Imprinting to Chemical Cues:
The Basis for Home Stream Selection in Salmon

Abstract. *Juvenile coho salmon were exposed to morpholine or phenethyl alcohol (p-alcohol) for 1½ months and then released in Lake Michigan. During the spawning migration 18 months later, morpholine and p-alcohol were metered into separate streams, and the number of morpholine- and p-alcohol–exposed fish returning to each stream was determined. Seventeen other locations were also monitored. The majority of the fish exposed to morpholine were captured in the stream scented with morpholine and most fish exposed to p-alcohol were captured at the p-alcohol-treated stream. This field study demonstrates that coho salmon imprint to and utilize chemical cues for homing.*

The odor hypothesis for salmon homing states that a juvenile salmon imprints to unique organic chemical odors of its natal stream and subsequently uses this cue to locate its stream during the spawning migration (*1*). We report here on field studies that document homing to two different organic chemicals by coho salmon [*Oncorhynchus kisutch* (Walbaum)]. The methods used were modified from previous work (*2–10*).

The basic procedure proposed by Hasler and Wisby (*1*) was to expose (imprint) young salmon to a synthetic chemical in

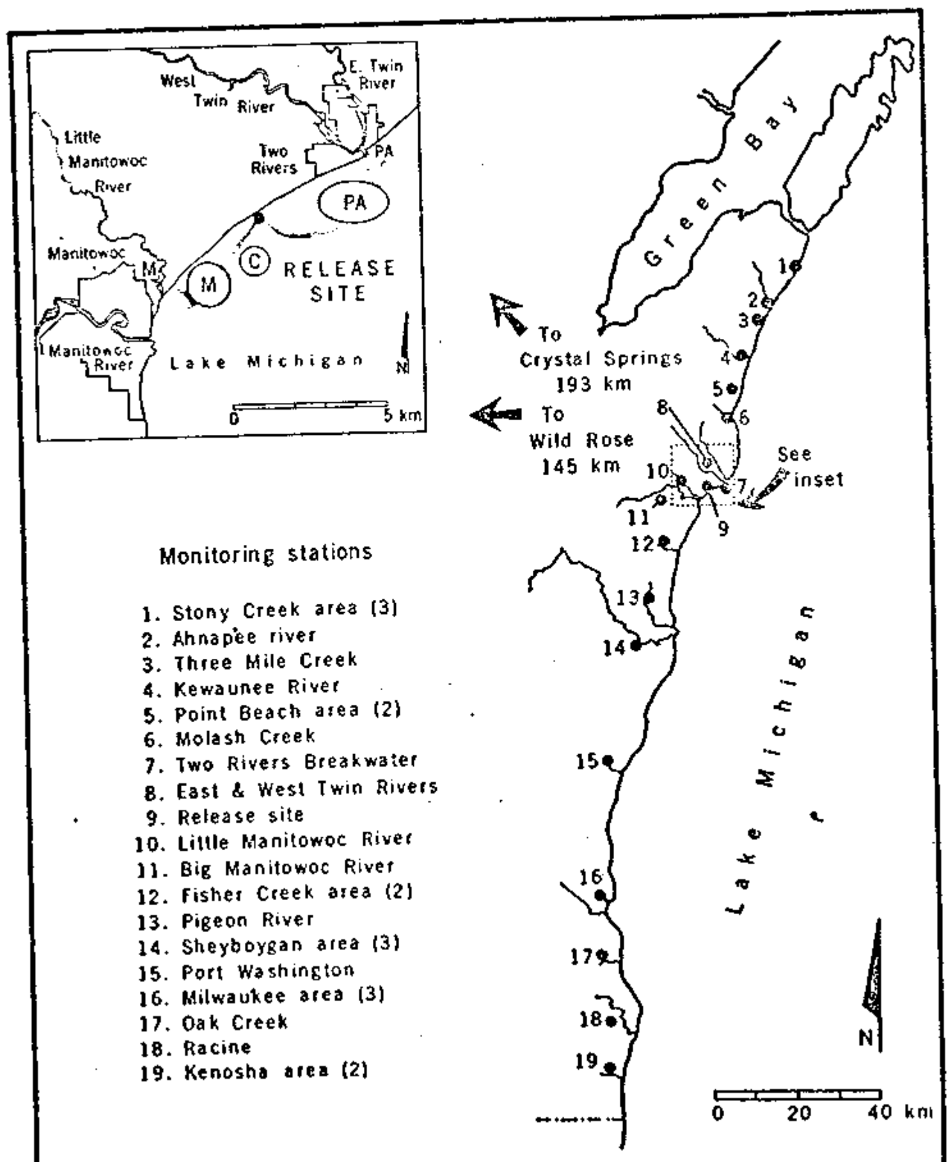

Fig. 1. Research area, Wisconsin shore, Lake Michigan. Numbers in parentheses represent the number of streams in the general area of the monitoring station that were surveyed. Inset shows detail of the release site, the morpholine-scented Little Manitowoc River (M) and the phenethyl alcohol–scented breakwater area at Two Rivers (PA).

place of natural home stream odors, to determine if they would return to a stream scented with that chemical. Morpholine (C_4H_9NO), a heterocyclic amine, and phenethyl alcohol ($C_8H_{10}O$) (p-alcohol) were selected because they can be detected by salmonids at low concentrations and are not known to occur in natural waters (3–6, 11). One group of fish was exposed to morpholine at a concentration of 5×10^{-5} mg/liter, and a second group was exposed to p-alcohol at 1×10^{-3} mg/liter. A third (control) group was left untreated. This experiment was conducted in 1973 with 5,000 fish in each group and again in 1974 with 10,000 fish per group.

Coho salmon were hatched and raised for 1½ years under identical conditions at a Wisconsin State Fish Hatchery at Wild Rose (1973) or Crystal Springs (1974) (Fig. 1). At this age the fish were divided into three groups of equal size, marked with different fin clips, and held in separate tanks (40 m long by 2.5 m wide by 1 m deep). The same water source, artesian spring water, was supplied to all tanks. This hatchery water was considered neutral because it was not connected directly with Lake Michigan and, therefore, could not provide the homing adult fish with cues about the location of a tributary stream. A multichannel peristaltic pump was used to meter morpholine into one tank and p-alcohol into a second.

The fish were exposed to the imprinting chemicals from 1 April to 13 May (1973 and 1974) during their presmolt and smolt stages (*10, 12–14*). (Smolts are about 16 months old, the age at which they leave their home tributary and migrate downstream.) All three groups of fish were then transported to and released in Lake Michigan between Manitowoc and Two Rivers, Wisconsin (Fig. 1), the streams that were to be scented during the spawning migration. Each of these streams was 4.7 km from the release site. The fish were released into the lake rather than a stream in order to reduce the possibility of their learning alternate cues about the test streams.

During the fall spawning migration 18 months later, morpholine was metered into the Little Manitowoc River and p-alcohol into a breakwater area at Two Rivers (Fig. 1) in order to simulate a home stream for exposed fish. The concentrations produced, calculated on the basis of mean flow rates, were 5×10^{-5} mg/liter and 5×10^{-3} mg/liter, respectively. Because of the variable flow rate of the test streams and the constant rate of morpholine and p-alcohol addition, the actual stream concentration ranged between 3×10^{-4} and 1×10^{-5} mg/liter for morpholine and 2×10^{-2} and 1×10^{-3} mg/liter for p-alcohol.

Both test streams were monitored for returning fish. In addition, 17 other locations were also surveyed in order to determine if a significant number of imprinted fish were straying into nonscented streams (Fig. 1). We hypothesized that if salmon use a chemical imprinting mechanism for homing, fish exposed to morpholine would return to the morpholine-scented stream, and fish exposed to p-alcohol would return to the stream treated with p-alcohol. Unexposed salmon served as controls to determine if fish would return to scented streams independent of chemical cues.

Streams were monitored by creel census surveys, electrofishing, and gillnetting. Sampling effort was not strictly comparable between sites because techniques that worked well at one place were not effective at others. The amount and type of effort spent in monitoring each area, and the exact locations and numbers of recoveries of fish from both experiments are recorded in Table 1.

The data for both experiments show that most of the morpholine fish returned to the morpholine-scented stream and most p-alcohol fish returned to the p-alcohol stream. In addition to the test streams, 17 locations were surveyed extensively, and we determined that treated fish returned specifically to the scented streams.

In 1974, of the total number of morpholine-treated fish recovered, 94.1 percent were found in the morpholine-treated stream. In 1975, 97.6 percent were recovered there (*15*). In 1974, 90.5 percent of all p-alcohol-treated fish recovered were captured in the area at Two Rivers scented with p-alcohol, and in 1975, 92.6 percent were captured there (*15*). By contrast, large numbers of control fish were captured at other locations.

Treated fish probably did not stray randomly or use alternate cues to return to scented streams because (i) they were

Table 1. Total number of morpholine-treated (M), phenethyl alcohol–treated (PA), and control (C) salmon captured at individual locations during 1974 and 1975. Data from the Little Manitowoc River (morpholine-scented) and the Two Rivers area (p-alcohol-scented) are printed in boldface type. Fishing effort is summarized by type and number of collecting trips at each location.

Location	1974						1975					
	Number recovered			Number of trips			Number recovered			Number of trips		
	M	PA	C	Creel census	Gill net	Electro-fishing	M	PA	C	Creel census	Gill net	Electro-fishing
1 Stony Creek (3)	1		4	90	13	13			12	40		
2 Annapee River		2	7	138	3	5	6	1	37	224	4	14
3 Three Mile Creek	2	1	1	27	5	5			2	26		
4 Kewaunee River				71		5				9		
5 Nuclear Power Plants (2)	1		4	123					2	3		
6 Molash Creek			2	8						1		
7 Two Rivers Breakwater	**3**	**118**	**15**	**184**	**3**	**1**	**3**	**192**	**12**	**126**	**14**	**1**
8 East & West Twin Rivers		15	7	123		9	3	8	21	17		14
9 Stocking Site	1		7	90	1				1	30		
10 Little Manitowoc River	**207**	**6**	**24**	**189**		**8**	**452**	**14**	**52**	**135**		
11 Big Manitowoc River	2	3	31	44		5		1	26	7		
12 Fisher Creek (2)			3	44					1	2		
13 Pigeon River				23								
14 Sheboygan River (3)	1		3	75		1			3	10		
15 Port Washington				38								
16 Milwaukee area (3)				65								
17 Oak Creek		1	7	306		5				180		
18 Racine			1	11								
19 Kenosha (2)				14								

"neutral" water during their life history, (ii) they were released km from the streams, and (iii) unexposed fish were recovered at several locations. We conclude, therefore, that fish treated with morpholine and p-alcohol used chemical cues to return respectively to streams treated with those substances. The fish learn (imprint to) the morpholine or p-alcohol during a short period of time during the smolt stage, and they retain these cues for 18 months without being again exposed to the chemicals. As this study was conducted in the field, it provides direct evidence that coho salmon use this mechanism for homing. These studies confirm the odor hypothesis for salmon homing.

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References and Notes

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2. Recent studies indicate that coho salmon use chemical cues for homing. In four experiments with coho salmon (*3–6*) and three with steelhead trout (*5, 7*), significantly larger numbers of fish chemically imprinted to morpholine than untreated fish returned to a morpholine-scented stream. When morpholine was not added to the stream during the spawning migration, treated and nontreated salmon returned in equal low numbers. Behavioral experiments (*3–5*) show that morpholine-exposed fish tracked with ultrasonic transmitters stopped in an area scented with morpholine and passed through the same area when morpholine was not present. Electrophysiological studies (*8*) indicated a significant difference in the amplitude of the EEG response recorded from the olfactory bulb between morpholine-exposed and control salmon to morpholine. In addition, Jensen and Duncan (*9, 10*) report that coho salmon marked and held in a natural water supply for 48 hours while smolting returned specifically to that water to spawn.
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9. Jensen and Duncan (*10*) transplanted coho salmon smolts from their original hatchery on the Columbia River to a spring-fed fish-holding facility on the Snake River. The fish were held for 48 hours and then released into the Snake River. During the spawning migration, marked fish were recovered near the springwater discharge 0.8 km downstream from the release point but not from other locations. To determine if the fish were actually homing to the water in which they had been held as smolts, water from the holding facility was pumped through a floating trap. As a control, river water was pumped into the trap on alternate days. No fish entered the trap when river water was used, but 399 fish were captured during periods when springwater was used. Springwater from the fish-holding facility was thus the orienting stimulus; fish were able to learn the characteristics of this water within 2 days.
10. A. Jensen and R. Duncan, *Prog. Fish-Cult.* **33**, 216 (1971).
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12. During the smolt stage, salmon undergo physiological and behavioral changes (*5*). Coho salmon taken from their original home stream or hatchery prior to the smolt stage and transplanted to a second stream subsequently returned to the second stream to spawn (*9, 13*). This indicates that the smolt stage is a sensitive or critical period when fish learn the cues that identify their natal tributary or river of release. This learning process probably terminates soon after they become smolts. Peck (*13*), for example, transplanted coho salmon older than smolts and determined that large numbers strayed into other streams.
13. R. Donaldson and G. H. Allen, *Trans. Am. Fish. Soc.* **87**, 13 (1957).
14. J. W. Peck, *ibid.* **99**, 591 (1970).
15. The number of treated salmon recovered in their respective streams probably represents a good sample of the total number returning. About 0.5 to 5.0 percent of naturally produced fish or fish raised and released from a fish hatchery return to their home stream. The breakwater at Two Rivers presented greater difficulty compared to the Little Manitowoc River in terms of sampling. This may account for the difference in the total number of recoveries for each group. The reason for the difference in the number of recoveries within groups in 1974 and 1975 is that twice as many marked fish (5,000 versus 10,000) were released in each group in 1975.
16. We thank T. Whitney, K. Hughes, C. Gosse, J. Heitz, W. Boes, and S. Drzewcicki for assistance in the field. R. Poff, R. Daly, and the Wisconsin Department of Natural Resources provided technical and logistical support. We also thank D. Madison, P. Hirsch and P. Johnsen for advice in these studies, and R. Stein and S. Bartell for reviewing the manuscript. Supported by National Science Foundation grants GB7616 and GB343 to A.D.H., University of Wisconsin Sea grant, Department of Commerce grant NOAA 2-35209, and the Wisconsin Department of Natural Resources.

5 January 1976

Residual Learning Deficit After Heavy Exposure to Cannabis or Alcohol in Rats

Abstract. *Acute oral administration of cannabis extract to rats (tetrahydrocannabinol dose, 10 milligrams per kilogram) impaired maze learning. The impairment was more marked after ten daily doses of the same size. After 1, 2, or 3 months' pretreatment with the same daily dose, followed by a 25-day drug-free period, no residual learning impairment was found. However, 6 months of daily administration of cannabis (tetrahydrocannabinol, 20 milligrams per kilogram) or alcohol (6 grams per kilogram) produced significant residual impairment of learning of maze and motor coordination tasks, 2 months or more after the last drug administration.*

It has long been recognized that chronic heavy use of alcohol may give rise to an organic brain syndrome characterized by slowing and interruption of mental processes, difficulty with abstract thought, and impairment of memory and learning ability (*1*). Walker and Freund (*2*) reported that rats kept on alcohol-containing diets for 6 to 12 months showed impaired avoidance learning when tested a month or more after the end of treatment.

Many clinical reports from India, North Africa, and elsewhere have referred to a similar "dementia" in long-term heavy users of hashish (*3*, p. 114). Clinical descriptions of a similar state have recently appeared in the North American and European literature, ranging from moderate impairment of verbal learning and recall (*4*) to a full clinical picture which in some cases was thought to indicate organic brain damage (*5*). Campbell *et al.* (*6*) described air encephalographic findings of enlargement of the cerebral ventricles and cortical atrophy in ten young patients who had used cannabis heavily in addition to smaller amounts of other drugs for at least 6 months.

The interpretation of these findings is complicated by the frequent presence of multiple drug use, malnutrition, infections, and other incidental factors, as well as by the difficulty of distinguishing between chronic intoxication and residual postintoxication effects. We have therefore examined the effects of cannabis, acutely and chronically, as well as those of chronic ethanol, on performance of learning tasks in rats, under conditions in which the confounding factors were excluded.

An ethanolic extract of preassayed marihuana leaf material was heated to convert all the tetrahydrocannabinolic acid to tetrahydrocannabinol (THC) (*7*). The THC content of the extract was assayed by gas-liquid chromatography (*7*), and the appropriate dose was then dissolved in 0.2 ml of olive oil for administration to the rats.

For the acute experiment, 18 animals were reduced to 80 percent of their free-feeding weight, and pretrained in the Rabinovitch-Rosvold modification of the Hebb-Williams closed-field maze (*8*). This test has been shown to be sensitive to cortical ablation and to drug-induced learning deficits.