EXPERIENTIA

Vol. 32 – Fasc. 9 Pag. 1091–1222 15. 9. 1976

GENERALIA

Chemical Stimuli and Reproduction in Fish

Introduction

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In studying animal communication human observers focus on sensory modalities that are predominant in their own lives. Thus, ethologists have elaborately investigated visual and auditory communication systems whereas other modalities, such as chemoreception, have attracted less attention. Bird navigation and homing are controlled by various modalities and the fact that many problems in this field are still unsolved may at least partially be due to our reluctance to consider 'unconventional' sensory modalities and cues. In addition, ethologists tend to overlook other modalities once an animal, such as a cichlid fish, has been classified as, for example, mainly visual and its communication system has been 'explained' in terms of visual cues. However, in many cases animals communicate in several modalities (cf. Tavolga's contribution) even though exclusion of a single modality may not severely limit their behavior.

This collection of contributions regarding the role of chemical stimuli in reproductive behavior of fish should stimulate research in such 'neglected' modalities. As will be demonstrated in these articles, chemical stimuli have a variety of functions in the reproductive behavior of fish. They may guide salmons to their breeding ground, serve the recognition of young by a cichlid parent, and control aggressive and sexual motivations in gobiid, anabantid and cichlid fish. However, only a few species have so far been studied, and the chemical nature of the stimuli involved is generally unknown. Due to their long-term persistence, chemical stimuli may be particularly significant in tonic control of behavioral and physiological states in animal communities.

Chemical Cues for Homing Salmon

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It has long been established that salmonids return from the open waters of lakes or oceans with great specificity to their homestream to spawn (reviewed by Hasler[1] and Harden-Jones[2]). This migration is usually divided into two phases: 1. open-water migrations and 2. migrations near or in the homestream. We will only deal with the second phase here. Early speculations by Buckland[3] and later by Scheer[4] suggested that the uniqueness of the chemical charateristics of the homestream enables the adults to recognize this homestream upon returning from the sea. In a more advanced formulation of this idea, Hasler and Wisby[5] proposed the olfactory hypothesis. The use of olfaction for homing requires that: 1. each stream must have a characteristic and persistent odor perceptible by the fish, 2. fish must be able to discriminate between the odors of different streams, and 3. fish must be able to retain an 'odor memory' of its homestream during the period which intervenes between downstream and homing migration. At least

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[1] A. D. Hasler, *Underwater Guideposts – Homing of Salmon* (University of Wisconsin Press, Madison 1966), p. 155.

[2] F. R. Harden-Jones, *Fish Migration* (St. Martin's Press, New York 1968), p. 325.

[3] F. Buckland, *Natural History of British Fishes* (Unwin, London 1880), p. 420.

[4] B. T. Scheer, Q. Rev. Biol. *14*, 408 (1939).

[5] A. D. Hasler and W. J. Wisby, Am. Nat. *85*, 223 (1951).

seven lines of behavioral and electrophysiological evidence support the olfactory hypothesis and provide evidence about one or more of these three postulates.

Laboratory studies with conditioned fish have demonstrated an acute sense of smell in fish, with threshold values for many chemicals of at least 10^{-6} mg/l (reviewed in HASLER[1], TEICHMANN[6] and KLEEREKOPER[7]). Thus odors (e.g., stream odors) are perceptible at low concentrations.

HASLER and WISBY[5] succeeded in training groups of bluntnose minnows to distinguish between the waters of two Wisconsin creeks that differed in the geology and vegetation of their drainage basins. Trained animals could no longer detect differences between these waters when the nasal sacs were cauterized. Similar results were found in other studies (MCBRIDE et al.[8], WALKER[9]).

In many sensory impairment experiments by investigators in North America, Europe and Japan (reviewed by STASKO[10]), the intact olfactory sense seemed necessary for correct homing to occur. For instance, in one experiment (WISBY and HASLER[11]), coho salmon displaced downstream were unable to relocate their homestream reliably after their nares were occluded. Other studies demonstrated that blinded salmonids home nearly as well as control fish. Therefore, vision appeared to be relatively unimportant for relocating the original stream during the upstream migration. The effects of sensory impairment operations are not known (see partial discussion of this point in HARDEN-JONES[2]).

Behavioral experiments with unconditioned adults captured in the homestream (IDLER et al.[12], MCBRIDE et al.[8], SUTTERLIN and GRAY[13], and FAGERLUND et al.[14]) have shown increased activity of adult salmon when homestream water was introduced into their tanks containing neutral water. Others waters were not as effective.

Transplantation studies indirectly support the olfactory hypothesis. Fingerling salmon, taken from their homestream or hatchery shortly before the beginning of their downstream migration and transplanted to a second stream, returned to the second stream to spawn (CARLIN[15,16]; DONALDSON and ALLEN[17]; PECK[18]; JENSEN and DUNCAN[19]; see also review by RICKER[20]). These studies have demonstrated that the memory of the characteristics of the homestream are not inherited and that there exists in several species a rapid learning process at the time the juvenile salmon begin their downstream migration. In another study (PECK[18]), in which salmon were released after the smolting stage had been completed, a great deal of straying was observed. This suggests that imprinting is completed sometime during the smolt stage.

A sixth line of evidence is electroencephalographic studies on homing salmon. This work is reviewed in a later chapter (see HAHN). These studies indicate that salmon can discriminate between homestream waters and that there is some correlation between the intensity of the EEG response and the identity of the homestream water for a particular salmon. However, the usefulness of the technique for salmon studies is in doubt (COOPER and HASLER[21], BODZNICK[22]).

Finally, there have been studies designed to demonstrate odor imprinting and a long-term olfactory 'memory' of the homestream through the use of synthetic odors. HASLER and WISBY[5] proposed a decisive experiment to test the olfactory hypothesis. They proposed that coho salmon be imprinted in a hatchery to a synthetic chemical which does not occur in nature, is neither a repellant nor an attractant, and is perceived in low concentrations. Smolting coho salmon thus imprinted would be marked and allowed to migrate to open water. At the time of their spawning migration, attempts would be made to decoy the adults into a tributary downstream from the imprinting site by introducing the imprinting chemical cue into the water. Morpholine was found to be appropriate for this test (WISBY[23]).

Variations on these experiments have been conducted from 1970 until the present. In each experiment a large group of coho salmon smolts was exposed to morpholine at about 5×10^{-5} mg/l, while a second group was left unexposed. Both groups were then stocked at locations along Lake Michigan. During the adult spawning migration 18 months later morpholine was dripped into a stream to simulate a homestream for the returning fish. Data were collected on three aspects: census, behavioral, and electrophysiological.

[6] H. TEICHMANN, Ergebn. Biol. *25*, 177 (1962).

[7] H. KLEEREKOPER, *Olfaction in Fishes* (Indiana University Press, Bloomington 1969), p. 222.

[8] J. R. MCBRIDE, U. H. FAGERLUND, M. SMITH and N. TOMLINSON, Can. J. Zool. *43*, 245 (1964).

[9] J. C. WALKER, M. S. Thesis, University of New Brunswick (1967).

[10] A. B. STASKO, Ann. N.Y. Acad. Sci. *188*, 12 (1971).

[11] W. J. WISBY and A. D. HASLER, J. Fish. Res. Board Can. *11*, 472 (1954).

[12] D. R. IDLER, H. R. MCBRIDE, R. E. JONES and N. TOMLINSON, Can. J. Biochem. Physiol. *39*, 1575 (1961).

[13] A. M. SUTTERLIN and R. GRAY, J. Fish. Res. Board Can. *30*, 985 (1971).

[14] U. H. M. FAGERLUND, J. R. MCBRIDE, M. SMITH and N. TOMLINSON, J. Fish. Res. Board Can. *20*, 1457 (1963).

[15] B. CARLIN, J. Cons. perm. int. Explor. Mer. *147*, 89 (1959).

[16] B. CARLIN, Lecture Series, the Atlantic Salmon Ass. Montreal 1968, p. 22.

[17] R. DONALDSON and G. H. ALLEN, Trans. Am. Fish. Soc. *87*, 13 (1957).

[18] J. W. PECK, Trans. Am. Fish. Soc. *99*, 591 (1970).

[19] A. JENSEN and R. DUNCAN, Progr. Fish-Cult. *33*, 216 (1971).

[20] W. E. RICKER, in *The Stock Concept in Pacific Salmon* (Eds. R. C. SIMON and P. A. LARKIN; MacMillan Lectures in Fisheries, University of British Columbia, Vancouver 1972), p. 231.

[21] J. C. COOPER and A. D. HASLER, Fish. Res. Board Can. Tech. Rep. *415*, 44 (1973).

[22] D. BODZNICK, Comp. Biochem. Physiol. *52A*, 487 (1974).

[23] W. J. WISBY, Ph. D. Thesis, University of Wisconsin, Madison 1952), p. 41.

For four experiments a highly significant number of exposed compared to unexposed fish was captured at the artificially scented stream: 216 vs. 28, 437 vs. 49, 647 vs. 65 and 439 vs. 55 (Scholz et al.[24,25], Cooper et al.[26-28]; Madison et al.[29]). Fish released as smolts 13 km north of the simulated homestream homed in a similar manner to those released near the homestream. During a third year, morpholine was not added to the stream during the spawning migration and exposed and non-exposed fish returned in equal low numbers (51 vs. 55). These results confirm the existence of odor imprinting and long-term memory in coho salmon.

To obtain more direct information on homing to the imprinted chemical cues, imprinted fish equipped with ultrasonic transmitters were released along the shoreline of Lake Michigan and tracked into an area scented with morpholine (Madison et al.[29], Scholz et al.[24,25]). 20 imprinted fish tracked into this area always stopped migrating and milled around for up to 4 h before leaving (presumably when the morpholine scent had been dissipated by water currents). When no odor was present in this area, 13 imprinted fish moved through without stopping. Non-imprinted fish (14 tracks) moved through the area without stopping. Imprinted fish tracked through the area when a compound closely related to morpholine was used (N-β-hydroxyethylmorpholine) moved through the area without stopping. These results indicated that fish exposed to morpholine recognized the odor and used it for homing in an experimental situation closely approximating natural conditions.

In electrophysiological experiments (Cooper and Hasler[26,30]), EEG responses to morpholine were tested from imprinted and non-imprinted fish captured in the simulated homestream. There was a significant difference in the amplitude of the EEG signals to morpholine for imprinted compared to non-imprinted salmon for 4 experiments. Significant differences were not seen when other substances (N-β-hydroxyethylmorpholine or 1-methionine) were tested. Thus, electrophysiological experiments provide evidence that early exposure to morpholine significantly influences the subsequent responses of these fish.

Experiments on rainbow trout by behavioral (Cooper and Scholz[27]) and physiological (Cooper and Hasler[26]) means indicated that this species also can be imprinted to morpholine and use this scent for homing to a simulated homestream.

In another test of the olfactory hypothesis using the artificial imprinting technique (Scholz et al.[31]), one group of fish was exposed to morpholine, a second group to phenethyl alcohol (PEA) and a third left unexposed. These groups were marked and released into Lake Michigan halfway between two streams. During the spawning migration morpholine was released in one stream and PEA into the other. Census results showed that significantly higher numbers of morpholine-imprinted fish (compared to PEA-imprinted fish) returned to the morpholine-scented stream, while PEA-imprinted salmon homed to the stream scented with this odor. These results verify the original hypothesis that an odor is the basis for identifying 'home' water.

In summary, laboratory and field experiments along seven lines show that odors are the basis for homestream recognition. Juvenile salmon appear to imprint during the smolting to the odor of their homestream, recall this memory during the subsequent adult migration, and use this information to relocate the homestream in the second part of the homing migration.

[24] A. T. Scholz, J. C. Cooper, D. M. Madison, R. M. Horrall, A. D. Hasler, A. E. Dizon and R. Poff, Proc. 16th Conf. Great Lakes Res., Huron, Ohio 1974, vol. 16, p. 143.

[25] A. T. Scholz, R. M. Horrall, J. C. Cooper, A. D. Hasler, D. M. Madison, R. J. Poff and R. Daly, Wisconsin Dept. Nat. Res. Fish. Mgmt. Rep. *80*, 45 (1975).

[26] J. C. Cooper and A. D. Hasler, J. Fish. Res. Board Can, *33*, 688 (1976).

[27] J. C. Cooper and A. T. Scholz, J. Fish. Res. Board Can., *33*, 826 (1976).

[28] J. C. Cooper, A. T. Scholz, R. M. Horrall, A. D. Hasler and D. M. Madison, J. Fish. Res. Board Can., *33*, 703 (1976).

[29] D. M. Madison, A. T. Scholz, J. C. Cooper, R. M. Horrall, A. D. Hasler and A. E. Dizon, Fish. Res. Board Can. Tech. Rep. *414*, 35 (1973).

[30] J. C. Cooper and A. D. Hasler, Science *183*, 336 (1974).

[31] A. T. Scholz, R. M. Horrall, J. C. Cooper and A. D. Hasler, Science *192*, 1247 (1976.

Chemical Stimuli in Reproductive Behavior in Fish: Communication

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The importance of chemical stimuli in the behavior of fishes has been widely documented in such areas as schooling, alarm responses, detection and discrimination of plant odors, and in migration. Recent reviews and compilations have given ample testimony to the high degree of sensitivity and discrimination of che-