AN ABSTRACT OF THE THESIS OF

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Title: Behavioral Response of Juvenile Coho Salmon (Oncorhynchus kisutch) to Olfactory Cues

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Dr. Carl B. Schreck

A behavioral response assay was developed to evaluate the olfactory ability of juvenile coho salmon (Oncorhynchus kisutch) over the period of parr-smolt transformation and to evaluate the effects of certain pollutants on olfactory acuity. Coho salmon parr could detect and avoid concentrations of the amino acids L-serine and DL-alanine as low as 1×10^{-7} M in January. The lowest concentrations of L-serine and DL-alanine avoided by smolts in late April - early May was 1×10^{-5} M and 1×10^{-6} M, respectively, while it was 1×10^{-6} M and 1×10^{-5} M for smolts tested in June. Exposure to copper temporarily blocked the ability of juvenile coho to detect and avoid DL-alanine at 1×10^{-5} M. Exposure to 2,4-D, however, had no measurable effect on olfactory ability of the fish. Juvenile coho salmon were not attracted to odors of roe, milt, bile, liver or mucus from adult coho salmon. Catechol and B-phenylethylamine are proposed as potential candidates for artificial imprinting chemicals since they can be

detected by coho salmon at concentrations of 5×10^{-8} M and 4×10^{-8} M, respectively, but are neither naturally attractive nor repulsive to fish at these concentrations.

Behavioral Response of Juvenile Coho Salmon (*Oncorhynchus kisutch*) to Olfactory Cues

by

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INTRODUCTION

Adult anadromous salmonids tend to return to spawn in the stream from which they migrated as smolts (see review by Hasler, 1966; Harden-Jones, 1968), although some straying does occur. Nicholas et al. (1982) estimated that 43% of the coho salmon spawning in Yaquina River, Oregon, tributaries in 1980 were strays from a private salmon hatchery on Yaquina Bay. The straying of hatchery-produced salmonids has the potential for two deleterious effects: 1) fewer fish returning to the hatchery for spawning, resulting in a decreased egg-take, and 2) the hatchery fish may spawn with wild fish in the river which could result in production of fewer smolts and thus fewer adults (Reisenbichler and McIntyre, 1977). This study was conducted to investigate several aspects of salmonid olfaction which may affect the straying of hatchery-produced adult salmonids.

The freshwater phase of the spawning migration of salmonids is primarily guided by olfaction (Hasler et al., 1978). Young salmon imprint to the odor characteristics of their homestream as smolts (Jensen and Duncan, 1971) and then use this information to return to the homestream to spawn as adults. Since imprinting ability must be especially keen when the fish are smolts, it is possible that the olfactory ability of the salmon is greater at this time than as parr.

Straying of adult fish may be due to an inability of the smolts to detect and learn the unique odor environment of the homestream, or

failure of the adult to recognize the odors upon returning. Hara (1972) showed that pollutants such as copper and mercury could block olfaction of coho, Oncorhynchus kisutch, and sockeye salmon, O. nerka. Exposure to substances which interfere with olfaction may cause straying of adult salmon. In the Pacific Northwest, salmonids may be exposed to forest herbicides while residing in streams. The effects of herbicides on fish olfaction are not known.

One approach to reduce straying of adult fish from the hatchery is to use a technique known as "artificial imprinting." Artificial imprinting involves exposing smolting salmonids to a synthetic chemical odorant and then releasing them to grow and mature in the ocean. During the adult spawning migration, these salmon will return to the site scented with this chemical (Scholz et al., 1975). This technique has been primarily used experimentally with morpholine and phenethyl alcohol as odorants (Hasler et al., 1978), but possibly could be used in production hatcheries to reduce adult straying.

Another approach to reducing the straying of fish may be to use naturally produced odorants to attract fish into the hatchery. Nordeng (1977) proposed that anadromous salmonids home to odors produced by conspecifics which are residing in the homestream.

My goal was to improve the homing accuracy of hatchery-reared salmon through a better understanding of salmonid olfaction. The experiments reported here were conducted to answer the following four questions: (1) Is olfactory sensitivity of coho salmon heightened during parr-smolt transformation? (2) Do certain pollutants affect

olfactory ability? (3) Can other chemicals, in addition to those already proposed, be used for artificial imprinting? (4) Are odors of select organs or parts of salmon attractive to other coho salmon?

METHODS

Juvenile coho salmon were held in 1 m diameter flow-through fiberglass tanks supplied with 12 C well water at Smith Farm laboratory in Corvallis. All tests were conducted in this well water between 0800 and 1600 hours. Fish were fed to repletion daily with Oregon Moist Pellets. Food was withheld from the fish 24 h before testing.

Olfactory Sensitivity

To determine whether olfactory sensitivity of coho salmon is heightened during the parr-smolt transformation, the ability of juvenile coho salmon from Fall Creek Fish Hatchery, Oregon, to detect and avoid odors was evaluated in two Y-mazes (Fig. 1). Well water entered the head of the right and left channels of the maze at 8 l min^{-1} and the head of the middle channel at 15 l min^{-1}. The middle channel was used to aid in the separation of flows from the right and left channels, as shown by tests with rhodamine dye, and was screened so that no fish could enter. The right, middle, and left channels were covered to exclude light, and the holding area (that part of the maze into which the test fish are introduced) was illuminated. The right and left channels were separated from the holding area by gates which could be raised and lowered by a drawstring unbeknownst to the fish. A funnel-type trap was placed in the opening of the right and left channels to allow the fish to enter, but generally prevent them from exiting.

Fig. 1. Top view of Y-maze used to determine behavioral responses of fish to various odors. Arrows indicate direction of water flow.

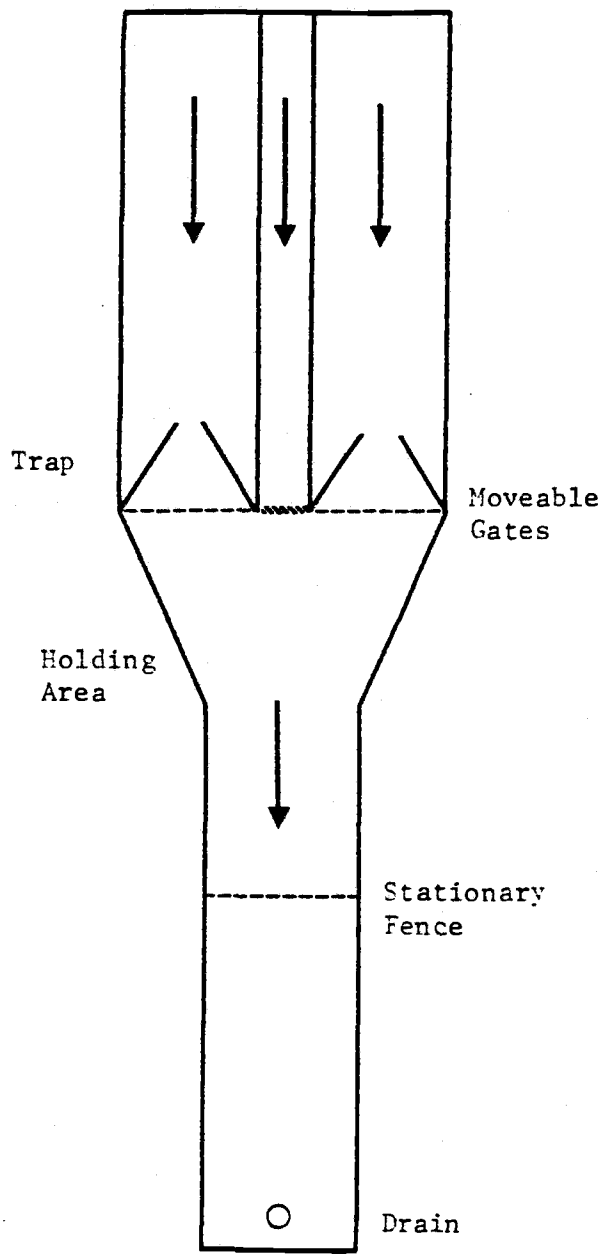

Figure 1.

A trial consisted of introducing 10 fish into the holding area of the maze and letting them habituate for 30 min. An odorant was then allowed to drip into the head end of one of the channels, selected randomly before each trial, for 10 min before the gates to both channels were raised. The fish were allowed 5 min to choose to enter either channel. The gates were then lowered and the number of fish in the scented and unscented channels and the holding area were recorded. Six trials were conducted for each odor concentration. Control trials were conducted in exactly the same manner with the exception that pure well water was added to one channel rather than scented well water. Each fish was tested only once during each period as a parr and again as a smolt.

The odorants used to determine olfactory sensitivity were the amino acids L-serine and DL-alanine. Coho salmon have been shown to avoid L-serine at low concentrations (Idler et al., 1956). Avoidance of an amino acid concentration in the Y-maze was assumed to be a measure of the fish's ability to detect the amino acid at that particular concentration. An odorant solution was prepared immediately before each trial by dissolving the appropriate amount of crystalline amino acid in well water. This odorant solution was supplied to one channel of the Y-maze by a mariotte bottle which allowed the scented water to drip into the Y-maze at a constant rate.

The preference behavior of the fish in the Y-maze was analyzed by chi-square test. For control trials, the number of fish entering

either channel of the Y-maze was compared to the 1:1 ratio expected by random choice. For each concentration of odorant, the chi-square value was determined by comparing the ratio of fish in the scented and unscented channels of the Y-maze to that ratio obtained in the control trials. The lowest concentration of odorant that the fish avoided was considered the threshold concentration and assumed to be a measure of the olfactory sensitivity of the group of fish at that particular time.

The fish were tested as parr in January-February (mean fork length, 125 mm; mean weight, 22.0 g) and as smolts in late April-early May (157 mm, 45.0 g) and in June.

Pollutant Treatments

The effect of exposure to copper and the forest herbicide 2,4-D (2,4-dichlorophenoxy acetic acid) on the olfactory ability of juvenile coho salmon from Oregon's Fall Creek Hatchery was evaluated in the Y-mazes. The fish were exposed to the pollutant and then tested in the Y-maze following the same procedure as used for determining olfactory sensitivity. DL-alanine at a concentration of $1x10^{-5}$ M was the odorant used, as this concentration was found effective in the olfactory sensitivity tests. Fish avoiding the scented channel, based on the chi-square analysis, were considered not affected by, or recovered from, the pollutant exposure.

Two groups of 80 post-smolt coho salmon were exposed to either copper ($CuSO_4$) (0.47 μM in August) or 2,4-D (10 mg l^{-1} in September)

for 24 h. The exposures were in 60 cm diameter fiberglass tanks receiving a continuous flow of well water. The pollutants were continuously added to the tank via mariotte bottles. After the 24 h exposure, the fish were removed from the exposure tank in groups of 10 and placed in the Y-maze to be tested for avoidance of DL-alanine. After the initial Y-maze test, the fish were held in a 60 cm diameter tank supplied with pure well water until tested again in the Y-maze 4, 7, and 14 days after the exposure period, or until the fish avoided the scented channel.

In another test, four groups of 20 post-smolt coho salmon were exposed to copper (3.1 mM in August) for 1.5 min to simulate a hatchery treatment for external bacteria as recommended by Leitritz and Lewis (1980). The fish were placed in a bucket containing a fresh 15 l solution of $CuSO_4$ for 1.5 min and then placed into one of two Y-mazes, in groups of 10, to be tested for avoidance of DL-alanine. After the initial Y-maze test, the fish were held in a 60 cm diameter tank supplied with clean water until tested again 4, 7, and 14 days after exposure, or until the fish avoided the scented channel. Control fish which were not exposed to the pollutant, but otherwise received the same treatment as exposed fish, were tested in the Y-maze on the same schedule as copper-exposed fish.

Conspecific Odors

To test the possibility that odors from coho salmon are attractive to other coho salmon, the response of juvenile Fall Creek

Hatchery coho to odors produced by adult coho salmon was evaluated in the Y-maze using the procedure described previously for determining olfactory sensitivity. Bile, roe, and mucus were obtained from freshly killed maturing coho salmon captured in salt water at Oregon Aqua Foods, Inc. (OAF), Newport, Oregon. Liver, roe, and milt were obtained from mature coho salmon in fresh water at Salmon River Hatchery, Oregon, that had just been killed for spawning. Care was taken to avoid contamination of the odor sources by other odorants, such as human odors or other fish products.

Bile, milt, and mucus were stored at -21 C. The whole roe and macerated livers were allowed to soak in water (500 g tissue l^{-1} for 18 h at 5 C to extract water-soluble components. The water was then decanted and frozen at -21 C until used. Immediately before each trial, the odor source was thawed and the appropriate dilution was made in the mariotte bottle. The concentrations of odors tested were 3 mg l^{-1} for bile, 30 mg l^{-1} for mucus and milt, and 15 mg l^{-1} for liver extract and roe extract.

Detection of Synthetic Imprinting Chemicals

To determine the possibility of using two odorants, catechol ($C_6H_6O_2$) and β-phenylethylamine ($C_8H_{11}N$), for imprinting salmon in a hatchery, juvenile coho salmon from Eagle Creek National Fish Hatchery Oregon, were tested in the Y-maze during June and July to establish their ability to detect these odorants at concentrations which are neither attractive nor repulsive. Trials were conducted

in the Y-maze following the procedure of the olfactory sensitivity test to determine a concentration of each chemical which was neither attractive nor repulsive to the fish.

To determine that the fish could indeed detect the odor at this "neutral" concentration, 75 juvenile coho salmon were placed in one 60 cm diameter fiberglass tank and exposed to the chemical for 14 days at the "neutral" concentration during July. The chemical was continuously added to the tank via a mariotte bottle to achieve a constant concentration of chemical in the exposure tank. After the 14 day exposure, the fish remained in the exposure tank for 4 additional days without the chemical before testing in the Y-maze. The fish were tested in the Y-maze with the chemical at the same concentration that was present in the exposure tank. If the fish are able to detect the odor at this concentration, they should enter the channel of the Y-maze with the odor, simulating "homestream recognition" and preference.

This detection assay was verified using morpholine at 6×10^{-10}M, a concentration that coho salmon are known to detect, but is neither attractive nor repulsive to fish previously unexposed to morpholine (Scholz et al., 1975).

RESULTS

In the behavioral avoidance tests in which the fish did avoid the odorant, more than 50% of the fish making a choice to enter either channel of the Y-maze consistently entered the unscented channel. In the control trials, the number of fish entering either channel in a given trial consistently differed by no more than three fish. Thus, the total number of fish in the scented and the total number in the unscented channels, respectively, for the six trials were pooled for each concentration and the control tests. The number of fish not entering either channel was usually 12% to 30% of the total number tested for each concentration.

Olfactory Sensitivity

The fish did not prefer one channel of the Y-maze over the other (Fig. 2a, b). During the control trials in which unscented water was added to one channel, the ratio of the number of fish in the channel with the unscented water to the number of fish in the other channel was not significantly different than 1:1 for parr ($X^2 = 0.02$), smolts in April ($X^2 = 0.20$) or smolts in June ($X^2 = 0.02$). There was no difference between the number of parr and smolts that did not move into either channel of the Y-maze.

The threshold concentrations for the avoidance of L-serine by coho parr was 1×10^{-7}M ($X^2 = 11.60$; $P < 0.05$), smolts in late April-early May was 1×10^{-5}M ($X^2 = 4.15$; $P < 0.05$), and smolts in June was 1×10^{-6}M ($X^2 = 4.85$; $P < 0.05$) (Fig. 2a). The threshold concentrations for the avoidance of DL-alanine by coho parr was 1 x

Fig. 2. Total percent of juvenile coho salmon avoiding various concentrations of amino acids in a Y-maze as parr in late January-early February, and smolts in late April-early May and June, based on those fish moving into a channel. Data from all six trials at each concentration were pooled. a. Amino acid is L-serine. b. Amino acid is DL-alanine. *P < 0.05, based on chi-square values determined by comparing total number of fish in scented and unscented channels of the Y-maze to control trials using pure well water. "C" denotes control trials. "N" denotes the total number of fish entering the channels of the Y-maze at each concentration.

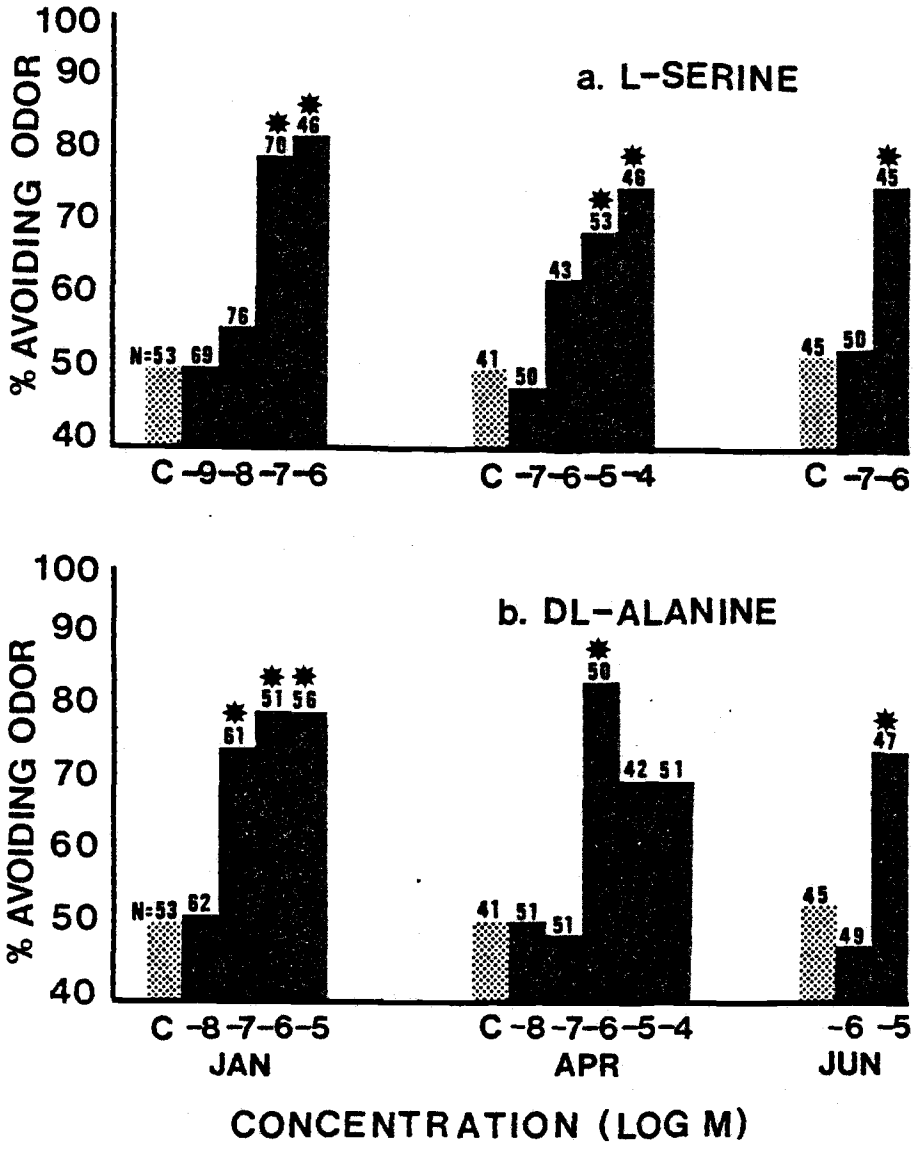
a. L-SERINE
% AVOIDING ODOR
100
90
80
70
60
50
40
N=53
69
76
78
46
41
50
43
53
46
45
50
45
C -9-8-7-6
C -7-6-5-4
C -7-6
b. DL-ALANINE
% AVOIDING ODOR
N=53
62
61
51
56
41
51
51
50
42
51
45
49
47
C -8-7-6-5
JAN
C -8-7-6-5-4
APR
-6 -5
JUN
CONCENTRATION (LOG M)

Figure 2.

10^{-7}M (X^2 = 7.37; $P < 0.05$), smolts in late April-early May was 1 x 10^{-6}M (X^2 = 11.36; $P < 0.05$) and smolts in June was 1 x 10^{-5}M (X^2 = 5.48; $P < 0.05$) (Fig. 2b).

Pollutant Treatments

In control tests (fish not receiving pollutant exposure), the fish avoided DL-alanine at 1 x 10^{-5}M on all days tested (Fig. 3).

Exposure to copper at 0.47 µM for 24 h inhibited the ability of juvenile coho salmon to avoid DL-alanine at 1 x 10^{-5}M in the Y-maze for 7 days following the exposure (Fig. 3). By day 14 following the exposure the fish had regained the ability to detect and avoid DL-alanine.

Coho salmon exposed to copper at 3.1 mM for 1.5 min did not avoid DL-alanine immediately after the exposure, but did avoid the amino acid 4 days following copper exposure (Fig. 4).

Exposure to 2,4-D at 10 mg l^{-1} for 24 h did not decrease the ability of coho salmon to detect and avoid DL-alanine at 1 x 10^{-5} M. The fish avoided the amino acid (X^2 = 25.78; $P < 0.05$) immediately after the herbicide exposure.

Conspecific Odors

Unscented water dripped into the Y-maze was neither attractive not repulsive to juvenile coho salmon. Juvenile coho salmon were not attracted to any of the conspecific odors tested in the Y-maze (Table I). One of the odors tested, roe from coho from OAF was avoided by the Fall Creek Hatchery juvenile coho salmon.

Fig. 3. Effect of 24 hour exposure to 0.47 μM copper on behavioral response of juvenile coho salmon to 1 x 10^{-5}M DL-alanine in a Y-maze and recovery from the exposure. *P < 0.05, based on chi-square values determined by comparing total number of copper-exposed fish in scented and unscented channels of the Y-maze to control trials without copper exposure. "N" denotes the total number of fish entering the channels of the Y-maze during each day of testing.

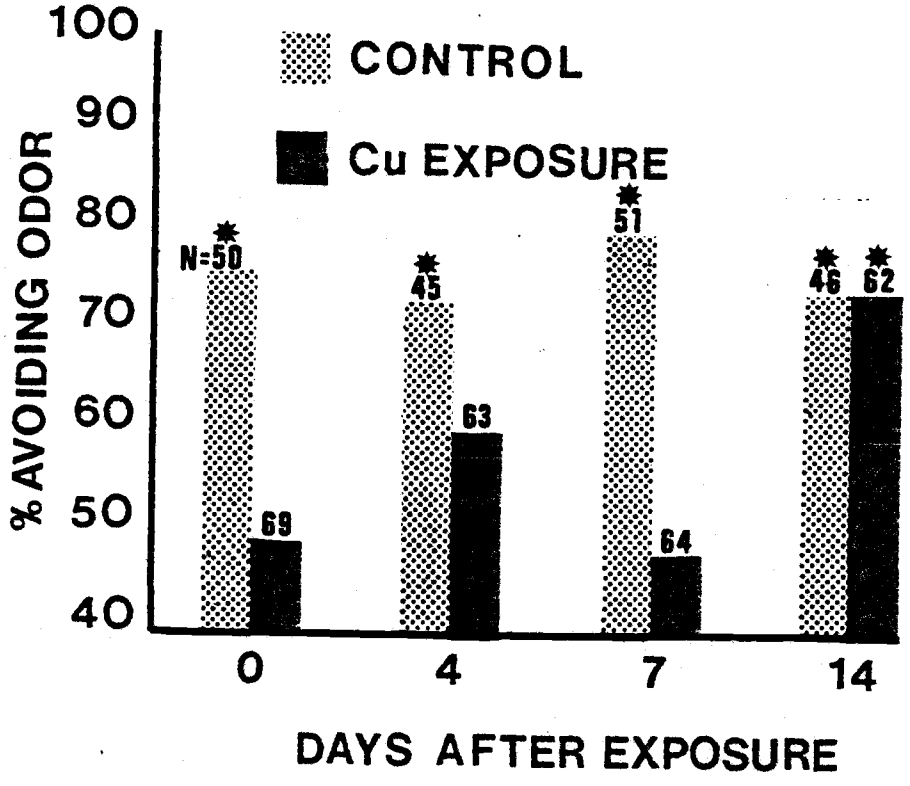
100
90
80
70
60
50
40
% AVOIDING ODOR
CONTROL
Cu EXPOSURE
N=50
69
45
63
51
64
46
62
0
4
7
14
DAYS AFTER EXPOSURE

Figure 3.

Fig. 4. Effect of 1.5 minute exposure to 3.1 mM copper on behavioral response of juvenile coho salmon to 1 x 10^{-5}M DL-alanine in a Y-maze and recovery from the exposure. *$P < 0.05$, based on chi-square values determined by comparing the total number of copper-exposed fish in the scented and unscented channels of the Y-maze to control trials without copper exposure. "N" denotes the total number of fish entering the channels of the Y-maze during each day of testing.

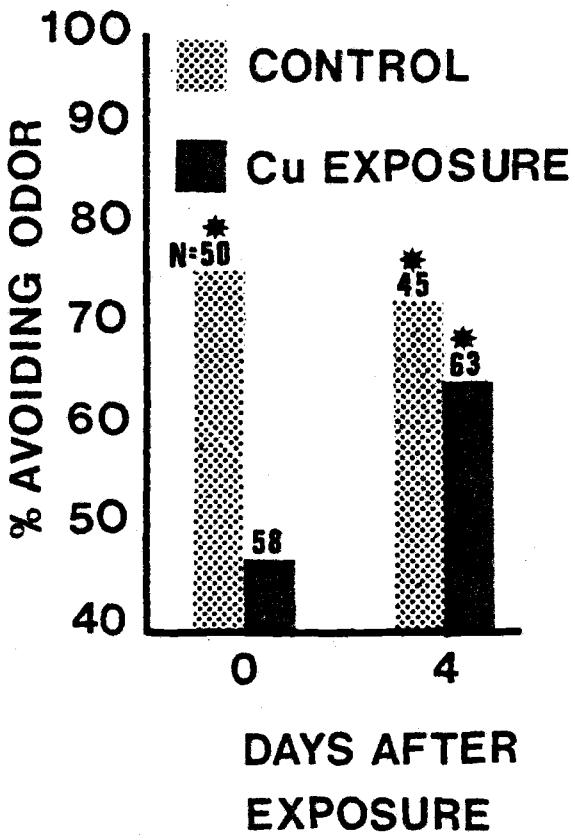
100
90
80
70
60
50
40
% AVOIDING ODOR
CONTROL
Cu EXPOSURE
N=50
58
45
63
0
4
DAYS AFTER
EXPOSURE

Figure 4.

Table I. Total number of juvenile coho salmon, tested 10 fish at a time, entering channel of Y-maze containing odors from adult salmon from Oregon Aqua-Foods and Salmon River Hatchery, and entering the channel without the odor in a preference test. Chi-square (χ^2) values were determined by comparing the total number of fish in the scented and unscented channels of the Y-maze to control trials using pure well water. Ho: number in scented = number in unscented.

ODOR	Conc., mg l^{-1}	N	Scented	Unscented	χ^2
ORE AQUA					
Roe	15	100	20	60	6.13*
Mucus	30	80	33	36	0.01
Bile	3	60	20	29	0.33
SALMON R.					
Roe	15	60	22	25	0.01
Milt	30	60	23	20	0.41
Liver	15	60	18	35	1.64
CONTROL		60	21	24	0.20

* Significant at $P < 0.05$

Detection of Synthetic Imprinting Chemicals

Juvenile coho salmon exposed to morpholine at 6 x 10^{-10}M for 2 weeks were slightly attracted to that concentration of odor in the Y-maze 4 days after the exposure (Table II).

Coho avoided catechol at concentrations of 5 x 10^{-5}M and 5 x 10^{-6}M, but did not avoid and were not attracted to concentrations of 5 x 10^{-7}M or 5 x 10^{-8}M (Fig. 5a). Four days after a 2 week exposure to catechol at 5 x 10^{-8}M, the fish were slightly attracted to that concentration of odor in the Y-maze (Table II).

β-phenylethylamine was avoided by coho at concentrations of 4 x 10^{-5}M and 4 x 10^{-7}M. Fish only weakly avoided a concentration of 4 x 10^{-6}M of β-phenylethylamine and did not avoid the chemical at 4 x 10^{-8}M (Fig. 5b). Following the 2 week exposure to β-phenylethylamine at 4 x 10^{-8}M, the coho were slightly attracted to the chemical in the Y-maze (Table II).

These results indicate that juvenile coho salmon can detect catechol and β-phenylethylamine at concentrations of 5 x 10^{-8}M and 4 x 10^{-8}M, respectively, which are not attractive nor repulsive to fish experiencing the odor for the first time.

Table II. Responses of juvenile coho salmon to odors in a Y-maze 4 days after a 2 week continuous exposure to an odor concentration which was not attractive nor repulsive before the exposure. Chi-square (χ^2) values were determined by comparing the total number of fish in the scented and unscented channels of the Y-maze to control trials using pure well water. Ho: number in scented = number in unscented.

Odor	Conc., M	N	Number of fish in scented channel	Number of fish in unscented channel	χ^2
Morpholine	6×10^{-10}	60	25	13	2.52[a]
Catechol	5×10^{-8}	74	38	24	1.73[a]
β-phenylethylamine	4×10^{-8}	74	37	21	2.45[a]
Unscented water		60	26	27	0.02

[a]Significant at $P < 0.25$

Fig. 5. Total percent of juvenile coho salmon avoiding odorants at various concentrations in a Y-maze, based on those fish moving into a channel. Data from all trials at each concentration were pooled. a. Odorant is catechol. b. Odorant is β-phenylethylamine. *$P < 0.05$, based on chi-square values determined by comparing total number of fish in scented and unscented channels of the Y-maze to control trials using pure well water. "N" denotes the total number of fish entering the channels of the Y-maze at each concentration.

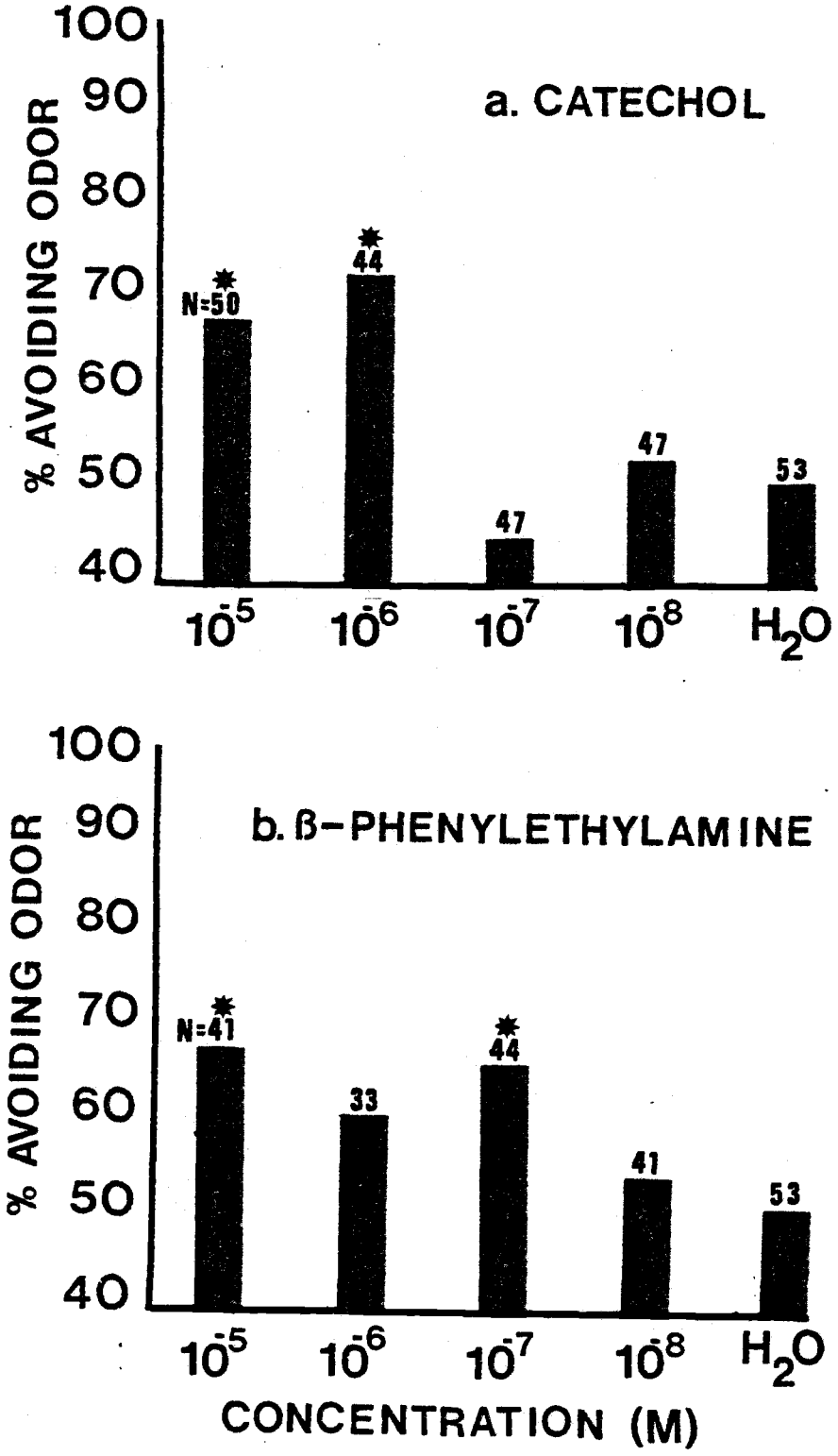
a. CATECHOL
% AVOIDING ODOR
100
90
80
70
60
50
40
N=50
44
47
47
53
10^{-5}
10^{-6}
10^{-7}
10^{-8}
H_2O
b. ß-PHENYLETHYLAMINE
% AVOIDING ODOR
100
90
80
70
60
50
40
N=41
33
44
41
53
10^{-5}
10^{-6}
10^{-7}
10^{-8}
H_2O
CONCENTRATION (M)

Figure 5.

DISCUSSION

The behavioral tests conducted in the Y-maze proved to be a simple rapid method to investigate the olfactory ability of juvenile salmonids. A Y-maze has also been used to determine water source preference of sockeye salmon fry (Bodznick, 1978), and chinook salmon (O. tschawytscha) adults (Whitman et al., 1982), and avoidance responses of juvenile coho salmon to aromatic hydrocarbons (Maynard and Weber, 1981) and rainbow trout (Salmo gairdneri) fry to herbicides (Folmar, 1976).

The amino acids L-serine and DL-alanine are effective odorants for these tests as the fish consistently avoided the odors in the range of 10^{-5} to 10^{-6}M. Idler et al. (1956) showed that adult salmon in a river avoided dilute concentrations of a mammalian skin rinse and that L-serine is a repellent present in the rinse. I found that juvenile coho salmon will avoid L-serine and DL-alanine in laboratory tests, and the two amino acids have similar response thresholds (ca. 10^{-7}M).

The threshold concentrations for the avoidance of L-serine and DL-alanine for juvenile coho in the behavioral tests are comparable to threshold concentrations found electrophysiologically in coho salmon, supporting my assumption that if the fish can detect the amino acid then they will avoid it. Hara (1972) estimated the threshold concentration for the detection of L-serine and L-alanine by coho salmon to be between 10^{-6}M and 10^{-7}M. These thresholds were

based on electroencephalographic responses recorded from the olfactory bulb when the nares were perfused with water containing the amino acid. L-alanine was slightly more effective as an olfactory stimulant than L-serine (Hara, 1972). My tests showed no consistent measurable difference in threshold of L-serine and DL-alanine.

Imprinting to chemical characteristics in the homestream is suspected to occur at the time coho undergo the parr-smolt transformation (see review by Hasler et al., 1978), and I thus reasoned that the olfactory ability of the smolt should be greater than that of the coho parr. The threshold concentrations for the avoidance of L-serine and DL-alanine changed between the parr and smolt stage; however the threshold concentration increased during the smolt stage for both amino acids. An explanation for this observation may be that there was a behavioral change during smolting in which the fish do not avoid the amino acids at low concentrations, even though the odors are detected. This decrease in avoidance behavior may be beneficial at a time when the fish need to remain in the stream and learn the odor characteristics of the stream. Smoltification is accompanied by behavioral changes (Hoar, 1976), in which the coho salmon changes from a territorial stream-dwelling parr to a schooling seaward migrating smolt. It is also possible that there may be no actual change in olfactory sensitivity when the smolt imprints to the water source, only a commitment of the odors to long term memory which will be recalled when the adult enters freshwater on its spawning migration.

The fish showed visual signs of smoltification, such as a loss of parr marks and an increase in silvery coloration, in April and June when they were tested as smolts. The smolts were not less inclined to swim against the current and into either arm of the Y-maze than were parr. There was no significant difference in the ratio of the total number of fish not moving into either arm of the Y-maze as parr or smolts.

In this study an exposure to copper at 0.47 μM for 24 h blocked olfaction of coho salmon as measured by the ability to avoid DL-alanine at 1 x 10^{-5}M. Hara (1972) found that a 10 s exposure of the nasal cavity of coho salmon to a 0.43 mM solution of $CuSO_4$ completely blocked the electroencephalographic (EEG) response to olfactory stimulants, including L- and D-forms of serine and alanine. This toxicological effect of the copper was reversible; the EEG response recovered after a 30 min rinsing of the nasal cavity with freshwater. A 12 h exposure of coho salmon to 1.6 μM $CuSO_4$ also blocked the EEG response to olfactory stimulants (Hara, 1972). Lorz and McPherson (1977) found that exposure of coho salmon yearlings to sublethal concentrations of copper (0.16 - 0.47 μM) caused a reduction in gill Na^+, K^+-activated ATPase activity, survival in seawater, and the percentage of downstream migrants in a natural stream.

A recommended hatchery practice for treatment of external bacterial infections of salmon and trout is a 1-2 min exposure to $CuSO_4$ at 3.1 mM (Leitritz and Lewis, 1980). The olfactory ability of juvenile coho salmon subjected to this treatment was blocked, but only for a brief period.

Copper causes deleterious effects on salmonid olfaction and thus exposure to copper should be avoided at times when olfactory sensitivity is very important. For example, a $CuSO_4$ treatment for external bacteria during smoltification could result in the fish not imprinting to the odor source to which they are expected to return as adults. If adult salmon encounter copper during the spawning migration, olfaction may be blocked and might stray as returning adults.

Exposure to 2,4-D did not cause any measurable effect on the olfactory ability of juvenile coho salmon. Although there was no olfactory impairment, caution should be taken to avoid exposure to this or any other organic chemical during smoltification. It is possible that smolts could imprint to such a chemical and then not be able to locate the source during the homing migration as an adult. Lorz et al. (1979) exposed yearling coho salmon to 2,4-D at concentrations up to 200 mg l^{-1} for 144 h and found no mortality during the exposure, no effect on the gill Na^+-K^+ATPase activity nor seawater survival.

Natural fish odors have been implicated as olfactory cues for homing of adult salmonids (Nordeng, 1971, 1977; Selset and Doving,

1980). Nordeng (1977) proposed a pheromone hypothesis for homing of adult salmonids in which odors such as bile acids or mucus produced by juvenile salmon residing in streams are the olfactory cues which guide the adult salmon to the homestream during the spawning migration. In this study there was no natural attraction found by juvenile coho to odors produced by adult coho salmon. This does not disprove the pheromone hypothesis for homing, but suggests that some developmental stages of the salmonids are not attracted to odors produced by conspecifics.

A possible explanation for the straying of adult salmon from its homestream or hatchery is that the fish do not recognize the odors emanating from this site as the same odors to which it imprinted as a smolt. Imprinting of salmon to synthetic chemicals may be a means to reduce straying of hatchery produced adults from the hatchery. If the salmon imprint to a synthetic odorant that does not occur anywhere else in the drainage basin, the returning adult should have little difficulty recognizing the return site scented with this odorant and it could not confuse it with any other location in the drainage.

Odorants to be used for "artificial imprinting" of salmon should not be naturally attractive nor repulsive to fish at the concentration used, so that fish not imprinted to the chemical will not be affected by its presence. Also, fish need to be able to detect the odorant at the concentration present in the stream if it is to serve as an olfactory cue for homestream identification. The two chemicals

screened for use as imprinting odorants, catechol and β-phenylethylamine meet these requirements when used at concentrations of 5×10^{-8}M and 4×10^{-8}M, respectively. Although the results of the detection assay for these two chemicals did not show a strong response of the fish to the odor, the fish probably did detect the chemicals at these concentrations. The fish did not show a strong response to morpholine at 6×10^{-10}M either, a concentration which coho salmon are known to detect (Hirsch, 1977; Sandoval, 1980). Before these chemicals are used for imprinting with salmonids, the ability to detect these odors at these concentrations and lower concentrations should be verified using another assay, such as a cardiac conditioning assay as used by Hirsch (1977), Johnstone (1980), and Sandoval (1980).

Although I only considered aspects of olfaction as influences of the homing success of salmonids, there are other factors which may have a role in straying. Variables such as size of fish at release and time of release are known to be important in the survival of juvenile salmon to adults (Wallis, 1968), and may also affect the straying of adults. Stresses encountered at the time of release may also contribute to reduced survival and/or straying.

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APPENDICES

Appendix I. Cardiac Conditioning

A cardiac conditioning assay was tested as a technique to determine olfactory sensitivity of juvenile coho salmon. A similar assay was used by Sandoval (1980) to determine minimum concentrations of various odors that coho salmon could detect. This assay consists of a series of paired presentations of an odor and a mild electric shock. After a series of these paired presentations, the fish should associate the odor with the shock. A momentary decrease in the heart rate of the fish during the presentation of the odor, but before the shock, indicates that the fish can detect the odor. Once the fish is conditioned to respond to the odor at the initial concentration, the concentration is successively lowered until the fish no longer responds to the addition of the odor. The lowest concentration to which the fish responds is the threshold concentration.

The testing apparatus consisted of a plexiglass tube, 5 cm diameter by 25 cm long, fitted with rubber stoppers at both ends in which polyethylene tubing was inserted. A constant flow of water through the tube was supplied from an overhead reservoir. Two silver wire electrodes (0.13 mm diameter) were implanted in the cardiac region of the fish and sutured into place while the fish was anesthetized. The fish was then placed in the plexiglass tube and allowed to recover for 12-16 h. The heart rate was recorded on a Gould strip chart recorder. The odor was injected with a syringe into the water inflow tube and reached the fish in the plexiglass tube in approximately 3 s. Four to 6 s after the odor

had reached the fish, a mild electric shock (1.5 V, 0.003 mA) was delivered to the caudal region of the fish. Table I.1 shows variations of the conditioning tests attempted.

Initially, a 5 ml volume of odorant (morpholine) was injected into the system to obtain a concentration of 6×10^{-9} M. The fish detected this injection, as indicated by a decrease in heart rate after the injection but before the shock. As a control to be sure the fish was responding to the odor and not some other stimulus, 5 ml of water taken directly from the inflow tube was injected. The fish responded to this injection of water, indicating that the fish detected some stimulus other than, or in addition to, the odor.

Smaller volumes of odor and water controls were tried and it was found that the fish could detect an injection of 0.1 ml of water.

to see if the fish were responding to a change in pressure caused by the injection, an open system was developed in which the injection would not cause an increase in pressure in the tube. This open system consisted of the inflow water running into an open funnel and then through tubing to the plexiglass tube with the fish. The odor and water controls were injected into the water in the funnel. The fish responded to either injections of 0.1 ml of odor and water controls.

Throughout these trials with different injection techniques and injection amounts, very few fish (less than 10% of those tested) responded to any stimulus as indicated by a momentary decrease in

heart rate. With this great lack of success, this assay was abandoned for use in this study.

Table I.1. Cardiac conditioning procedures attempted in development of assay.

Chamber	Heart Rate Electrodes	Odor Presentation	Heart Rate Response	
			Odor	Control
plexiglass box 14x37x9.5 cm	external steel plates	gravity feed	no heart beat recorded	
plexiglass tube 5x25 cm	external steel rods	syringe injection in inflow tube	no heart beat recorded	
plexiglass tube	implanted silver wire	syringe injection in inflow tube, 1 ml	yes	yes
plexiglass tube	implanted silver wire	syringe injection in inflow tube, 0.1 ml	yes	yes
plexiglass tube	implanted silver wire	syringe injection into funnel at top of inflow tube, 0.1 ml	yes	yes

Appendix II. Orientation Conditioning

An orientation conditioning system was tested to determine whether it could be used as a odor detection assay. Sandoval (1980) used a similar assay to evaluate the ability of juvenile coho salmon to discriminate between several olfactory cues. The objective of orientation conditioning is to modify the behavior of a group of fish during a series of trials to enter a specific area of a trough when a scent is present and to avoid that area when the scent is not present.

A group of juvenile coho salmon were placed in a Y-maze which was modified by blocking one channel such that the trough was essentially straight with an inflow section (arm) and outflow section (leg). The arm of the trough was illuminated by a single 40 watt light bulb and the leg was not lighted. This arrangement was used to ensure that the fish would avoid the arm unless a scent was added, at which time the fish would enter the arm to receive a food reward.

once each day, the scent was dripped into the arm of the trough via a mariotte bottle (S+ trial). After the scent had been dripping for 10 min, the gate to the arm was closed and the fish in the arm were fed and counted. Three min after closing the gate, the gate was reopened and the fish were allowed to leave the arm section. At another time each day the fish were observed for 10 min when no scent was added (S- trial), and then the arm gate was lowered and the fish in the arm section were counted. A positive difference between the number of fish in the arm during S+ and S-

trials indicated that the fish were detecting and responding to the scent.

To test the importance of acclimation procedure in the trough to the conditioning of the fish, four different procedures were used. Fifteen fish were added to each trough and allowed to acclimate for 1 week prior to testing according to the following procedures:

A. Fish had access to the entire length of the trough and were fed in the arm section while the scent was added;

B. Fish were kept in the arm of the trough and were fed in the arm section while the scent was added;

C. Fish were kept in the leg of the trough and were fed in the leg section while the scent was added;

D. Fish were kept in the leg of the trough and then herded by the observer into the arm while the scent was added and then fed.

None of these acclimation procedures were effective in teaching the fish to enter the arm while the scent was present. Acclimation procedure A was adopted for use in succeeding experiments for two reasons. First, this procedure closely resembled the actual testing procedure, with the exception that the gate was left open and the food was added earlier in the acclimation process. Procedure A also resulted in a greater total number of fish entering the scented arm during conditioning trials than the three other acclimation procedures.

Social hierarchies can become established in the trough and interfere with the conditioning of the fish to a stimulus. In one of the troughs containing 15 fish, one fish would always enter the arm during S+ trials and would chase away the other fish which tried to enter the arm at this time. This dominant fish was removed from the trough and within 3 days another fish would exhibit the behavior of a dominant fish. When the first dominant fish was returned to the trough, it quickly reestablished its dominance over the other fish in the trough. This dominance behavior of a single fish will prevent definite conclusions about olfactory detection in a system as used in this study since it will be difficult to know whether the majority of the fish do not respond to the odor because they do not detect the odor, or because of aggressive actions of a dominant fish even though the odor is detected.

To try to avoid problems of dominance hierarchies developing in the trough, the effect of group size on response to conditioning trials was examined. One, 2 and 4 fish were placed in the troughs, allowed to acclimate for 3 weeks according to procedure A described above, and then conditioning trials were conducted for 16 days.

The results from this experiment to evaluate group size indicate that there was no difference in the ability to learn to respond to a scent in trials employing 1, 2 or 4 fish. After 16 days of conditioning trials none of the fish in any of the three troughs consistently entered the arm of the trough on trials when the scent was present.

The lack of consistent response of the fish to the scent makes this orientation conditioning assay, as used in this investigation, unsuitable for use in tests of olactory ability.