02 p.p.m. at each attenuation step.

Table 2.--Responses of a group of 100 juvenile sockeye salmon[1] in tests with eugenol[2] and control tests with ethanol[3].

Concentration[4] (p.p.m.)	Eugenol			Control		
	No. of tests	No. of positive responses	No. of negative responses	No. of tests	No. of positive responses	No. of negative responses
4.50 - 5.00	2	2	0	1	0	1
3.50 - 4.00	2	2	0	-	-	-
2.50 - 3.00	5	5	0	4	1	3
2.00 - 2.40	9	9	0	1	0	1
1.50 - 1.90	6	6	0	1	0	1
1.00 - 1.40	5	5	0	1	0	1
0.50 - 0.90	9	9	0	1	0	1
0.36 - 0.40	13	13	0	1	0	1
0.30 - 0.34	13	11	2	3	0	3
0.24 - 0.28	23	18	5	7	1	6
0.16 - 0.22	31	16	15	12	0	12

1/ Prior to the tests the fish had received 60 associations of eugenol with food.

2/ Eugenol in solution with ethanol was made up to a volume of approximately 5 cc. for each test.

3/ In each control test ethanol was equal in volume to the eugenol solution in the following test.

4/ Between 2.50 and 5.00 p.p.m. the concentration was reduced by 0.50 p.p.m. at each attenuation step. Between 0.50 and 2.40 p.p.m. the concentration was reduced by 0.10 p.p.m. at each attenuation step. Between 0.16 and 0.40 p.p.m. the concentration was reduced by 0.02 p.p.m. at each attenuation step.

decreased geometrically. It has long been held that, as a stimulus decreases geometrically, the intensity of the sensation decreases arithmetically (Fechner's Law). Perhaps then there is a relationship between the concentration of the chemical, the intensity of the sensation, and the time required for the fish to respond.

The results of the tests indicated that the perceptual threshold for eugenol in sockeye salmon of this age was no lower than 0.18 p.p.m. Neurath (1949) using conditioned responses found that the threshold for eugenol in Phoxinus laevis was 0.06 p.p.m. Juvenile sockeye have been found to react to natural food extracts at 0.01 p.p.m. (McBride et al., 1962), and young coho salmon were reported by Wisby[2/] to detect morpholine at 0.000001 p.p.m. In these two studies a conditioned response technique was not employed.

Although no direct evidence was obtained that juvenile sockeye detected eugenol by olfaction, Phoxinus laevis was able to detect this chemical by the olfactory sense alone (Neurath, 1949). It seems likely that the responses of salmon to eugenol may also be mediated through this sensory system.

In view of the relatively high threshold concentration at which juvenile sockeye were found to detect eugenol, the suitability of this chemical for testing the conditioning and decoying technique suggested by Hasler and Wisby is questionable. Conditioning young salmon to eugenol solution in hatchery raceways might be practical. In small streams the amount of chemical required to condition young sockeye would tend to make the cost of prolonged application prohibitive.

2/ Wisby, W. J. (1952) Olfactory responses of fishes as related to parent stream behavior. Ph. D. thesis, Univ. of Wisconsin, Madison, 42 pp. (Typewritten)

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