LONG-TERM OLFACTORY "MEMORY" IN COHO SALMON, *ONCORHYNCHUS KISUTCH*[1]

Many experiments have correlated the importance of olfaction and the precise homing of sexually mature salmon. As juveniles, the fish are presumably imprinted on the natural odors of their natal-area water (Hasler, 1966). The odors apparently serve as cues to guide the adult's return. Thus, some type of "odor memory" must persist from the time of the downstream journey of the smolt to the return of the sexually mature adult. For introduced Lake Michigan coho salmon, *Oncorhynchus kisutch*, this is either ½ year (precocious males) or 1½ years.

The existence of long-term olfactory memory persisting over this time period has only been inferred. Idler et al. (1961) and Fagerlund et al. (1963) found that already homed salmon made unconditioned behavioral responses to home water. Hara, Ueda, and Gorbman (1965), Ueda, Hara, and Gorbman (1967), and Oshima, Hahn, and Gorbman (1969a) found specific EEG (electroencephalographic) responses to home-stream water. Hara (1970) in his review of this EEG technique states: "This electric response [from the olfactory bulbs] is specific in the sense that it cannot be evoked by water from spawning sites of other groups of breeding salmon." The EEG and behavioral studies strongly suggest long-term memory of the juvenile's stream experience. However, since these workers used homed adults that had recently experienced home water, the data are evidence only of an odor memory lasting from the time of removal from the home stream to the time of testing.

We tested coho salmon that were exposed to a synthetic odoriferous substance for 1 month during smoltification and then removed from any conceivable influence of this substance for 10 months. Ten months later these fish and controls were examined for olfactory bulbar EEG responses (after Hara et al., 1965) to the imprinting substance.

Materials and Methods

On 7 April 1970, approximately 2,500 hatchery-raised coho salmon smolts (1½ years old) were put into each of two contiguous 25-m sections of a raceway at a Wisconsin State fish hatchery at Crystal Springs. We marked the fish to eventually distinguish the upper section control subjects from the lower section experimentals. A small drop (⅓ m) prevented water in the lower section from reentering the upper section. Immediately below the drop a dilute concentration of morpholine was introduced by infusion pump at a rate to maintain a steady-rate concentration of 10^{-5} ppm. This value is one order of magnitude above an avoidance threshold of unconditioned coho salmon fingerlings (Wisby, 1952). On 5 May 1970, 1 month after initiation of the morpholine treatment, all but 50 fish from each raceway section were trucked to Lake Michigan and released as part of another experiment. The 100 remaining fish were moved to a hatchery near Madison, Wis., and held together in a single outside raceway for 10 months prior to EEG tests.

Our testing procedure was generally similar to that used by Hara et al. (1965) to examine olfactory bulb responses to home-stream water. The subject was paralyzed with gallamine triethiodide (2 mg/kg), restrained, and the gills perfused with tap water. One of the olfactory bulbs was exposed, and an electrode (Transidyne General, model 415[2]) was placed on the surface near the rear margin. The responses evoked by perfusion of the ipsilateral naris were amplified (Bioelectric Instruments, model DS2c) and recorded on a two-channel oscillograph (Hewlett Packard, model 7712B) for later analysis. This oscillograph was equipped with an integrating preamplifier for efficient quantification of bulbar activity. Therefore, all responses reported later

[1] This work was completed under financial support from the National Science Foundation (Grant No. GB 7616X) to Prof. Hasler and the University of Wisconsin Sea Grant Program which is a part of the National Sea Grant Program maintained by the National Oceanic and Atmospheric Administration of the U.S. Department of Commerce.

[2] Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

are expressed as the sum of the positive areas under the response wave form.

Beginning on 25 February 1971, one fish was examined per day with 1% and 0.01% morpholine stimuli. Fourteen fish were used. Each test was started at approximately 1000 hr. Every subject was tested with the morpholine concentrations and the responses compared with responses to 0.06 N NaCl. Stimuli were randomly ordered and presented for 10 sec followed by 75 sec of tap water rinse. The stimulus series was then repeated seven times.

Results

Fish that had been exposed to 10^{-5} ppm of morpholine as smolting juveniles evidenced significantly higher bulbar EEG activity over controls when tested with 1% and 0.01% concentrations of morpholine (Table 1). Responses to 1% morpholine gave a Mann-Whitney value of $U = 5$ (Siegel, 1956) with probability of 0.006 that the control group and the experimental group were drawn from the same treatment population. Responses to the 0.01% level were less markedly, but still significantly, different ($U = 11$, $P = 0.049$).

Discussion

Exposure to low concentrations of morpholine produced a sensitization which lasted at least 10 months. But, we did not attempt to determine whether this observed sensitization was exclusively to morpholine or to other stimulatory products. Casual observation of our data did not reveal the experimental subjects to be more responsive to NaCl than the controls; but this comparison was difficult to make in our experimental design because of the changing relationship between response amplitudes and background activity levels. (Hence, the necessity of continual comparison of morpholine response with NaCl response, our reference.) Even if overall olfactory responsiveness is increased as a result of pretreatment, sensitization to morpholine is still proportionally greater (experimental vs. control) 10 months later. We hypothesize that exposure to morpholine imprinted the fish during one of the critical periods in the life of the coho salmon, a period when the fish is undergoing physiological changes in preparation for entering a marine environment.

Independent evidence indicates the existence of a critical period. The Wisconsin Department of Natural Resources allows approximately 1 month of imprinting during the period of smoltification before releasing the fish into the river system. Imprinting for less time or at different stages of the life cycle seems to result in more straying (Peck, 1970).

Morpholine was chosen as the imprinting substance since the responses of fingerling coho salmon to it have been investigated (Wisby, 1952). Consequently, the concentration could be chosen with knowledge of the performance parameters of the coho salmon. It was necessary to be above threshold but not so high as to cause enthusiastic avoidance or sublethal damage. Because of the vagaries of the flow measurements

TABLE 1.—Morpholine-elicited EEG responses of morpholine-imprinted coho salmon compared with those of controls. E designated subjects were imprinted with morpholine at a concentration of 10^{-5} ppm; C designated were controls. Median EEG responses of each fish to 1% morpholine and 0.01% morpholine stimuli are ranked (ties carry averaged ranks) and Mann-Whitney U values and probability values are shown for each treatment level.

1%														
Group	C	C	C	C	C	C	E	E	E	E	E	C	E	E
Median[1]	63.5	100	140	220.5	230	250	255	266.5	287	333	351.5	600	915	917
Rank	1	2	3	4	5	6	7	8	9	10	11	12	13	14
						$U = 5$	$P = 0.006$							
0.01%														
Group	C	C	E	C	C	C	E	E	C	E	E	C	E	E
Median[1]	0	0	0	0	0	25.6	38.5	41.5	45	45	50	50	67	77.5
Rank	3	3	3	3	3	6	7	8	9.5	9.5	11.5	11.5	13	14
						$U = 11$	$P = 0.049$							

[1] Median response = $\left(\frac{\text{response morpholine}}{\text{response 0.06 N NaCl}} \times 100\right)$ for eight trials.

in the raceway, one order of magnitude above threshold was chosen. Nevertheless, Wisby (1952) reported some avoidance at this concentration. We did not, however, observe any avoidance where the substance was metered into the raceway. It should be noted that concentrations at the delivery tube could be as high as 100 ppm before mixing took place. But, since the substance was delivered immediately below the falls caused by the separating dam, mixing was assumed to be rapid and complete.

Although fish were treated with very low concentrations of the morpholine, EEG responses were not evident until 100 ppm was reached in responsive fish. But, other workers have also found a relative lack of sensitivity with electrophysiological methods. Home-stream responses disappeared with dilution of home-stream water to 5% (Oshima, Hahn, and Gorbman, 1969b), and Sutterlin and Sutterlin (1971), recording from the olfactory epithelium of Atlantic salmon, *Salmo salar*, found no response to morpholine at 0.9 ppm. Yet Wisby (1952) got clear behavioral manifestations of perception at concentrations of 10^{-6} ppm.

Acknowledgments

Special thanks go to Ronald Poff and Jerry Kryka of the Department of Natural Resources, Wisconsin for providing fish and hatchery space.

Literature Cited

FAGERLUND, U. H. M., J. R. MCBRIDE, M. SMITH, AND N. TOMLINSON.
1963. Olfactory perception in migrating salmon. III. Stimulants for adult sockeye salmon (*Oncorhynchus nerka*) in home stream waters. J. Fish. Res. Board Can. 20:1457-1463.

HARA, T. J.
1970. An electrophysiological basis for olfactory discrimination in homing salmon: A review. J. Fish. Res. Board Can. 27:565-586.

HARA, T. J., K. UEDA, AND A. GORBMAN.
1965. Electroencephalographic studies of homing salmon. Science (Wash., D.C.) 149:884-885.

HASLER, A. D.
1966. Underwater guideposts—homing of salmon. Univ. Wis. Press, Madison, 155 p.

IDLER, D. R., J. R. MCBRIDE, R. E. E. JONAS, AND N. TOMLINSON.
1961. Olfactory perception in migrating salmon. II. Studies on a laboratory bio-assay for home-stream water and mammalian repellent. Can. J. Biochem. Physiol. 39:1575-1584.

OSHIMA, K., W. E. HAHN, AND A. GORBMAN.
1969a. Olfactory discrimination of natural waters by salmon. J. Fish. Res. Board Can. 26:2111-2121.
1969b. Electroencephalographic olfactory responses in adult salmon to waters traversed in the homing migration. J. Fish. Res. Board Can. 26: 2123-2133.

PECK, J. W.
1970. Straying and reproduction of coho salmon, *Oncorhynchus kisutch*, planted in a Lake Superior tributary. Trans. Am. Fish. Soc. 99:591-595.

SIEGEL, S.
1956. Nonparametric statistics for the behavioral sciences. McGraw-Hill, N.Y., 312 p.

SUTTERLIN, A. M., AND N. SUTTERLIN.
1971. Electrical responses of the olfactory epithelium of Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. 28:565-572.

UEDA, K., T. J. HARA, AND A. GORBMAN.
1967. Electroencephalographic studies on olfactory discrimination in adult spawning salmon. Comp. Biochem. Physiol. 21:133-143.

WISBY, W. J.
1952. Olfactory responses of fishes as related to parent stream behavior. Ph.D. Thesis, Univ. Wisconsin, Madison, 42 p.

ANDREW E. DIZON

Southwest Fisheries Center
National Marine Fisheries Service, NOAA
P.O. Box 3830
Honolulu, HI 96812

ROSS M. HORRALL

Marine Studies Program
University of Wisconsin
Madison, WI 53706

ARTHUR D. HASLER

Laboratory of Limnology
University of Wisconsin
Madison, WI 53706