Proc. 16th Conf. Great Lakes Res. 1973:
143-153. Internat. Assoc. Great Lakes Res.

OLFACTORY IMPRINTING IN COHO SALMON: HAVIORAL AND ELECTROPHYSIOLOGICAL EVIDENCE

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Abstract. The olfactory hypothesis of salmon homing states that salmon imprint to the chemical odors of their homestream and use these odors to relocate the same stream during their spawning migration. To test this hypothesis, fingerling coho salmon were exposed to a chemical odor (morpholine) not known to be associated with natural river odors. An equal number of fish were not exposed (controls). Both groups were stocked into Lake Michigan without having any river experience. During the spawning migration, morpholine was dripped into a river near the location where fish were released. It was hypothesized that if the salmon were imprinting to morpholine as the homestream odor, then only the exposed group of fish would recognize the river as the homestream, since it contained morpholine.

Three separate methods (census, ultrasonic tracking and recording EEG responses from the olfactory bulb of 2 salmon brains) were used to test this hypothesis. Results from the census of returning fish demonstrated that a significantly higher number of morpholine-imprinted fish, as opposed to non-imprinted fish, returned to the morpholine-scented stream to spawn. Results from behavioral experiments with fish equipped with ultrasonic transmitters and followed under natural migratory conditions indicated that imprinted fish stopped migrating in an area where they encountered morpholine odors. Imprinted fish moved through the area without stopping when no odor was present. Similarly, there was a characteristic strong specific response of imprinted spawning salmon to morpholine as evidenced by the EEG technique, while non-imprinted fish responded less strongly to morpholine. All three lines of evidence independently demonstrated that coho salmon imprinted to morpholine reacted to the chemical as adults, supporting the olfactory imprinting hypothesis. (Key words: Olfactory imprinting hypothesis; coho salmon; behavioral experiments; natural migration).

INTRODUCTION

It has long been demonstrated that anadromous salmonid fishes, after spending up to several years in the ocean, return with great specificity to their natal stream to spawn and die. How this remarkable biological phenomenon, termed "homing", is accomplished is still not completely understood, although a variety of sensory mechanisms have been suggested (Hasler 1966; Harden Jones 1968). Most workers agree that the fish probably use a combination of cues in order to find their way home, especially since there are two well defined aspects of the migration, orientation in the open ocean and inshore orientation including recognition of the main river, as well as the home tributary. During the final stages of migration, olfactory cues are considered to be of primary importance in guiding the fish's movements. The present study provides some new evidence supporting the olfactory hypothesis, especially with regard to odor imprinting.

REVIEW OF THE OLFACTORY HYPOTHESIS

Before discussing this new evidence, the authors would like to briefly review the basis for the olfactory hypothesis for salmon orientation and discuss

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some of the difficulties encountered by previous investigations. It has been hypothesized that each stream has a different chemical composition and thus unique odor characteristics which the salmon are able to remember and use to locate the homesteam during the spawning migration (Hasler and Wisby 1951; Wisby and Hasler 1954; Hasler 1966). The process of forming a permanent memory[4] to homestream odors during a critical period in the early stages of the life history of a salmon has been termed "imprinting" (Hasler and Wisby 1951; Brett and Groot 1963; Madison et al. 1972; Cooper and Hasler 1972). Behavioral and neurophysiological evidence supports this hypothesis (Hasler and Wisby 1951; Wisby and Hasler 1954; Groves et al. 1968; Hiyama et al. 1966; Idler et al. 1961; Fagerlund et al. 1963; Hara et al. 1965; Oshima et al. 1969a, 1969b; Hara 1970; Dizon 1971[5]). Unfortunately there is not enough space to discuss this work individually, but the authors would like to point out some common problems. For example, although behavioral studies have demonstrated certain perceptual capabilities of salmon, they have not clearly shown how these are related to the natural situation. Another underlying problem not controlled in much of the work reported is the factor of "recent experience" (Brett and Groot 1963; Oshima et al. 1969a). Many studies have looked at behavioral and physiological responses of migrating salmon captured in the homestream to various water samples. In many of these experiments, the largest response was usually to homestream water, indicating a retention of odor cues. It has been argued that some or all of the experimental results might be explained as a response to the odor of the water to which the salmon were most recently exposed (i.e., homestream water), instead of involving long-term memory. Oshima et al. (1969a) using electrophysiological techniques, for example, have demonstrated that salmon which had previously shown no response to University of Washington, College of Fisheries water exhibited responses when they had been held in this water for 67 hr. The fish responded more strongly to this water than to homestream water.

More data indirectly supporting the olfactory hypothesis have come from transplant experiments (Wickett 1958; Ricker 1959; Donaldson and Allen 1957; Carlin 1968). Fingerling salmon taken from their original homestream and transplanted to another stream a short period of time before the beginning of their downstream migration returned to the second stream to spawn. Two important conclusions have been drawn from these results. First, they have shown that the memory of the homestream is not inherited. Second, they have demonstrated the existence of a rapid learning process which occurs at the time the juvenile salmon begin their seaward migration (Carlin 1968; Donaldson and Allen 1957). Although it is commonly assumed that this rapid learning process is connected with olfactory imprinting, there is little direct evidence to establish this assumption, except for the work of Jensen and Duncan (1971).

ARTIFICIAL IMPRINTING TO CHEMICAL ODORS

Despite the difficulties encountered in past research, it was felt that the olfactory hypothesis, and more specifically, the chemical imprinting process, warranted further investigation because of its potential as a powerful tool in terms of fisheries management. The problem was how to find a better method to test this hypothesis under more natural conditions and how to treat the experiment in such a

[4]The term "memory" does not imply conscious thought.

[5]Dizon, A. E. "Ecological Aspects of the Evoked Olfactory Bulb Electroencephalograph of Fish with Special Reference to Homing Behavior in Salmon" (Ph.D. thesis, University of Wisconsin, Madison, Wisconsin, 1971).

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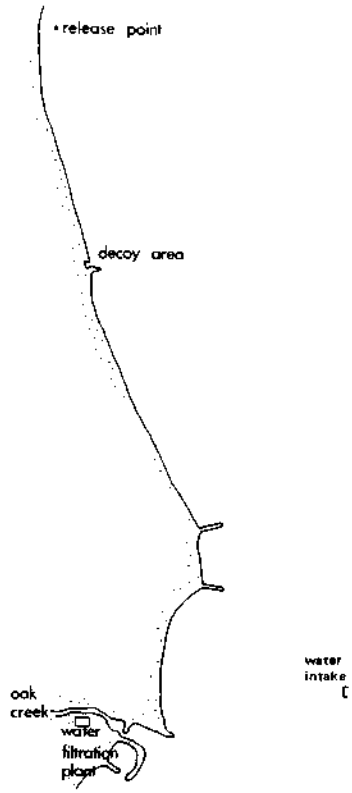

FIG. 1. Research area: South Mi

[6]Wisby, W. J. "Olfactory Response
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way as to eliminate alternative sensory mecahnisms. Most researchers believe that isolating the odors or some sort of decoy experiment would solve these problems. The approach the authors have taken has been to combine these suggestions.

In brief review, the olfactory hypothesis states that salmon imprint to the chemical odors of their homestream and use these odors to relocate the same stream during their spawning migration. To test this hypothesis, the authors followed the suggestion of Hasler and Wisby (1951) and attempted to imprint young salmon to chemical odors not normally associated with natural river odors and subsequently decoy the migrating adults into a stream artifically treated with the odor. Behavioral and neurophysiological techniques were used to test the specific response of returning salmon to the odor.

CENSUS

Methods

In 1971, 16,000 coho salmon (*Oncorhynchus kisutch*) fingerlings hatched and reared at Wild Rose Fish Hatchery were transported to South Milwaukee, Wisconsin and held in large tanks at the city's water filtration plant located adjacent to Oak Creek (Fig. 1). The fish were divided into two groups, 8000 of which were exposed to morpholine and 8000 which were not exposed (controls). Fin clips were used to distinguish between the two groups. Lake Michigan water was supplied to the tanks from an intake crib located about 1.50 km offshore ENE of Oak Creek, so that the water the fish were being held in was not associated with any future stream with which the fish would come into contact. Morpholine was dripped into one of the tanks at a steady-state concentration of 5×10^{-5} mg/l. Morpholine was chosen as the imprinting chemical because Wisby (1952)[6] found that it was a stable organic compound which is soluble in water and could be detected by coho salmon at low concentrations (10^{-6} mg/l). It was neither an attractant nor a repellent for coho at this concentration. The reason an organic compound was selected was because earlier work (Hasler and Wisby 1951; Idler et al. 1961) indicated that the identifiable component of stream odors was contained within the organic fraction of the water. The fish were imprinted for about 30 days in April and May.

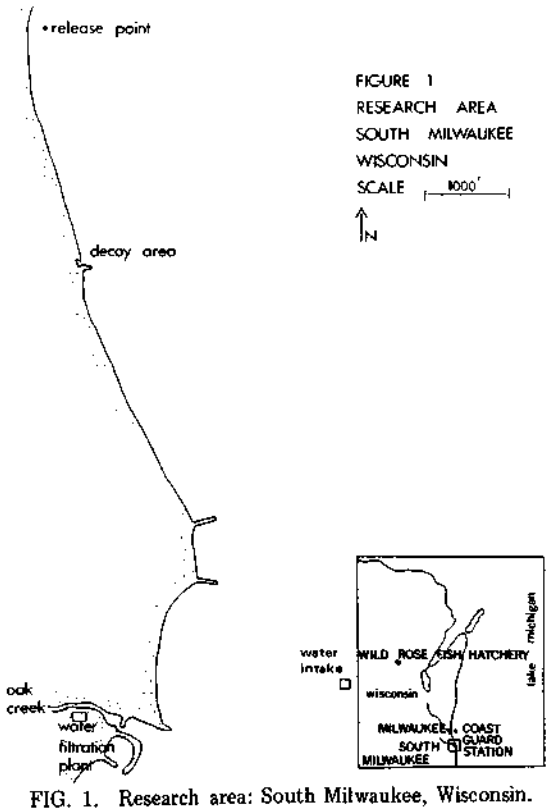

FIG. 1. Research area: South Milwaukee, Wisconsin.

[6]Wisby, W. J. "Olfactory Responses of Fishes Related to Parent Stream Behavior" (Ph.D. thesis, University of Wisconsin, Madison, Wisconsin, 1952).

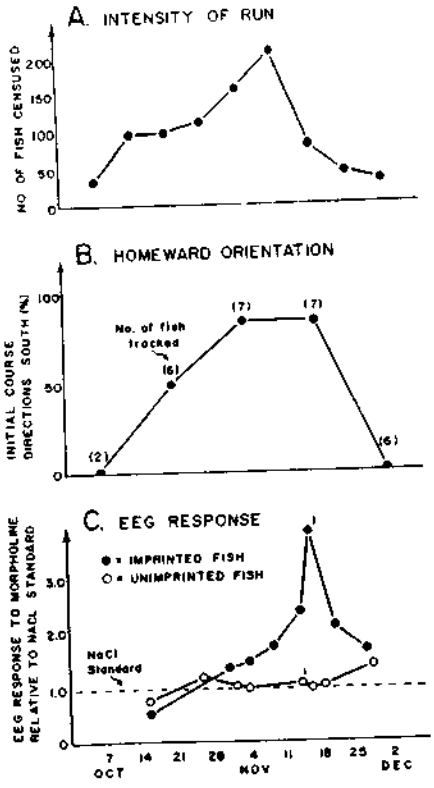

FIG. 2. Correlations between census, tracking and EEG studies.

(1) = mean of two fish tested on the same day.

About two weeks after the onset of smolting or the beginning of active downstream migration, presumably when odor imprinting was no longer occurring, the fish were released at the mouth of Oak Creek. During the spawning season in the fall of 1971 and 1972, the chemical was added to Oak Creek at approximately the same concentration to which the fish were exposed. Since the purpose of this method was to eliminate all exposure to the future "homestream" (Oak Creek), it was hypothesized that if salmon were imprinting to homestream odors (morpholine in this case) then only the morpholine-exposed fish would recognize Oak Creek as the homesteam and return there to spawn. Fyke nets, electroshocking, gill nets and creel census were used to survey the returning fish.

Results

Salmon released in the spring of 1971 were recovered in the fall of 1971 and 1972. In 1971, 31 morpholine-exposed sexually mature juvenile coho returned to Oak Creek compared to only 3 controls. During the 1972 adult run, 185 morpholine-exposed fish and 25 controls were captured. These results supported the existence of olfactory imprinting and long term memory of mainstream odors.

The number of salmon returning to Oak Creek over the course of the fall followed roughly a normal distribution, centered in the second week of November (Fig. 2a). The peak of this curve correlated well with the other aspects of the study.

BEHAVIORAL EVIDENCE FOR OLFACTORY IMPRINTING

Methods

The census of returning salmon gave only indirect information on the actual behavioral response of salmon to chemical odors. To obtain more direct information, imprinted fish equipped with ultrasonic transmitters were released along the shoreline of Lake Michigan and tracked into an area scented with morpholine.[7] The hypothesis was that if morpholine-imprinted fish were cueing on morpholine, they would remain in the scented area. The control experiments were conducted by tracking imprinted fish through the same area when no odor was present. To check for the possibility that fish were behaving differently when morpholine was present, not because they were imprinted to it, but be-

[7]The reason that ultrasonic tracking was used over more standard behavioral methods, such as experimental tanks or Y-mazes, was that it allowed the authors to conduct experiments under more natural conditions, since the fish's movements were not restricted.

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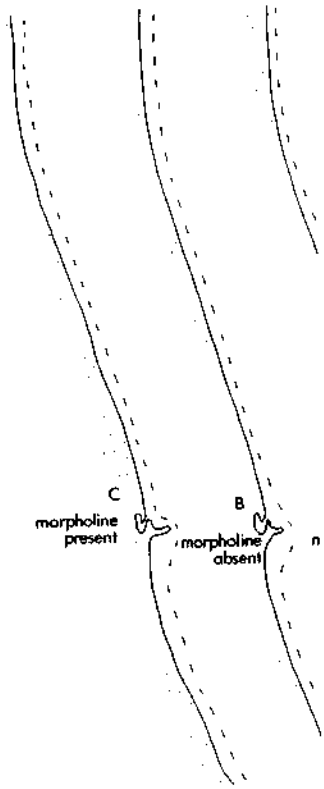

FIG. 3. Generalized ultrasonic tr
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cause it was a unique odor compared to normal shoreline odors, non-imprinted fish were tracked through the area when morpholine was present.

Salmon used for this study were captured in Oak Creek and identified as imprinted or non-imprinted fish by fin clips. After capture, to control for recent odor experience to morpholine, the fish were held in a tank supplied with Lake Michigan water between 1 and 148 hr (mean about 76 hr) before they were tracked. Thus, if fish responded to morpholine it was probably not because they were recently exposed to the chemical before tracking.

An ultrasonic transmitter (7.3 x 1.4 cm) was inserted down the esophagus into the stomach of the fish. Since coho stop feeding during the spawning migration, the effect of the transmitter was probably minimal. Tagged fish were transported by boat to the release point about 3.2 km north of Oak Creek along the shore of Lake Michigan. A directional hydrophone connected to receiving equipment on a tracking boat was used to follow the signal from the tagged fish. Positions were determined in relation to markers placed at 130 m intervals along the shoreline and tracks were plotted on a map (Figs. 3 and 4). The release site was selected because the authors assumed that fish released along the shore would follow the shoreline south back to Oak Creek. The decoy area was located between the release point and Oak Creek. If some cue other than olfaction was operating to get the fish back to the mouth of Oak Creek, then the fish would have to pass through the scented area before reaching Oak Creek. Since salmon normally migrate up rivers to spawn, the decoy site was chosen because a small stream ran into the lake at that point.

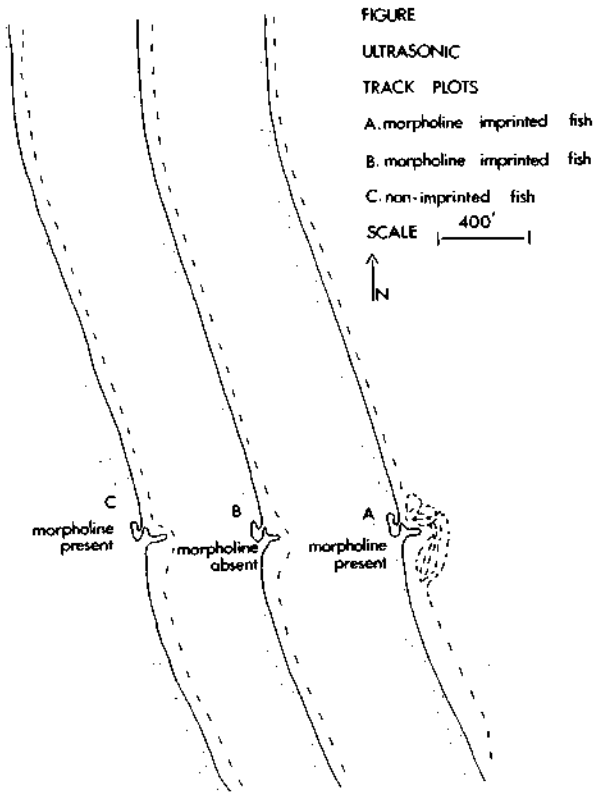

FIG. 3. Generalized ultrasonic track plots for morpholine-imprinted (a and b) and non-imprinted (c) fish.

Morpholine was dripped into the lake in a line extending from the mouth of the stream to about 75 m offshore, creating an "odor barrier" through which the fish had to swim.

Results

Twenty-two salmon were tracked past the decoy area. At the beginning of each track, fish milled in the release area, possibly some sort of adjustment period from handling and transport. Movement patterns between the release point and the decoy area were similar for all fish; migration was usually along the shoreline, typically within 30 m of shore. After imprinted fish encountered the line of morpholine (10 tracks) they always stopped migrating and milled in the area for up to four hours before continuing south (Figs. 3a and 4a). When no odor

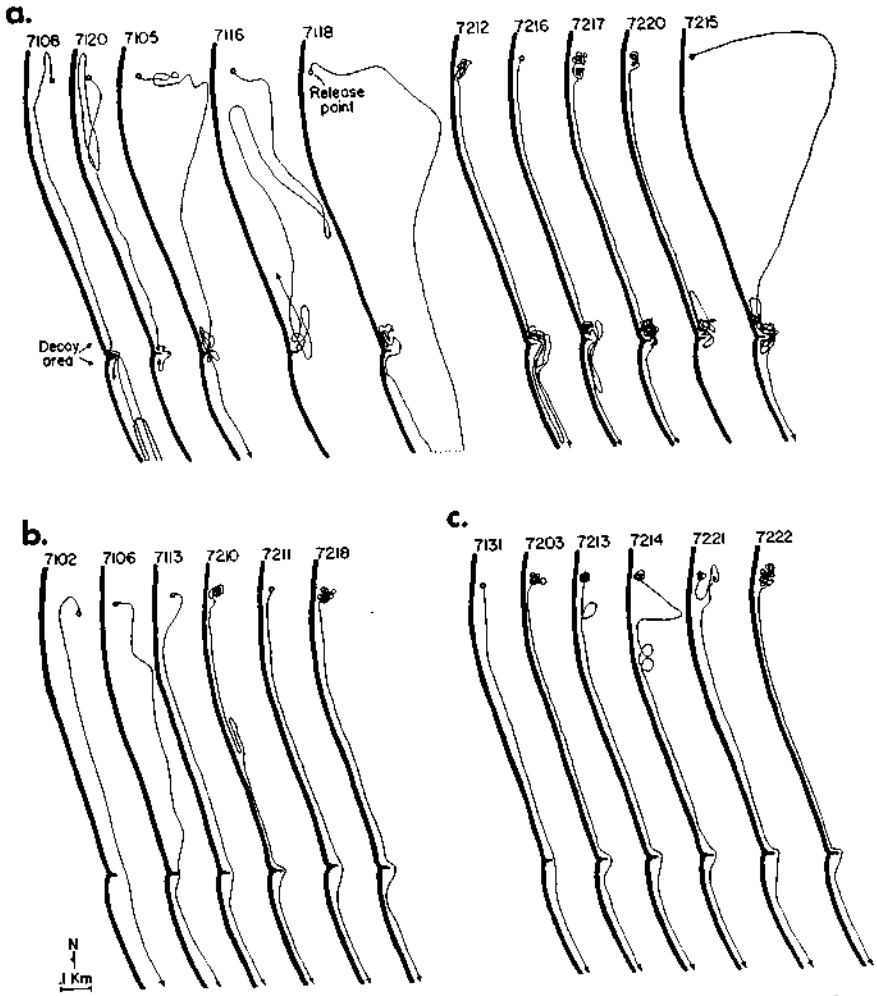

FIG. 4. Tracks of all salmon studied during behavioral experiments in 1971 and 1972 which moved south into the decoy area. Shown are the courses of (a) the imprinted salmon when morpholine was present in the decoy area, (b) the imprinted salmon when morpholine was absent from the decoy area and (c) the non-imprinted salmon when morpholine was present in the decoy area. The first two digits of each track number identifies the year during which the track was recorded.

was present, imprinted fish (6 tracks) moved through the area without stopping (Figs. 3b and 4b). Non-imprinted fish (6 tracks) moved through the area without stopping, even though morpholine had been added (Figs. 3c and 4c), suggesting that imprinted fish were not stopping in the area because morpholine was a unique shoreline odor. These results indicated that fish imprinted to morpholine recognized the odor and used it for homing in an experimental situation closely approximating natural conditions.

In addition to the fish tracked south, fish were tracked in other directions. These occurred mainly at the beginning and at the end of the run; thus there was a stronger tendency for homeward orientation (southerly movements) at the peak of the spawning season (Fig. 2b). (Note: The 22 fish tracked south were actually mixed results from fish tracked in 1971 and 1972. Nine fish

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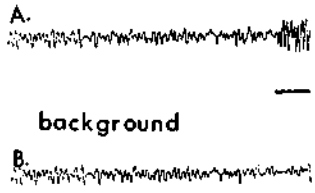
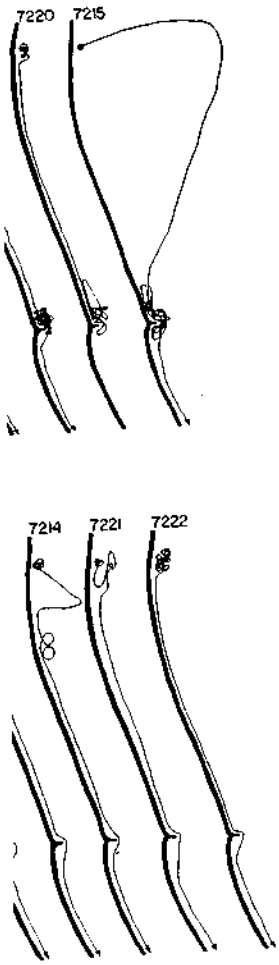


FIG. 5. EEG response



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moved south in 1971 and 13 moved south in 1972. A total of 30 fish were tracked in 1972. Only the 1972 tracks were included in looking at homeward tendency during the spawning season since corresponding EEG and census data was not collected in 1971 [see Madison et al. (1973) for more details]).

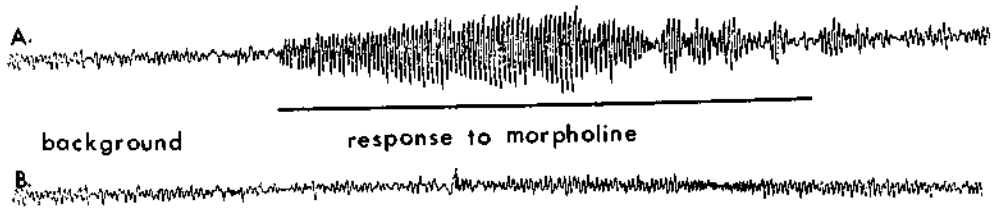
ELECTROPHYSIOLOGICAL EVIDENCE FOR OLFACTORY IMPRINTING

Methods

In addition to the behavioral experiments, the authors have used an electrophysiological approach to determine the response of salmon to homestream odors (morpholine) (Cooper and Hasler 1973a; 1973b). This technique involved recording olfactory bulb electroencephalographic (EEG) responses of morpholine imprinted and non-imprinted spawning salmon to morpholine. Earlier EEG work (Hara et al. 1965) demonstrated that the largest responses of homing salmon, tested with a variety of water samples, were usually to homestream waters. The authors hypothesized that if salmon were imprinting to morpholine, then morpholine-imprinted fish should have responded more strongly to morpholine than non-imprinted fish.

Imprinted and non-imprinted salmon were captured in Oak Creek, paralyzed with flaxedil (2 mg/kg body weight) and restrained in a holding box. Their gills were perfused with city tap water saturated with oxygen. A portion of the skull over the forebrain was removed with a dental drill to permit the insertion of an electrode into the olfactory bulb. EEG recordings (Fig. 5) were made with a Grass Polygraph and integrator. Heartbeat (EKG) was monitored as an indication of the fish's condition. Fourteen water samples were used, including morpholine (to test for chemical imprinting), .06 M NaCl (an easily defined stimulus with a characteristic repetitive response), Oak Creek water, Lake Michigan water (to test for recent odor experience) and some other organic compounds. Samples were presented in random order and introduced into the nares with a squeeze bottle.

For the purpose of standardizing the response of each trial, the integration of the responses to each test sample was divided by the integration of the NaCl record. The means of at least three trials and in most cases four trials per fish (usually the subject's heartbeat became erratic after four trials) are presented for 1% morpholine (Table 1). In addition, the means of fish tested only once or twice are also included in Table 1. The mean re-



FIGURE

A. EEG RESPONSE OF MORPHOLINE IMPRINTED FISH TO MORPHOLINE

B. EEG RESPONSE OF NON-IMPRINTED FISH TO MORPHOLINE

FIG. 5. EEG responses of (a) imprinted and (b) non-imprinted salmon to morpholine.

TABLE 1. Electroencephalographic responses (morpholine/NaCl responses) of imprinted (M) and non-imprinted (C) Coho salmon, *O. kisutch*. All three or more trials, except * = two trials, ( ) = one trial.

Date	10/16	10/16	11/4	11/16	11/2	11/14	11/19	11/14	10/26	10/31	10/31	11/15	11/28	11/2	11/4	11/27	11/9	11/21	11/14	11/15
Group	M*	C*	C	C*	C	C	C	C	C*	M	M	M	C	M	M	M*	M	M	(M)	M
Response	0.54	0.79	1.00	1.00	1.02	1.02	1.03	1.11	1.21	1.33	1.38	1.39	1.40	1.41	1.51	1.70	1.73	2.19	2.45	6.54
Rank	1	2	5	5	5	5	5	8	9	10	12.5	12.5	12.5	12.5	15	16.5	16.5	18	19	20

TABLE 2. Responses (morpholine/NaCl) of imprinted coho salmon to morpholine over time.

Date	10/16*	10/31	10/31	11/2	11/4	11/4	(11/14)	11/15	11/15	11/21	11/27*
Response	.54	1.33	1.38	1.41	1.51	1.73	2.45	1.39	6.54	2.19	1.70

TABLE 3. Electroencephalographic responses of imprinted and non-imprinted coho salmon (*O. Kisutch*) to 1% morpholine. Fish were imprinted at Crystal Springs Fish Hatchery in Antigo, Wisconsin during the smolting period and held at Nevin Fish Hatchery in Madison, Wisconsin for nine months prior to testing. (adapted from Dizon 1972).

Group	C	C	C	C	C	C	M	M	M	M	M	C	M	M
Response (morpholine/NaCl)	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

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sponses were ranked according to magnitude and then tested for significance by the Mann-Whitney Rank Test (Siegel 1956). Responses that were closer than 0.03 were considered the same rank.

Results

Eleven imprinted fish and 9 non-imprinted fish were tested using the EEG technique. The responses of the imprinted and control fish to morpholine were significantly different for the eight imprinted fish and six control fish tested at least three times ($U = 3$, $P \leq 0.001$). When the additional three imprinted and three control fish were added to the sample size, the responses were still significantly different ($U = 12$, $P \leq 0.01$). Morpholine-imprinted fish responded strongly to morpholine; control fish did not (Fig. 5). Imprinted fish did not respond to Oak Creek water without morpholine. Other chemicals typically produced no response.

The magnitude of the response to morpholine was roughly correlated with the number of salmon returning to the stream. The largest evoked potentials occurred at the peak of the run (Fig. 2c and Table 2).

Evidence against the recent exposure hypothesis has been found in the EEG experiments. Non-imprinted salmon did not respond to morpholine, even though they had been exposed to it in the stream. Furthermore, imprinted salmon held in a trap in Oak Creek for a week did not respond to Oak Creek water nor did fish held for several days in a tank supplied with Lake Michigan water respond to the Lake Michigan sample. In both instances, fish responded strongly to morpholine.

These results strongly supported the odor imprinting hypothesis[5].

TABLE 3. Electroencephalographic responses of imprinted and non-imprinted coho salmon (*O. Kisutch*) to 1% morpholine. Fish were imprinted at Crystal Springs Fish Hatchery in Antigo, Wisconsin during the smolting period and held at Nevin Fish Hatchery in Madison, Wisconsin for nine months prior to testing. (adapted from Dizon 1972).

Group	C	C	C	C	C	C	M	M	M	M	C	M	M
Response (morpholine/NaCl)	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

SUMMARY AND CONCLUSIONS

A Unified Approach for Studying Olfactory Imprinting

In summary, the authors have used three separate methods (census, ultrasonic tracking and EEG) to test if migrating coho salmon exposed to morpholine as juveniles recognized the odor and used it for homing. Results from the census experiment demonstrated that a significantly higher number of morpholine-imprinted fish, as opposed to non-imprinted fish, returned to Oak Creek to spawn. Results from the ultrasonic tracking study indicated that imprinted fish, followed under more or less natural migratory conditions, stopped migrating in an area where they encountered morpholine odors. Imprinted fish moved through the area without stopping when no odor was present. Similarly, there was a characteristic strong specific response of imprinted spawning salmon to morpholine, as evidenced by the EEG technique, while non-imprinted fish did not respond or responded less strongly to morpholine. All three lines of evidence independently demonstrated that coho salmon exposed to morpholine during the smolting period reacted to the chemical as adults, supporting the olfactory imprinting hypothesis. Recent exposure does not seem to have been an important factor compared to long-term memory as was indicated by both behavioral and electrophysiological studies.

[5]Dizon et al. (1973) were actually the first to use electophysiological methods to study the effect of imprinting to morpholine in coho salmon. For two groups of fish, one exposed to morpholine as fingerlings and the other left unexposed, there was a significantly different response to morpholine when these fish were tested with the EEG technique ten months later. Fish were ranked according to the strength of response (Table 3). Imprinted fish responded more strongly to morpholine than non-imprinted fish.

There was also a correlation between the peak of the spawning season, an increased EEG response of imprinted salmon to morpholine and a stronger tendency for initial homeward orientation of tracked fish. It seems reasonable to suggest that these observations are related to increased sensitivity to specific chemical odors possibly triggered by changing hormone levels. An hormonal basis for increasing sensitivity is especially attractive considering the rapid physiological changes the fish undergo during the course of the spawning season. Kleerekopter (1969) has also stated that it was clear that the acuity of the olfactory sense in fishes can be affected directly and indirectly by hormones. If the increased sensitivity hypothesis is true, it may go a long way toward explaining some of the variability in the results of previous investigations.

In conclusion, results from this unified approach indicated that coho salmon can learn, retain and use artificial chemical information to achieve successful homing. It seems unlikely that salmon possessing this ability would not use this mechanism in the natural situation.

ACKNOWLEDGMENTS

The authors especially thank and acknowledge the following for their assistance in the field: Sy Drzeweicki, Don Geiger and John Skorupski at the South Milwaukee Water Filtration Plant; Ed Mueller and his advanced biology students at South Milwaukee High School for their help; the authors' field assistants, Rod Smith, Steve Lewis, Terry Chapp, Peter Johnsen, Peter Hirsch, Jack Robinson, Tim Lewis, John Hildreth, Don Kender, Sandy Wangemann and John Shepardson; and the South Milwaukee Yacht Club. The authors also acknowledge the Department of Natural Resources Southern District Fish Managers Jim Holzer, Stan Druckenmiller and Wilbur Byum; Northern District Fish Managers Russ Daily and Paul Schultz and Hatchery Superintendents Gerry Kryka (Crystal Springs Fish Hatchery), Don Seskleba (Wild Rose Fish Hatchery) and Wes Warrick (Nevin Fish Hatchery). Henry Guckel and Al Scidmore of the University of Wisconsin Department of Electrical Engineering supplied the ultrasonic transmitters used in the tracking study. This project was supported by University of Wisconsin Sea Grant, Department of Commerce, NOAA 2-35209; Training Grant No. T900192 with the Federal Water Quality Administration; NSF Grants GB7616 and GB 343; the Wisconsin Department of Natural Resources and the City of South Milwaukee.

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