water conditioned by the presence of undamaged conspecific individuals is an attractant, and that water conditioned by injured individuals or by addition of a water extract of minced skin elicits a fright reaction. In our experiments, however, no special efforts were made to avoid injury to the skin of the fish during the extraction process. The extract contained loose scales, which indicated skin damage, and it is unlikely that we failed to extract an alarm substance that may have been present.

Another experiment has significance: Four adult top smelt were homogenized in sea water in a blender; the resultant slurry was filtered through cheesecloth and the sea-water suspension was introduced into the tank. We have no quantitative data from this experiment because formation of bubbles on the surface obscured the fish in the photographs, but our subjective impression was that the fish were attracted by this extract; certainly they showed no alarm reaction.

Our results conflict with those of Skinner *et al.* (*7*). We have learned that their experiments were carried out on 15 adult fish in a 190-liter aquarium, under which conditions they found it necessary to take precautions against false positive reactions. In an attempt to reconcile the disparities between our results and theirs, we placed ten juvenile-to-adult top smelt in a shielded 190-liter aquarium. After 48 hours, a top-smelt extract was added. This experiment was repeated several times without our observing any clear-cut changes in the behavior of the fish. We could evoke reactions similar to those observed by Skinner *et al.* only by strong mechanical stimulation—as by rapping on the glass. When the top smelt in our large tank were frightened by sudden movements or splashing, they made a short dash before going to the bottom where they hovered for several minutes. Therefore we question the interpretation that signs of distress such as jumping and severe seizures represent natural fright behavior in top smelt; we feel that they should be interpreted as an experimental artifact.

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Chemical Communication in Social Behavior of a Fish, the Yellow Bullhead (Ictalurus natalis)

Abstract. *Studies of behavior in yellow bullheads showed that they recognized individuals of their own species by means of pheromones. After training by reward and punishment, blinded bullheads were able to discriminate between the odors of two donor fish, but they lost this ability when deprived of their sense of smell. The main source of the intraspecific chemical stimuli involved in recognition is the mucus. A change in status after fighting was chemically communicated to other bullheads.*

The well-known fright reaction by certain fishes depends on their ability to smell a substance from the skin of another fish (*1*). In addition, pheromones have been postulated to aid in the schooling of fishes (*2*), and parent jewel fish (*Hemichromis bimaculatus*) recognize their own broods by means of chemical signals (*3*). Marler and Hamilton (*4*) summarize the role of pheromones (intraspecific chemical stimuli) in the behavior of vertebrates. Our work indicates that chemical communication may play a more important role in the behavior of fishes—particularly in those fishes whose behavior is not governed predominantly by visual clues—than previously ascertained.

The yellow bullhead, although visually deficient (*5*), possesses an acute external taste sense on which it relies in searching for food (*6*). Although the sense of smell is apparently not important in feeding, the olfactory anatomy of the species indicates a substantial acuity of smell (*7*). Blind bullheads kept in large aquariums exhibited complex social behavior (territories, dominance hierarchies, intricate agonistic displays, and fights). These facts suggested to us that olfaction and its relation to the social behavior of the species should be investigated.

Our experiments were designed to ascertain whether or not one bullhead could discriminate between two others by means of its chemical senses alone. Immature fish of approximately 100 g and 20 cm in total length were collected from local lakes, blinded with Phemerol (*8*), and quarantined for several weeks. They were placed in separate 19-liter aquariums. Each tank contained a clay pot which served as a shelter. The fish were divided into test and "donor" groups, with 50 ml of the tank water of the latter acting as the stimuli to be discriminated. Each test fish was trained, according to a nonrandom but scrambled schedule by food reward (chunks of beef liver) and electric shock (*9*), to discriminate between two donor fish which were moved from one aquarium to the other to avoid discrimination on the basis of tank odors unrelated to a fish. Water from tanks of donors was gently poured over the bubble train of the airstone in the test aquarium.

As fish learned to discriminate between the odors of two donors, their responses varied appropriately. When the positive odor was introduced, they rose rapidly to the surface, at the front of the aquarium, and gulped as if in search of food. When the negative odor was introduced, they fled to the pot where they were safe from electric shock. In the 5-second interval between the introduction of the negative odor and the shock, some of the fish would even threaten the odor before retreating (Fig. 1).

Ten animals learned to discriminate between water from positive and negative donors in about 30 trials (range, 19 to 32; mean, 26.2 trials). They were subjected to a total of 937 tests, of which 42 (or 4.5 percent) showed incorrect responses. All fish were able to remember the learned discrimination for at least 3 weeks without retraining.

We then cauterized the nares of three of these test animals and of two with no previous training. Gross microscopic examination after the operation showed that the olfactory epithelium had been destroyed. No regeneration took place during the period of the experiments. Stress induced by the cautery was short-lived; the animals began to feed after several hours. After a period of 1 week, the three experimental animals were given 408 trials in which they compiled 197 correct scores (48.3 percent). Training was attempted with the naive fish but was not successful after 32 trials. With these same fish, 114 more tests produced 50 correct scores (43.9 percent). Thus, these tests demonstrated that the fish deprived of their olfactory sense have no ability to discriminate between two other fish.

We then tested the slime of the original donor fishes rather than their ambient water. An experimenter (with rubber gloves) gently lifted the donor fish out of its aquarium by means of a nylon net and wiped the fish's dorsal surface lightly with a piece of wet cheesecloth. The cloth was then placed in a 50-ml vial filled with water in which no fish had resided. The remainder of the experiment proceeded as previously described.

In 32 such trials, one of seven test fish made two errors to a negative stimulus. In the remaining 30 correct performances, we noted that the responses were less intense to the neutral water enriched by the donor slime than to the donor water itself. The lessened intensities of reaction to mucus in water where no fish had resided might be ascribed to a concentration of mucus, to the lack of certain fecal residues, or to the absence of urine (which freshwater fish excrete copiously), or to all of these factors. Stress products have been reported in fish urine (*10*).

Behavior observations had suggested to us that stress influenced chemosensory recognition. Several pairs each of a dominant and a submissive bullhead were made to share 190-liter aquariums. When the dominant fish was removed from such an aquarium, isolated overnight, and returned the next day, the submissive fish did not attack it as it would have attacked a stranger. However, when the dominant fish was returned after having experienced a losing encounter with another bullhead, the originally submissive one immediately attacked it.

To test more rigorously the effects of stress, six donor fish were subjected to mild electric shocks at regular intervals for several hours. After this gentle-to-moderate stress, the donors were recognized by the test fish correctly and without fail. To introduce a variation in stress, donors were made to fight with larger, neutral fish. They lost the fights and, as a result, were altered chemically in such a manner that the test fish did not discriminate between them. These experiments corroborated our observations that natural stress altered the pheromone system, but we have not yet resolved whether the changed chemical nature of a stressed fish is due to its altered slime or to other substances, perhaps in the urine.

In nature, bullheads do not live in 19-liter aquariums; therefore, one might suspect that the demonstrated olfactory capacity for distinguishing between other fish is not important in their lives in lakes and ponds. Only field studies, difficult to devise and execute, primarily because of the highly nocturnal habits of bullheads, should tell us whether or not individual intraspecific recognition is important in nature, as it is suspected to be for many animals (*4*).

An attempt was made to approach natural conditions in large aquariums of up to 7600 liters where bullheads were observed. Whether their vision was intact or not, they reacted to the introduction of small amounts of water from the aquariums of other bullheads. Subsequent observations and experiments in which opaque barriers were raised between previously separated animals suggested that pheromones had a part in their behavior. Interactions of a bullhead with others all pointed to reliance on individual chemosensory recognition and to the existence and importance of a complex chemical communication system outside the breeding season.

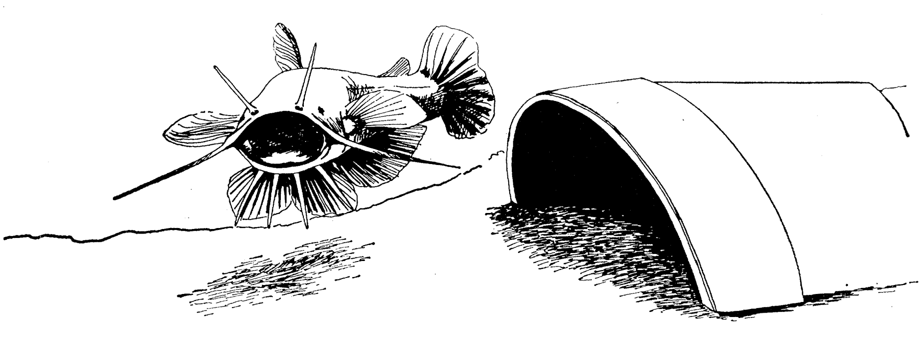

Fig. 1. Blind bullhead attacking the odor of its negative donor immediately before fleeing into the pot. [Drawing from a photograph]

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Primitive Microfossils or Not?

There is increasing interest in possible organic remains in the early Precambrian strata of the earth, and even in celestial material. A variety of minute forms have been shown by Barghoorn and Tyler (*1*) and others to have such distinctive morphology, as well as composition preserved within rocks of such low permeability (after lithifaction) as flint, that there seems little doubt that they are the remains of life some 2 billion or more years ago.

More recently, microscopic forms from meteorites which may suggest comparable remains of primitive life have been found, and perhaps soon similar forms from the moon may be discovered. Some of these, which can be demonstrated not to result from contamination, seem to show a morphology so similar to the preservable remains of simple forms of life known to the microbiologist (or to the organ taxa of the palynologist) that they have