NOTES

OLFACTORY ELECTROENCEPHALOGRAPHIC RESPONSES OF HOMING COHO SALMON, *ONCORHYNCHUS KISUTCH,* TO WATER CONDITIONED BY CONSPECIFICS[1]

Experimental determination of the home stream of migrating salmon is an important technique for elucidating possible orienting cues. Responses are observed in the fish that are specific to the home-stream water and weaken with increasing dilution.

Two techniques have been employed with some success: Idler et al. (1961) and Fagerlund et al. (1963) found that homed salmon made consistent unconditioned behavioral responses to the water to which they homed. Hara, Ueda, and Gorbman (1965) could electrophysiologically demonstrate unconditioned reponses of the olfactory bulb to the home stream. Hara (1970) in his review states that "this electric response is specific in the sense that it cannot be evoked by water from spawning sites of other groups of breeding salmon."

We undertook an investigation of lake-run coho salmon, *Oncorhynchus kisutch,* to determine whether these fish exhibited specific olfactory bulb responses to home water. We tested salmon from two different home streams, examining responses to waters both on and off the migration routes. Furthermore, our field situation allowed us to assess the effects of the returning adult spawners upon the olfactory quality of the home stream.

Materials and Methods

The experimental fish were hatched from eggs obtained from a Michigan fish hatchery and raised in a Wisconsin hatchery for 1.5 yr. In the spring of 1969 approximately 1 mo prior to smolting, they were placed in small impoundments in tributaries of the Ahnapee River and the Kewaunee River (Kewaunee County, Wis.) to which numerous fish have returned in previous years. After initiation of smolting, they were allowed to leave the ponds and make their way downstream to the rivers and thence to Lake Michigan (Figure 1). In the fall of 1970, the mature fish were trapped in weirs on their return up the respective tributaries. The EEG (electroencephalographic) experiments were conducted within 1 km of the trap at the Ahnapee site.

Our experimental procedure was similar to that used by Hara et al. (1965). The fish was paralyzed with gallamine triethiodide (2 mg/kg), and water was perfused continually over the gills. An electrode was placed near the rear margin of the exposed olfactory bulb. The responses, evoked by perfusion of the ipsilateral naris, were amplified (Bio-electric Instruments, model DS2C[2]) 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. Responses reported later are expressed as the sum of the positive areas under the response wave.

Our procedure differed from that of previous workers in two ways:

1. A monopolar stainless steel microelectrode (Transidyne General, model 415100K) was used instead of a bipolar one. The indifferent electrode was clipped to the contralateral operculum.

2. Instead of direct introduction of stimuli to the naris via a wash bottle, a perfusion apparatus allowed one of four samples to be interspersed between a continuous tap water rinse (1 ml/s). Every 75 s, test water (10 ml) was introduced into the interrupted tap water flow. The median response to each stimulus was then
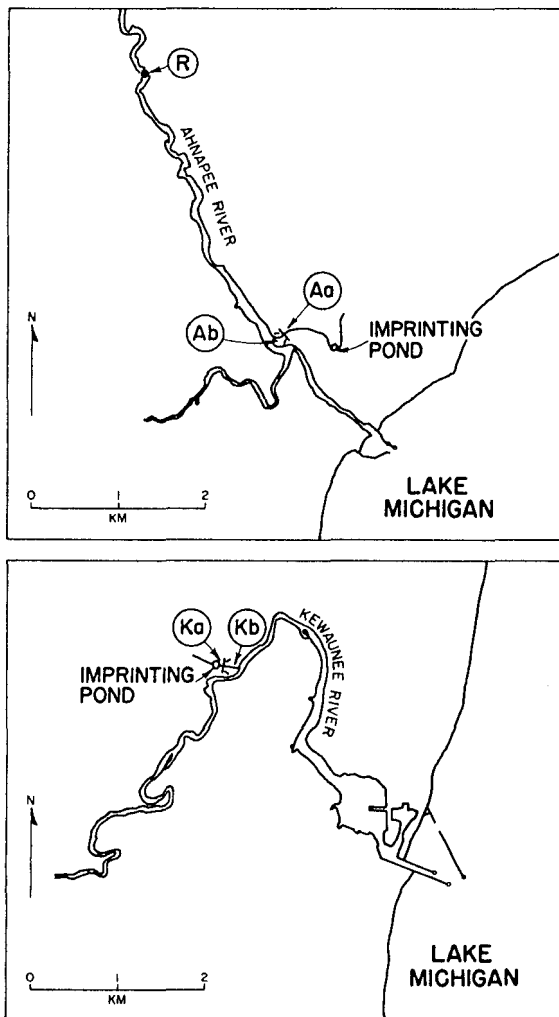
FIGURE 1.— Map of area. Water from Lake Michigan (L) was collected upwind of river mouth.

used in the later analysis. A control series with the same stimulus in each of the four holders demonstrated that delivery through any one of the four sample holders gave comparable results.

Water samples for testing responses of coho salmon were collected from the two home streams (a tributary to the Ahnapee River (A) and a tributary to the Kewaunee River (K)), the Ahnapee River (R) 4 km above the entrance of the home-stream tributary, and Lake Michigan (L) (Figure 1). Samples from the home streams were taken above (Aa, Ka) and below

(Ab, Kb) barrier fish traps so that Aa and Ka were home-stream waters while Ab and Kb were home-stream water plus the scents from the returning migrants. Both home streams (A, K) had similar physical features and coho salmon stocking histories; but the tributary to the Kewaunee River (K) contained a greater proportion of well water than stream water compared with the tributary to the Ahnapee River (A).

All sample presentations were made in groups of four to each fish, and responses were quantified as a ratio to the response to water from station Ab. This water sample was included as one of the four presentations for all fish and was chosen as a base because it evoked intense responses. Nineteen Ahnapee fish were tested: 11 with water samples from Aa, Ab, R, and L; 3 with Aa, Ab, R, and water from an unrelated source; 1 with Aa, Ab, and 2 samples from unrelated sources; and 4 with water from Aa, Ab, Ka, and Kb. Six fish from Kewaunee were tested with water from Aa, Ab, Ka, and Kb. (The water samples from other sources showed no consistent patterns and will not be discussed.)

Results

For the Ahnapee fish, samples from the terminus of the return migration evoked greater responses than the sample from the origin (L). But this result is probably not an example of Hara's (1970) "specific electric" response to the home stream: (1) One-half of the "stimulus strength" was added at the trap, although water collected above the trap (Aa) must be considered home stream (Table 1). (2) Agreement on order of responses to each water (Ab>Aa>R>L) was good among fish [$W = 0.87$, $k = 11$, $N = 4$, $P<0.01$ Kendal coefficient of concordance test (Siegel, 1956)], but the absolute difference in responses between presumably nonhome water from the Ahnapee River (R) and home water (Aa) was small. (3) Six fish collected from the Kewaunee River and four controls from the Ahnapee site were tested with water collected at Ab and Aa and from the corresponding stations from Kewaunee, Kb, and Ka. Kewaunee fish responded to their home-stream water more

TABLE 1.—Median EEG response ratios of coho salmon[1] from two different spawning sites to waters collected both on and off their migration route.

Water sample collection point	Number of fish	Quartile Responses		
		1st	2d	3d
Ahnapee fish:				
Aa	19	0.40	0.50	0.67
R	14	.14	.37	.47
L	11	0	.07	.21
Kb	4	.16	.28	.35
Ka	4	.04	.10	.18
Kewaunee fish:				
Aa	6	.36	.46	.55
Kb	6	.19	.25	.55
Ka	6	0	.06	.12

[1] Response ratio $\left[\dfrac{\text{response to sample}}{\text{response to Ahnapee tributary below (Ab)}}\right]$.

strongly if the water was taken from below the traps (Kb) than from above the traps (Ka). Furthermore, Kewaunee fish responded to Ahnapee home-stream water more than to their own (Table 1). Yet the Kewaunee fish did home correctly — three of the six Kewaunee fish carried fin clips unique to that site (the other three were unmarked).

Discussion

Hara (1970) in his recent review of electrophysiological studies of olfactory discrimination by homing salmon, concluded that "each spawning area has its own specific stimulant, or special combination of stimulants, recognized and responded to by the anadromous salmon." He acknowledged both that EEG responses of returning salmon adults can indicate a recognition of a chemical factor from conspecifics and that recent exposure of mature salmon to nonhome-stream water can result in strong EEG responses to that water (Oshima, Hahn, and Gorbman, 1969). Therefore olfactory responses to the home stream could result from (1) natal imprinting (a long-term memory), (2) recent exposure to the water (a short-term memory), (3) recognition of chemical factors from conspecifics, (a genetic (?) memory), and (4) the presence of stimulatory products not in any way specific to the home stream, transient, and incidental to the salmon's appearance, past or present, in the stream (responses independent of memory).

Our work appears to be the first to separate responses to conditioned home-stream water (other conspecifics were present) from responses to "pure" home-stream water. Conditioning of water by conspecifics appeared to double the EEG response to the home stream.

Perception of factors in the home stream other than those contributed by conspecifics is indicated in the greater responses of the Ahnapee coho salmon to "pure" home-stream water (Aa) than to other waters (R, M) not strongly conditioned by conspecifics. But this result does not necessarily imply natal imprinting (long-term memory) or recent exposure (short-term memory). Perception of nonspecific odoriferous products is indicated since (1) responses to water from the home stream (Aa) and nonhome river (R) by Ahnapee fish differ little, and (2) Kewaunee fish responded more to "pure" water from the Ahnapee tributary than to their own home-stream water.

The consistence of ranking of the four test waters (Ab > Aa > R > L) by 11 fish indicates some degree of reliability of the electrophysiological technique. Nevertheless, there seems to be an inherent lack of precision associated with evoked bulbar recordings. Sutterlin and Sutterlin (1971) found that EEG responses varied greatly among and within fish when compared to simultaneous olfactory epithelium recordings. Furthermore, our collection stations for water samples were subject to seiches (reversed current flow in the river and stream) and variable rates of river and stream discharge that made the water inconstant.

In conclusion, our experiments with lake-run coho salmon indicate the need to clearly differentiate between responses to "pure" home-stream water and water conditioned by presence of conspecifics. An intense and apparently specific response to home-stream water is not necessarily indicative of juvenile olfactory imprinting.

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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

A TEMPORARILY ANOXIC WATER MASS IN AN ALASKA ESTUARY

Anoxic marine waters have been reported to exist in the Black Sea, in many fjords in Norway, in British Columbia, and in a few tropical basins (Richards, 1965a). Anoxic estuaries in nontropical areas are usually characterized by the existence of a very shallow sill that restricts horizontal water movement and a strong pycnocline that restricts vertical water movement. When these conditions exist, and a water mass below the biological compensation depth is not mixed with water above or outside of its basin, the dissolved oxygen is eventually depleted and remains so until there is an intrusion of oxygenated waters. An anoxic condition may be temporary where the isolation of the water mass is temporary. A fortuitous observation of temporary anoxia in Little Port Walter on southern Baranof Island, Alaska, in October 1971 suggests the possibility of other temporarily anoxic estuaries in Alaska. This is the first reported occurrence of a naturally anoxic estuary in Alaska.

Powers (1963) provides a detailed description of the physical and hydrographic features of the Little Port Walter estuary (Figure 1).

> The estuary is located near the [southern] tip of Baranof Island in southeastern Alaska. . . . A stream, Sashin Creek, enters the inner end over a small waterfall which marks the limit of salt-water intrusion. At its seaward end the estuary connects with Chatham Strait and Port Walter Bay . . . .
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> The distance from the waterfall to Chatham Strait is 1.5 kilometers (km.); the distance across the widest part of the estuary is about 0.4 kilometer (km.). A peninsula . . . extends from the south shore and divides the estuary into two bays of approximately equal area, Inner Bay and Outer Bay. These are connected by a short channel . . . between [the peninsula] and the north shore.
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> The maximum observed depth of Inner Bay was 21 meters (m.) and of Outer Bay, 44 m. (referred to mean lower low water). The depth of . . . [the channel], where a shallow sill is present, was almost 5 m. Because of this sill, Inner Bay has the structural characteristics of a fiord; Outer Bay does not, since it connects