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Title: Odor Detection by Coho Salmon (Onchorhynchus kisutch): A Laboratory Bioassay and Genetic Basis

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Abstract approved: _______________

Dr. Carl Schreck

To assess the imprinting performance of juvenile salmon stocks transplanted to foreign water and to facilitate imprinting, I developed techniques for demonstrating odor detection, recognition, and discrimination in juvenile coho salmon (Oncorhynchus kisutch); screened phenol as a potential imprinting compound; and, in part, evaluated the genetic basis for olfactory sensitivity to home-stream water. Orientation conditioning, in which a fish is trained to orient in a forked maze to a scent, is a useful method for demonstrating odor recognition and discrimination. Cardiac conditioning is useful for determining minimum detection concentrations. Coho salmon recognize a mixture of two odors (morpholine and phenethyl alcohol) distinct from either odor individually. The fish can also discriminate between a single odor and a mixture containing that odor. Phenol is a good candidate for further testing as an imprinting compound. Coho salmon are not attracted or repelled by phenol at concentrations of less than 1×10^{-3} mg/L, but can detect it at 1×10^{-5} mg/L. Juvenile coho salmon can detect and orient to water from their hatchery as well as to water from a foreign hatchery diluted

1:1,000. Juvenile and smolting coho salmon can detect water from both their home and a foreign hatchery diluted 1:10,000. A 2 to 7 week acclimation period is needed before initiating the orientation conditioning if the fish are transported. Smolting coho salmon will not condition to follow scents upstream for food, but Fall Creek coho salmon cardiac conditioned better as smolts than as parr.

Odor Detection by Coho Salmon (*Oncorhynchus kisutch*): A Laboratory Bioassay and Genetic Basis

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Introduction

A feasible approach to reducing the amount of straying in fish used to repopulate streams or for hatchery production is to improve homing accuracy of migrating adults. Homing accuracy may be improved by "artificial imprinting" (Hasler *et al*. 1978; Allen *et al*. 1978) or by working within the genetic limitations of fish stocks (Raleigh 1971).

Only morpholine and phenethyl alcohol have been proved as odor cues for "imprinting" coho salmon (*Onchorhynchus kisutch*) and steelhead (*Salmo gairdneri*) smolts (Scholtz *et al*. 1978; Hasler *et al*. 1978). Streams scented with morpholine have attracted from seven to eight times more coho salmon imprinted with morpholine than coho salmon not imprinted (Allen *et al*. 1978). Additional compounds may be needed when migration routes of imprinted fish overlap.

Although imprinting to environmental stimuli is sufficient to return some transplanted fish, the addition of parent's genotype from the recipient stream increases returns of migrating adults significantly (Bams 1976). This implies genetic control of homing. However, no data were found on the ability or genetic basis of stream odor detection, recognition and discrimination by coho salmon before or during imprinting. No information was found on the impact of transportation on the ability of coho salmon to learn and recognize stream odors.

My purpose was to develop a rapid bioassay for demonstrating that juvenile coho salmon can detect, recognize and discriminate odors at concentrations that are neither attractive or repulsive. I assumed coho salmon can detect stream odors but are not attracted or repelled by these odors. Therefore attraction or avoidance of fish to an odor is not a test of detection. The bioassay was used to screen a phenolic compound as a potential imprinting cue and to determine if the ability to detect home stream water differs between juvenile stocks and has any genetic basis.

Methodology

A three-step bioassay, composed of behavioral response, cardiac conditioning and orientation conditioning, was developed to demonstrate the behavioral significance of odors that neither attract nor repel juvenile fish. The behavioral response bioassay was used to determine the concentration of a compound not attracting or repelling fish. Cardiac conditioning was used to determine if the fish could detect the odor at a neutral (non attractive or repulsive) concentration, and orientation conditioning was used to determine if the fish could recognize and discriminate the odor.

Phenol (C_6H_5OH) was chosen to be tested as potential imprinting compound. Phenol is soluble in water, similar in density to water, and stable in stream conditions. Bluntnose minnows (Pimephales notatus) can detect and discriminate phenol from other phenolic compounds at low concentrations (Hasler and Wisby 1950). Behavioral response and cardiac conditioning experiments were conducted to determine a candidate concentration for field test.

For the genetic aspect of the study two stocks of coho salmon, Fall Creek Hatchery (FC) and Sandy Hatchery (S) Oregon, were split into two subgroups of genetic peers. One subgroup of each stock was reared in its respective hatchery and the other at a third location, Smith Farm, and tested as follows:

	Stock Sandy Hatchery		Stock Fall Creek	
Rearing location	Sandy Hatchery	Smith Farm	Fall Creek	Smith Farm
Test water Conditioning	FC & S	FC & S	FC & S	FC & S

The following assumptions were adopted for this experiment:

> The fish can detect an odor at a test concentration if the fish will modify its behavior according to the presence or absence of the odor.
>
> The performance of the fish during the tests is an indication of the fish's imprinting and homing performance to the odor. That is to say a fish will not imprint or home to an odor it cannot detect, recognize or discriminate.
>
> The influence of genetic inheritance on imprinting ability would manifest itself in the fish's ability to detect, recognize or discriminate an odor.

Tests were conducted at Oregon State University's Smith Farm laboratory and Nash Hall constant temperature room. Smith Farm is supplied with well water (12°C; pH 6.2; 80 mg $CaCO_3$/L). The Nash Hall facility is supplied with chilled, declorinated tap water (9°C; pH 6.5; 40 mg $CaCO_3$/L).

Coho salmon, brood year 1977, from Sandy Hatchery were used as test animals for methodology development and phenol screening. Juvenile coho salmon from both Sandy and Fall Creek Hatcheries were used for the genetic aspect of the study. The hatcheries were selected because each has an established coho salmon run that has received essentially no contamination from other stocks for several years and they are located in separate drainage basins.

Morpholine and phenethyl alcohol were used as scents for validating the methodology used during the experiments. These compounds were chosen because of demonstrated detection by coho salmon (Scholtz et al. 1975; Hasler et al. 1978). The concentrations used were those found to be effective for imprinting coho salmon. Water samples for the genetic comparisons were collected from the respective hatcheries in the mid-section of the raceways. The samples, collected for the study, were stored as described by Hasler and Wisby (1951), Fagerlund et al. (1963), and Bodznick (1975) and thawed as needed for the test.

Behavior response

A behavior response bioassay similar to the bioassay described by Wisby (1952) was used to determine the neutrality of a compound with respect to avoidance and attraction of coho salmon. Thirty coho salmon were placed into a forked trough and allowed 24 hours of acclimation before testing was initiated. During test trials, the fish were allowed to choose between two arms of the trough, one treated with a scented drip while the second arm was treated with a non-scented water drip. Scenting of arms was randomized to avoid habituating the fish. During the trials, the drips were started and the lights in the trough turned off. At 15 minute intervals the gates at the lower end of each arm were closed, the lights turned on and the number of fish in each arm recorded. Several trials were conducted for each concentration of each compound.

The behavior chamber used for the bioassays was a 2.42m fork-shaped trough (Figure 1). The trough was divided into three sections, arms, fork, and leg. The arms and leg were 1.06m and the fork was 0.3m in length. Water flowed (2L/min.) into the upper end of each arm and out the lower end of the leg. Plexiglass gates, operated by draw-string, were located at the lower end of the arms and upper end of the leg to control movement of the fish. Fish in the arms were fed by feeding tubes operated remotely to minimize disturbing the fish. The troughs were screened with black plastic to isolate the fish from outside activity. Viewing ports of one-way mirror were located at the upper end of the arms. A 12 hour light-dark cycle was maintained in the troughs by an electric timer.

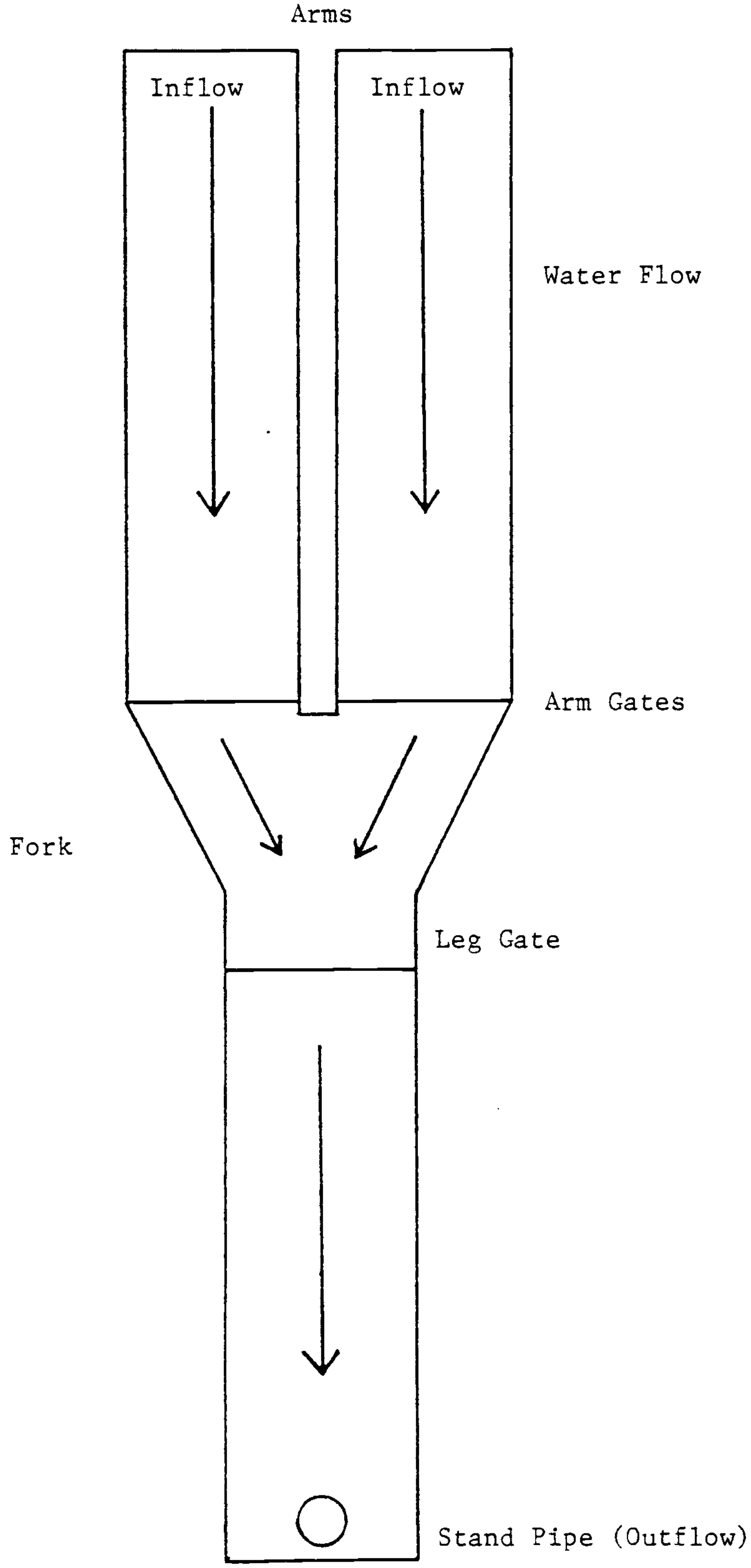


Figure 1: Top View of the forked trough used for the behavior aspect of the study.

The test scents were supplied (1 ml/sec.) by a mariotte bottle located just outside the plastic screening. The influent water was scented at the upper end of the arms. The formula by Scholtz et al. (1975) was used to calculate scent drip concentration.

Cardiac conditioning

The cardiac conditioning procedure involves monitoring a fish's heart rate during a series of paired presentations, called trials, of a scent and mild electric shock. If the fish can detect the odor, it will recognize it as a warning of the shock to come and its heart rate will decrease momentarily (Rommel and McCleave 1973; McCleave et al. 1974). A cardiac conditioning trial consisted of recording a fish's heart beat for 25 seconds, scenting the water, and recording the heart beat for an additional 25 seconds. The fish was then given a mild electric shock for one second, after which both scent and shock were discontinued. The test fish's heart beat was monitored by external electrodes to reduce handling stress before testing. Reliability of external electrodes was determined by running electrocardiograms (ECG) concurrently with external and implanted (as described by Heath 1972) electrodes for 11 fish. Identical results from both implanted and external ECGs were obtained.

A plexiglass chamber (14cm x 37cm x 9.5cm) as described by Spoor et al. (1971) was used for the cardiac conditioning experiments. Perforated stainless steel plates extending into both ends of the chamber were used as external cardiac electrodes. ECGs were amplified by Gould EEG amplifiers and recorded on a polygraph strip chart recorder. The high and low filter setting on the amplifiers were adjusted to dampen "noise" due to respiratory movements. A mercury wetted relay was used to short signals from the chamber to the amplifier during shock periods to prevent damaging the polygraph recorder and amplifier.

A 0.88mA to 2.81mA (2.01mV to 3.89mV) shock was delivered to the chamber by wire electrodes attached to the sides of the chamber. A constant water flow through the chamber, approximately one turn-over per 2 minutes, was maintained by a head box. The cardiac chambers were placed in a copper screen cage to filter electrical noise. The cage was covered with black plastic to isolate the fish from outside disturbances. The test scents were supplied by a mariotte bottle located immediately outside the black plastic (Scholtz et al. 1975). Influent water was scented at the inlet to the chamber. Foam rubber pads were placed under the cardiac chambers to dampen floor vibations.

The fish were placed into chambers 12 hours before testing was initiated. Six sets of eight trials each were conducted on each fish. The sets were separated by a 40 minute resting interval. An interval of 2.5-3.5 minutes was allowed to flush the scent from the chamber between successive trials. The irregular interval was used to minimize temporal conditioning by the fish.

The difference between the average heart rate (beats per 2.5 seconds) just before the scent was added, called reference beat, and the average heart rate during the presence of the scent but before the shock, called test beat, was used as an indicator or a cardiac response. A decrease in heart rate indicates a positive response, i.e., the fish was conditioned (Rommel and McCleave 1973; McCleave et al. 1974).

The set of eight trials with the greatest number of trials showing a decrease in heart rate was used to evaluate whether a fish was conditioned and was referred to as the "test set." In cases where two sets were the same, the second set was used as the test set. Test sets were used to account for different affects of electric shock on the fish's ability to detect odors and respond to conditioning and its heart's ability to respond and to account for possible variable learning capacity of fish of differing sizes. The mean change in heart rate for the eight trials during the test set was compared to zero by a one-tailed t-test. A fish with a significant (α = 0.05) decrease in heart rate during the test set was considered conditioned. The ratio of the number of conditioned fish to the number of non-conditioned fish for a test was evaluated using the chi square test.

Orientation conditioning

The orientation conditioning methodology is based on modifying the behavior of a group of fish during a series of trials to follow a scent to one end of a maze and avoid that end when the scent is not present. Positive, reward, conditioning was used to condition the fish to the odors. Negative (punishment) conditioning was tried but the fish would not respond (Appendix I).

Although the forked troughs (Figure 1) were used, one of the two arms in each trough was blocked and not used during the conditioning experiments. An earlier effort was made to condition the fish to choose between a scented and non-scented arm but the results were inconclusive (Appendix I).

For the orientation conditioning, 25 fish were placed into the forked troughs. The troughs were immediately scented at the upstream end of one arm and the fish in the arm fed. The scent was terminated at ten minutes. Two ten minute trials, scent present (S+) and scent absent (S-) were conducted on each subsequent day (Bloomfield 1969). During the S+ trials, the maze was scented, and those fish moving from the leg end of the forked maze to the scented arm were given a food reward. During S- trials, the fish were observed for an equal period of time but were not given the scent or food. At the end of each trial, the arm gate (Figure 1) was closed, the fish in the arm were counted and the number recorded as an S+ or S- response. The sequence of S+ - S- trials were randomized to avoid temporal conditioning by the fish. A strict morning-afternoon time schedule was maintained to encourage an appetite in the fish before each test. The fish were allowed access to the entire trough except one arm throughout the testing period. A conditioned response was achieved when the fish moved into the arm during S+ trials and avoided the arm during S- trials (Bloomfield 1969).

Experimental Design and Results

Methodology Validation

Cardiac conditioning

Phenethyl alcohol (PEA) was used to validate the cardiac conditioning procedure. Eight fish were tested with PEA at 1×10^{-3}mg/L and four fish were tested with non-scented water as controls from November through December. Five of the eight fish tested with phenethyl alcohol at 1×10^{-3}mg/L were conditioned ($\alpha = 0.05$; Table 1). No fish tested with non-scented water (control) were conditioned. The ratio of the number of conditioned fish to the number of non-conditioned fish tested with phenethyl alcohol differed significantly from the ratio of conditioned to non-conditioned fish tested with non-scented water (χ^2=4.3; df=1; $\alpha = 0.05$).

Orientation conditioning

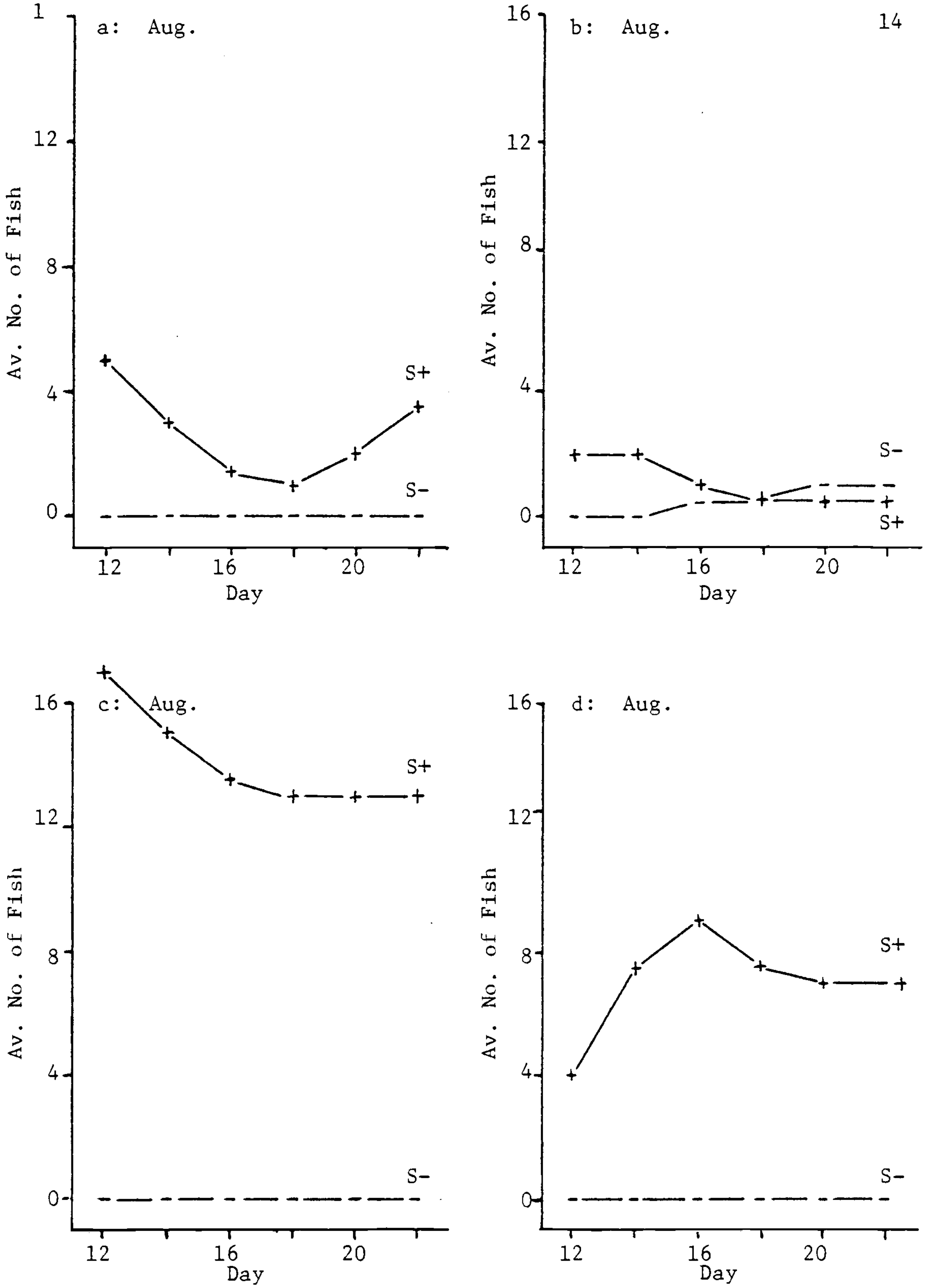
To validate the orientation conditioning bioassay, 24 post-smolt fish were placed into each of the four troughs on August 24 and treated with one of the following compounds: phenethyl alcohol (3×10^{-3}mg/L); morpholine (5×10^{-5}mg/L); a phenethyl alcohol and morpholine combination (3×10^{-3}mg/L and 5×10^{-5}mg/L respectively) and non-scented water (control), respectively.

The number of S+ and S- responses by the fish were averaged for 2 day periods and plotted against time for the last 12 days of the 24 day experiment for each compound tested (Figure 2) due to an error in experimental procedure. The relationships of the number of fish responding to the scented trials after a time showed the scented trial responses

TABLE 1. Coho salmon cardiac conditioned to phenethyl alcohol (PEA) at $1x10^{-3}$mg/L and non-scented water (control). The set number of eight trials with the greatest number of conditioned responses (CR) within that set, mean heart rate change ($\Delta \overline{HR}$) between the non-scented and scented periods during the set of 8 trials and calculated t- value are shown for each fish.

Test	Fish Number	Set Number	CR	$\Delta \overline{HR}$	Calculated t- Value	α = 0.05
PEA	1	6	7	-.112	2.385	Sig
	2	3	6	-.200	1.550	NS
	3	4	5	-.084	3.000	Sig
	4	5	6	-.097	2.487	Sig
	5	4	6	-.078	1.943	Sig
	6	5	6	-.107	1.814	NS
	7	1	6	-.072	1.600	NS
	12	3	7	-.372	2.735	Sig
Control	8	2	4	-.028	0.560	NS
	9	4	3	+.172	1.089	NS
	10	3	3	+.085	0.766	NS
	11	1	3	+.132	2.096	NS

Figure 2. Two day averages of fish responding to scented, S+, and non-scented, S-, trials from four groups of Sandy coho salmon treated with: a) morpholine, M, at $5x10^{-5}$mg/L; b) non-scented water (control), C; c) phenethyl alcohol and morpholine, P-M, at $3x10^{-3}$mg/L and $5x10^{-5}$mg/L respectively; and, d) phenethyl alcohol, P, at $3x10^{-3}$mg/L.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increased while the non-scented trial responses stayed the same or decreased. Since the S+ curves differ from the S- curves in all cases except the control, the phenethyl alcohol, morpholine, and phenethyl alcohol-morpholine combination treated groups were conditioned.

The responses of the three groups of fish treated with different scents were not compared because of the lack of replication. To determine odor specificity and cross-reactivity, each of the scent-treated groups was presented with the other two scents at the end of the experiment. Each group of fish was tested with a scent and given one reinforcement (S+) trial to their original odor and one non-reinforcement (S-) trial per day for 2 days before the next cross-reactivity trial. The phenethyl alcohol-morpholine, P-M, conditioned group did not respond to phenethyl alcohol, P, or morpholine, M, scents individually. Neither the phenethyl alcohol nor morpholine conditioned fish responded to a mixture of the two odors as follows:

	Positive (+) response and no (-) response of fish conditioned to:		
Test Odorant	M-P	P	M
M-P	+	-	-
P	-	+	-
M	-	-	+

Phenol Screening

Behavioral response

Three groups of 30 coho salmon each were used for the phenol avoidance-attraction trials. Each group was given four to six trials at each of five phenol concentrations in the behavioral bioassay (Table 2). Two control trials, non-scented drip in both arms, were conducted for each group before starting the scented trials. The number of fish in the two arms during the control trials was pooled by arm for the 3 groups. The total number of fish in the left arm did not differ significantly from the total in the right arm ($\chi^2 = 0.114$; df = 1; $\alpha = 0.05$). The number of fish in the scented and non-scented arms for the three groups was pooled for each concentration. A chi-square test was conducted to compare the total number of fish in the scented and non-scented arms for each concentration. The fish consistently avoided phenol at $1x10^{-1}$mg/L ($\alpha = 0.05$) and often avoided phenol at $1x10^{-2}$mg/L ($\alpha = 0.01$) (Table 2). No avoidance was noted at concentrations lower than $1x10^{-2}$mg/L. Fish not moving into either arm during the trials were not included in the analysis.

Cardiac conditioning

Five of six fish responded to phenol at $1x10^{-5}$mg/L and five of six fish responded to phenol at $1x10^{-4}$mg/L (Table 3). The test set selection criteria and data analysis described for the cardiac conditioning validation experiment were used to determine a conditioned response. The ratio of the number of conditioned fish to the non-conditioned fish for both phenol concentrations differed significantly from the ratio of the control group ($\chi^2 = 9.733$; df = 1; $\alpha = 0.05$).

TABLE 2. Chi-square (χ^2) values from comparing the total number of juvenile coho salmon in scented and non-scented arms of forked troughs and significance at $\alpha = 0.05$ for phenol avoidance-attraction trials. Fish not moving into either arm were not included in the analysis. H_0 = number in scented = number non-scented. The tests were conducted from April through May.

Phenol Concentration (mg/L)	Total Number Fish in Scented	Total Number Fish in Non-scented	χ^2	$\alpha = 0.05$
$1x10^{-5}$	111	137	2.726	NS
$1x10^{-4}$	88	89	0.006	NS
$1x10^{-3}$	115	119	0.068	NS
$1x10^{-2}$	103	146	7.426	Sig
$1x10^{-1}$	94	150	12.852	Sig

TABLE 3. Coho salmon cardiac conditioned to phenol at $1x10^{-5}$mg/L and $1x10^{-4}$mg/L. Number of the set (eight trials) with the greatest number of conditioned responses (CR), mean change in heart rate ($\Delta \overline{HR}$) and calculated t-value are shown for each fish. Tests were conducted during May.

Test	Fish #	Set Number	CR	$\Delta \overline{HR}$	Calculated t-value	α =0.05
Phenol $1x10^{-5}$mg/L	1	4	8	-.246	3.712	Sig
	2	5	6	-.096	3.840	Sig
	3	3	8	-.220	4.681	Sig
	4	2	7	-.240	2.162	Sig
	5	2	6	-.216	1.490	NS
	6	4	7	-.110	5.238	Sig
Phenol $1x10^{-4}$mg/L	1	2	7	-.244	3.342	Sig
	2	4	7	-.267	2.934	Sig
	3	5	7	-.153	4.250	Sig
	4	5	6	-.125	3.472	Sig
	5	6	6	-.139	3.390	Sig
	6	3	4	-.039	0.975	NS

Genetic Comparison

Cardiac conditioning

Fall Creek and Sandy Hatchery coho salmon stocks reared at Smith Farm were tested with Fall Creek Hatchery water diluted 1:1,000 and 1:10,000 (Table 4). A chi square test was used to compare the ratio of the number of conditioned fish to the number of non-conditioned fish of each group tested for each scent to the ratio of their respective control group (Table 5) and ratios between groups (Table 6).

The performance of the Fall Creek and Sandy Hatchery groups to water from both hatcheries did not differ significantly (Table 6). The number of Fall Creek coho salmon that were conditioned increased significantly between January and April (Table 5). The number of Fall Creek coho salmon responding to Fall Creek Hatchery scent at dilutions of 1:1,000 and 1:10,000 did not differ significantly from the number responding to the non-scented water in January but did in April. A chi square test of the ratios of conditioned to non-conditioned fish showed a significant increase in the number of Fall Creek coho salmon responding to Fall Creek water diluted to 1:10,000 between February and April (Table 6). The lack of experimental data precluded similar comparisons for Sandy Hatchery coho salmon.

Orientation conditioning

Fall Creek and Sandy Hatchery coho salmon stocks reared at both Smith Farm and their respective hatchery were tested with water from both hatcheries diluted 1:1,000. The number of S+ and S- responses for 2 day periods were averaged and plotted against time for each test

TABLE 4. Cardiac conditioning of Fall Creek (FC) and Sandy (S) Hatchery coho salmon reared at Smith Farm to water from both hatcheries diluted 1:1,000 and 1:10,000 and non-scented Smith Farm water (control). The date tested, fish stock, test water and dilution, number of fish tested (N), and number of fish conditioned (C), are shown for each test.

Test Date	Fish Stock	Test Water	Dilution	N	C
1/12	FC	FC	1:1,000	6	4
4/5	FC	FC	1:1,000	4	4
2/16	FC	FC	1:10,000	6	2
4/12	FC	FC	1:10,000	4	4
3/1	S	FC	1:1,000	4	4
4/5	S	FC	1:1,000	4	3
4/12	S	FC	1:10,000	4	3
4/23	FC	S	1:10,000	6	3
4/23	S	S	1:10,000	6	4
5/4	FC	Control		4	1
5/4	S	Control		4	0

TABLE 5. Chi-square (χ^2) values from comparing the responses (number of conditioned to number not conditioned) of Fall Creek (FC) and Sandy (S) Hatchery coho salmon, reared at Smith Farm and cardiac conditioned to water from Fall Creek (FC) or Sandy (S) Hatcheries, to the response of Fall Creek and Sandy coho salmon tested with non-scented water (control). Fall Creek and Sandy fish are compared to their respective control groups.

Date	Fish Stock	Test Water	Dilution	Response Conditioned	Response Non-conditioned	χ^2	α=0.05
5/4	FC	Control		1	3		
1/12	FC	FC	1:1,000	4	2	1.67	NS
4/5	FC	FC	1:1,000	4	0	4.80	Sig
2/16	FC	FC	1:10,000	2	4	0.10	NS
4/12	FC	FC	1:10,000	4	0	4.80	Sig
4/23	FC	S	1:10,000	3	3	0.57	NS
5/4	S	Control		0	4		
3/1	S	FC	1:1,000	4	0	8.00	Sig
4/5	S	FC	1:1,000	3	1	5.40	Sig
4/12	S	FC	1:10,000	3	1	5.40	Sig
4/23	S	S	1:10,000	4	2	4.45	Sig
5/4	FC	Control		1	3	a	
5/4	S	Control		0	4		

[a]Expected values for conditioned fish in conditioned to non-conditioned ratio are too small for χ^2 test.

TABLE 6. Chi-square (χ^2) values from comparing the responses (number conditioned to number not conditioned) of Fall Creek (FC) and Sandy (S) Hatchery coho salmon reared at Smith Farm and cardiac conditioned to water from Fall Creek (FC) and Sandy (S) Hatcheries.

Date	Fish Stock	Test Water	Dilution	Response Conditioned	Response Non-conditioned	χ^2	$\alpha=0.05$
1/12	FC	FC	1:1,000	4	2	a	
4/5	FC	FC	1:1,000	4	0		
1/12	FC	FC	1:1,000	4	2	1.34	NS
2/16	FC	FC	1:10,000	2	4		
2/16	FC	FC	1:10,000	2	4	4.45	Sig
4/12	FC	FC	1:10,000	4	0		
4/5	FC	FC	1:1,000	4	0	a	
4/12	FC	FC	1:10,000	4	0		
4/5	FC	FC	1:1,000	4	0	a	
4/5	S	FC	1:1,000	3	1		
4/12	FC	FC	1:1,000	4	0	a	
4/12	S	FC	1:10,000	3	1		
4/12	FC	S	1:10,000	3	1	0.63	NS
4/23	S	FC	1:10,000	3	3		
4/12	S	FC	1:10,000	3	1	0.08	NS
4/23	S	S	1:10,000	4	2		
4/23	FC	S	1:10,000	3	3	2.00	NS
4/23	S	S	1:10,000	4	2		
4/12	FC	FC	1:10,000	4	0	2.84	NS
4/23	FC	S	1:10,000	3	3		
3/1	S	FC	1:1,000	4	0	1.14	NS
4/5	S	FC	1:1,000	3	1		
1/12 & 4/5	FC	FC	1:1,000	8	2	0.14	NS
3/1 & 4/5	S	FC	1:1,000	7	1		
1/12 & 4/5	FC	FC	1:1,000	8	2	0.96	NS
2/16 & 4/12	FC	FC	1:10,000	6	4		

[a]Expected value too small for χ^2 test.

(Figures 3, 4, and 5). A visual inspection of the data indicates both Sandy and Fall Creek coho salmon reared at Smith Farm were conditioned to water taken from both hatcheries (Figures 3a: Nov., 3b: Nov., 3c, 3d, and 5a) during the period tested.

However, neither Fall Creek nor Sandy coho salmon reared at their hatcheries would respond to conditioning until a 7 week acclimation period at Smith Farm had passed (Figures 4 and 5). The Fall Creek coho salmon reared at Fall Creek did respond to conditioning following a 7 week acclimation period but the Sandy fish from Sandy did not. Fall Creek and Sandy coho salmon reared at Smith Farm tested at the same time (March) did not become conditioned. The two hatchery groups were placed in 1.0m circular tanks and held for an additional 2 weeks. At the end of that period the fish were tested with the same scent they received 2 weeks earlier. Neither group of fish held for 2 weeks were conditioned (Figure 5).

Conditioned fish were used to determine the effect of handling and crowding on the fish's response once conditioned. Two groups of Sandy coho salmon reared at Smith Farm, one conditioned to Fall Creek Hatchery water (Figure 3b) and the second a control, were transported in Smith Farm water for 2 hours on a truck and returned to their respective conditioning chambers. The same hauling protocol followed during the transport of the hatchery fish to Smith Farm was used. Both the conditioned and control groups were presented with their respective treatment at 4, 24, and 48 hours after being hauled. The conditioned fish did not respond properly to their scent until 48 hours (Table 7).

Figure 3. Two day averages of fish responding to scented (S+) and non-scented (S-) trials from Fall Creek (FC) and Sandy (S) Hatchery coho salmon reared at Smith Farm and tested with water from both hatcheries diluted 1:1,000: a shows the responses of FC fish to FC hatchery water in November and March; b shows the responses of S fish to S water in November and March; c shows the responses of S fish to FC water in December; and d shows the responses of FC fish to S water in January.

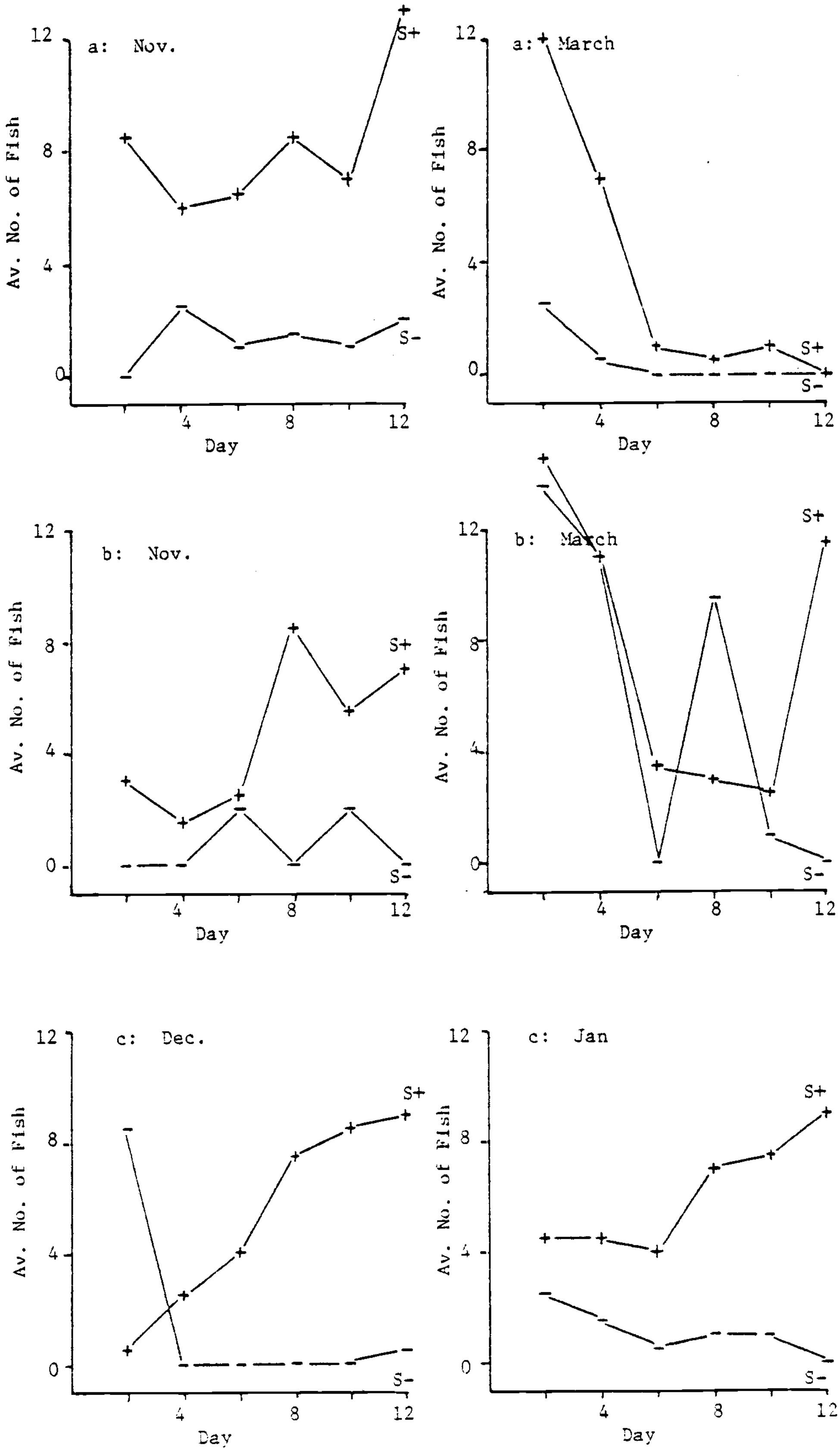
a: Nov.
a: March
b: Nov.
b: March
c: Dec.
c: Jan
Av. No. of Fish
Day
S+
S-
0
4
8
12

Figure 4. Two day averages of fish responding to scented (S+) and non-scented (S-) trials from Fall Creek (FC) and Sandy (S) Hatchery coho salmon reared at their respective hatchery and tested with water from both hatcheries diluted 1:1,000: a response of FC fish to FC water (no acclimation to Smith Farm) in November; b response of FC fish to S water (two week acclimation) in January; c response of S fish to S water (two week acclimation) in January; and d response of S fish to FC water (no acclimation) in December.

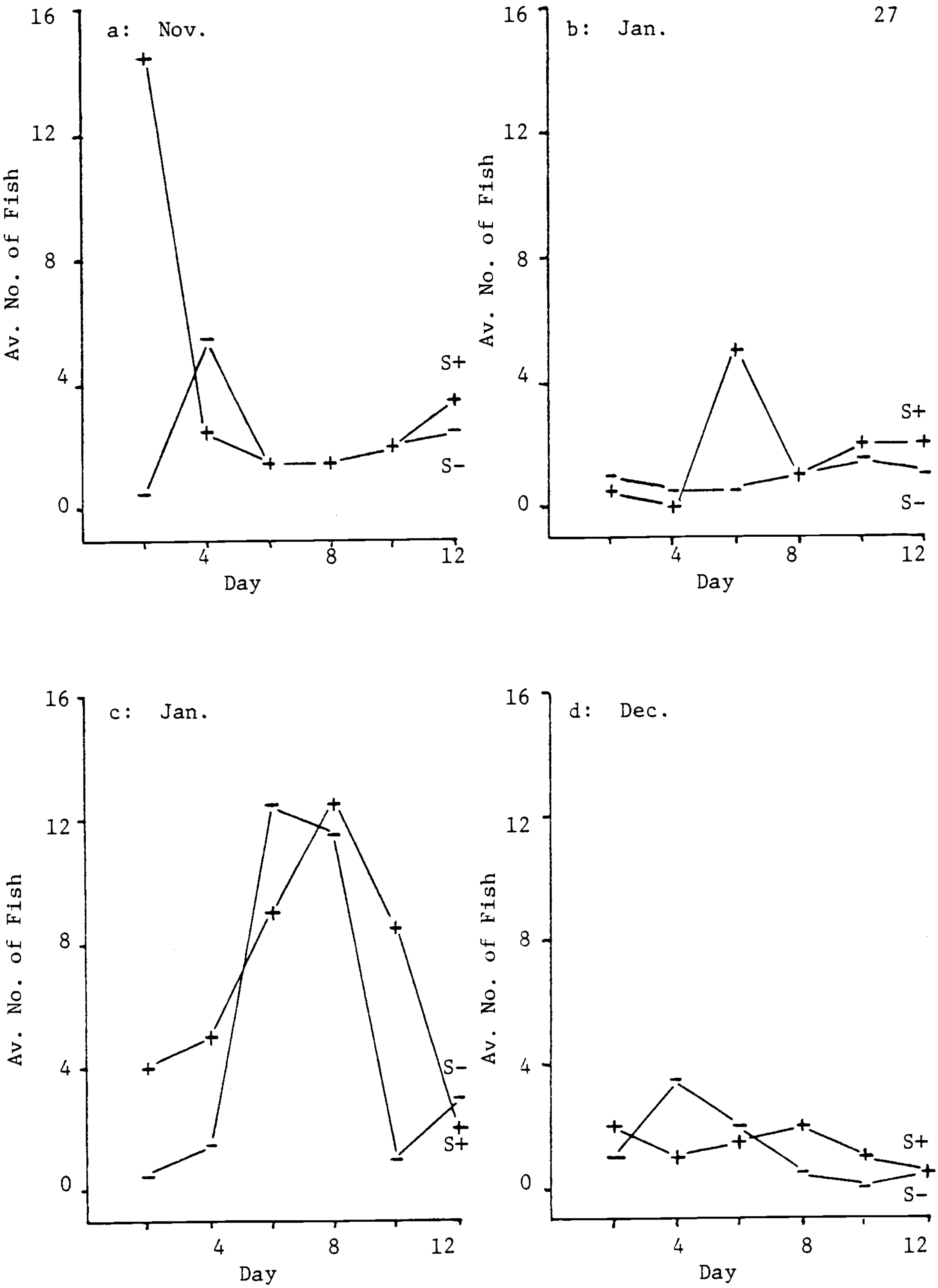
a: Nov.
b: Jan.
c: Jan.
d: Dec.
Av. No. of Fish
Day
16
12
8
4
0
4
8
12
S+
S-

Figure 5. Two day averages of fish responding to scented (S+) and non-scented (S-) trials from Fall Creek (FC) Hatchery coho salmon reared at and tested with water from FC Hatchery after 7 (a) and 12 weeks (b) acclimation to Smith Farm, and Sandy (S) coho salmon reared at and tested with water from S Hatchery after 7 (c) and 12 weeks (d) acclimation to Smith Farm.

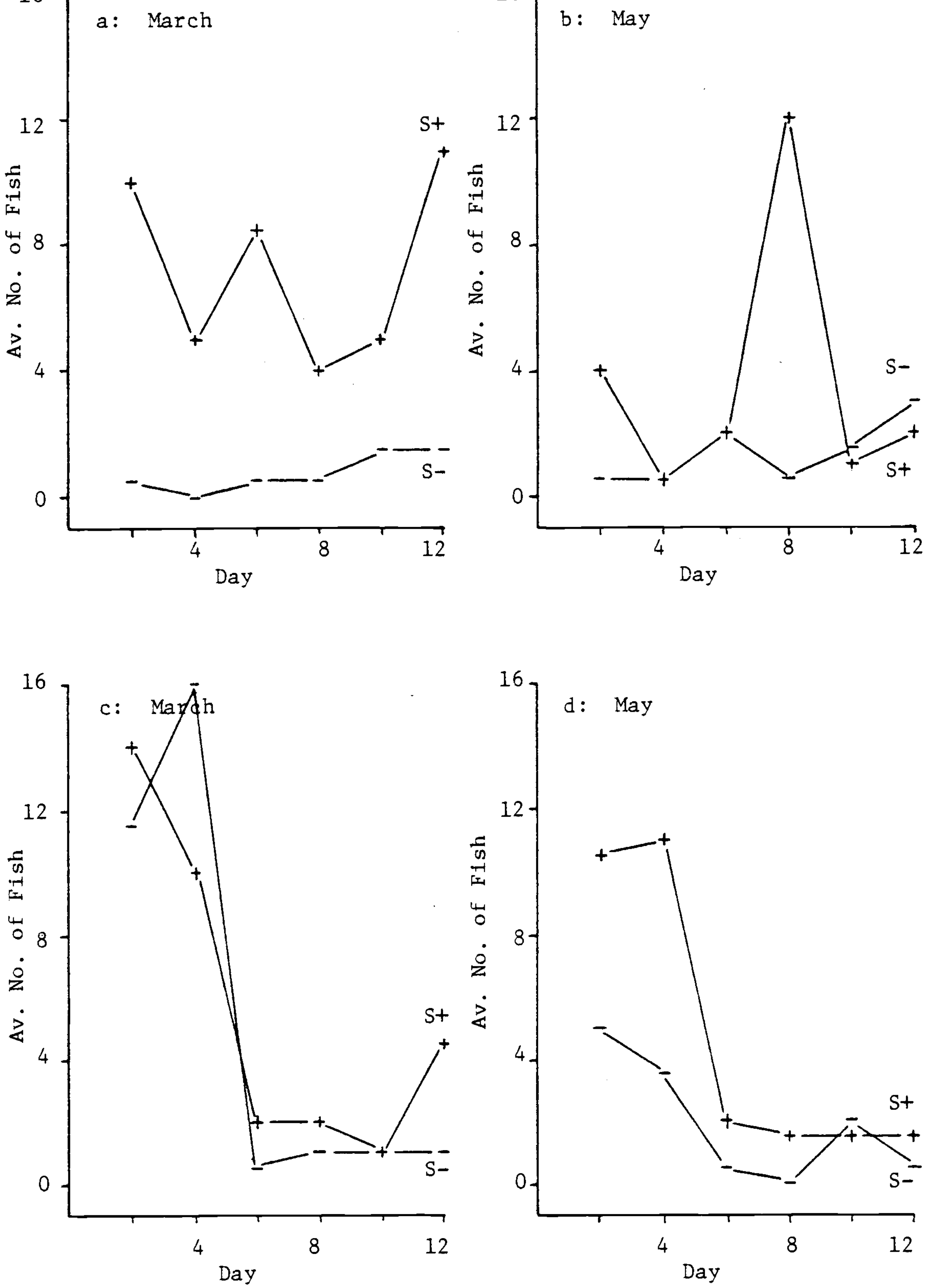
a: March
S+
S-
Av. No. of Fish
Day
0
4
8
12
16
b: May
S-
S+
c: March
d: May

TABLE 7. Number of fish from two groups of Sandy coho salmon, one conditioned to Fall Creek (FC) water diluted 1:1,000 and a control group following their respective test water through a maze during trials conducted after a 2 hour transportation challenge.

	Number of Fish Responding to Scent	
Hours from Challenge	Fish Conditioned to FC	Control Fish
-24	9	0
4	2	1
24	6	0
48	9	0

Discussion

I found cardiac and orientation conditioning are effective methods for comparing the olfactory discriminating ability of coho salmon. Cardiac conditioning was effective in determining odor detection for coho salmon as determined by the validation experiment. Cardiac conditioning also appeared efficacious in testing Atlantic salmon (Salmo salar) for sound (Hawkins and Johnstone 1978) and electric and magnetic fields (Rommel and McCleave 1973), and American eel (Anguilla rostrata) for dim light (McCleave et al. 1974) recognition.

During the validation experiment for the orientation conditioning method, the three groups of fish tested with phenethyl alcohol, PEA, morpholine, M, and a PEA-M combination produced results similar to the conditioned response criteria described by Bloomfield (1969). The group of fish tested with non-scented water did not produce these results. The data for the scent-tested groups is similar in form to the general fish learning curves reported by Agranoff and Davis (1968), Bitterman (1968), and Mackintosh (1969).

I found coho salmon are not attracted or repelled by concentrations of phenol less than 1×10^{-3}mg/L. Jones (1951) reported the minnow (Phoxinus phoxinus) showed no avoidance or symptoms of intoxication to a concentration of 4×10^{-2}mg/L. The coho salmon could detect phenol at 1×10^{-5}mg/L, as evidenced by results of the cardiac conditioning tests. Bluntnose minnows could be conditioned to phenol at 1×10^{-5}mg/L (Hasler and Wisby 1950).

Both stocks of fish reared at Smith Farm could detect water from both hatcheries diluted 1:10,000 in April as determined by cardiac conditioning experiments. Chinook salmon (*O. tshawytscha*) have imprinted and homed to hatchery water diluted 1:10,000 (Groves *et al*. 1968). The ratio of the number of fish conditioned to the number of fish not conditioned for tests conducted with the Fall Creek fish to Fall Creek water increased significantly from February to April. Hormone changes occurring during spring smolting may have affected the fish's olfactory ability. Fish mid-brain electrical potentials are perhaps "sensitized" by thyroxine (Gorbman 1969) which also potentiates the reactivity of the neurons in the olfactory bulb (Gorbman 1969). The pattern of spontaneous electroencephalograms (EEGs) from the olfactory bulb may depend on the level of circulatory thyroxine and the sensitivity and magnitude of response to an olfactory stimulus may also depend on thyroxine (Fontaine 1975). Levels of plasma thyroxine are believed to change in smolting coho salmon (Dickhoff *et al*. 1978). This hormone has also been implicated in regulation of downstream migration (Baggerman 1960, 1963).

Both Sandy and Fall Creek coho salmon could detect and learn to respond to water from both hatcheries diluted 1:1,000 as determined by the orientation conditioning method. The responses of the fish during the orientation experiment with hatchery water could not be compared for lack of replication and the uncertain effect of varying group size on learning (Gleason and Weber 1977). However, some differences in the performance by the groups are apparent. The response of a group of fish was affected by the stress from handling and changes in the environmental conditions experienced by the fish prior to conditioning, and the time of year or life history stage of the group.

The behavior of the hatchery-reared stocks (Figures 4 and 5) and the behavior of the fish used in the transporation experiment suggest that transporation, handling and change in environmental conditions adversely affected the fish's ability to be conditioned and their performance once conditioned. High levels of stress hormones have been reported in fish netted and held or handled (Fagerlund 1966; Mazeaud _et al_. 1977; Strange _et al_. 1978), and transportation can elicit a severe, sublethal hormonal stress response in coho salmon (Specker and Schreck, unpublished data). Bull (1936) recommended that the fish be allowed 2 weeks acclimation to research facilities before initiating a conditioning experiment. Denny and Ratner (1970) also suggested a period of acclimation to environmental changes before starting a conditioning experiment. The acclimation period probably depends on the severity of the stress placed on the fish. The Smith Farm subgroups did condition after being netted and counted into the forked troughs, while fish hauled for 2 hours needed from 2 to 7 weeks acclimation before conditioning.

The response of coho salmon during the orientation and cardiac conditioning experiments was affected by the time of year or life history stage of the fish. The Fall Creek and Sandy Hatchery coho salmon reared at Smith Farm could be conditioned in the fall but not in the spring in the orientation conditioning system. Juvenile coho salmon typically smolt and migrate to the ocean in the spring. Prior to smolting, the juvenile coho salmon are territorial (Hoar 1976; Woo _et al_. 1978). The behavior of the Smith Farm fish paralleled these characteristics before March, establishing and defending territories in the troughs. The fish become very aggressive at this stage, and during scented trials physical contact such as nipping was common. The behavior of the fish reared at Smith Farm and tested in March was more smolt like, as outlined by Hoar (1976) and characterized by losing territorial tendencies and forming schools. The fish tested in March grouped around the standpipe at the downstream end of the maze and rarely displayed aggressive acts, perhaps suggesting an inclination for migrating downstream.

Hatchery reared fish differed from their Smith Farm counterparts by conditioning in March. The discrepancy in behavior between the hatchery and Smith Farm fish may be contributed to size differences between the two groups and possible differences in degree of smolting. At this time the fish reared at Smith Farm (13.83cm ± 0.04) were slightly larger than peers raised at the hatchery (12.95cm ± 0.04). Larger coho salmon smolt earlier than smaller fish (Vanstone and Markert 1968) and migrate earlier (Gribanov 1962; Godfrey 1965). The Smith Farm-reared salmon may have smolted earlier due to increased size.

The time of year may have also influenced the fish's ability to detect or learn odors or both. There are seasonal differences in the learning ability of fish, perhaps attributable to changes in hormone levels (Shashava 1973). It is known that levels of certain hormones such as thyroxine change during the smoltification process (Fontaine 1975; Dickhoff _et al_. 1979; Dickhoff _et al_. 1978).

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Appendix

The following provides a description of the conditioning procedure and an evaluation of the results for each conditioning protocol tested in developing the orientation conditioning method. Coho salmon from Sandy Hatchery, Oregon, were used for these experiments.

Experiment I

Two forked troughs with water flowing into the head of the "arms" and out the bottom end of the "leg" were used for this experiment. The troughs were equipped with gates located at the upper end of the leg and the lower end of each arm. Two groups of 13 smolt-size coho salmon were placed into the leg of each trough and held for 7 days before the conditioning trials were begun. A trial involved metering the odor (PEA) into one of the arms, determined randomly each day, for 7 minutes before the gate at the upstream end of the leg was opened. The time interval allowing the odor to travel the full length of the trough had been determined by dye-markers.

The fish were allowed 1 minute to move up the trough and choose between the scented and non-scented arm before gates in the lower end of the arms were closed. The number of fish in each area (scented arm, non-scented arm or leg) of the trough were recorded. Those fish in the leg and non-scented arm were netted and placed into the scented arm. All fish were fed in the scented arm before the scent was terminated. The fish were crowded back to the leg end of the chamber and held until the following trial.

The results of these conditioning trials were inconclusive due to either the experimental technique or the effect of smolting on the behavior of the fish. The stress imposed on the fish by netting may have inhibited the fish's ability to learn. Smolt-size coho salmon were used for this experiment. Behaviorally, smolts move downstream. The smolting urge of the fish to move downstream may have inhibited their response to an upstream conditioning experiment or the migration urge may overpower any conditioning effort.

Experiment II

Four forked-shaped troughs as described in I above were used for this experiment. A group of 13 post-smolt coho salmon was placed in each trough. Phenethyl alcohol ($3x10^{-3}$mg/L), morpholine ($1x10^{-5}$mg/L) and a combination of phenthyl alcohol and morpholine ($3x10^{-4}$mg/L and $1x10^{-5}$mg/L, respectively) were used as odor-cues. Smith Farm water provided a control for this experiment. The coho in the four troughs were given either an odor-cue or control water during the test.

The fish were anesthetized (MS222 0.5mg/L) for weighing and measuring. They were then placed into the leg portion of the troughs and held there for 7 days before testing.

During a conditioning trial, scented water flowed into an arm that was chosen randomly each day. After 7 minutes the gates at the upper end of the leg and lower ends of the two arms were opened. A 9.5mA (4.8mV) current was passed through the leg and the non-scented arm 30 seconds after the gates opened. Following the electric shock, all the gates in the trough were closed and fish in the scented arm were fed. The number of fish in each area of the trough was recorded. The fish were crowded into the leg and held for the next trial. A morning and evening trial was conducted each day.

The behavior of the fish in the four troughs varied between and within troughs. Some fish displayed strange behavior, such as swimming in tight circles, or staying in the leg during the shock. Some fish moved into the arms regardless of scenting or shock.

The fish's behavior was influenced by the electric current. Some fish may have associated an act such as spinning with stopping the shock. Some fish located and stayed in areas of weak electric fields in the trough. A few fish would swim from the leg into the arms. However, the arm into which a fish swam seemed to have been based on the fish's first experience in that part of the trough. A fish choosing the arm that was not shocked during the first few trials may have considered that arm a refuge and preferred it during later trials. Conversely, a fish choosing an arm with shock during early trials might avoid it during later trials.

On day 11 of conditioning, one of the two gates was closed and not opened throughout the remainder of the experiment. The electric shock was also discontinued. The trough was scented, the gate closed, and the fish fed in the same manner as had been previously used.

The fish were less reluctant to move into the arm after the shock was discontinued. However, I could not distinguish fish following the scent for food from those fish that were not conditioned.

On day 18, the experimental procedure was changed so that one of the two daily trials was non-scented control water. The procedure for a non-scented (S-) trial was the same as for a scented (S+) trial except non-scented water was dripped into the arm. Fish in the arm were not fed. The sequence of S+ and S- trials was randomized to avoid temporal conditioning by the fish.

The number of fish swimming from the leg to the arm of the trough increased during scented trials. The number of fish swimming into the arm during non-scented trials decreased. This behavior was observed in fish exposed to each odor cue (phenethyl alcohol, morpholine, and phenethyl alcohol-morpholine combination). Very few fish swam into the arm of the trough treated with non-scented water only.

Experiment III

The forked troughs and treatment with four scents described in II above were used for these experiments. One of the two arms in each trough was blocked and not used during the experiment. A group of 25 juvenile coho salmon was placed into the forked portion of each trough. A scented trial was conducted within 10 minutes after the fish were placed into the troughs.

The procedure, S+ and S- trials, described above was used during the trials. However the fish were allowed access to the leg and one arm of the trough between trials.

The three groups of fish, phenethyl alcohol treated group, morpholine treated group, and phenethyl alcohol-morpholine combination treated group, became conditioned to their respective scent. The number of fish moving into the arm during S+ trials increased while the number of fish moving into the arm during S- trials decreased. The group of fish treated with non-scented odor during S+ trials did not show the same trend.