Alarm Reaction of the Top Smelt, Atherinops affinis: Reexamination

Abstract. *We did not observe the reported alarm reaction of the top smelt* Atherinops affinis *(Ayres) when presented with a methanol extract of whole top smelt; we interpret the reaction as an experimental artifact. Rather, a methanol extract of top smelt is an attractant, as is methanol extract of shrimp.*

The skin of the minnow *Phoxinus laevis* L. contains a substance that, when released by mechanical injury, alters the behavior of fish of the same species (*1*). The new behavior (forming a tight school, dropping to the bottom, refusing food, and dashing into cover) has been termed the alarm reaction (*Schreckreaktion*). The initial observations were extended (*2*, *3*) to a wide variety of fishes of the order Cypriniformes (*4*). According to the most recent review (*5*), the alarm reaction in fishes is restricted to members of the order Cypriniformes; Pfeiffer (*3*, *5*) tested 66 species of 32 families of fishes of other orders without finding such a reaction. However, an alarm reaction had been reported for the atherinid fish *Hepsetia stipes* (Müller and Troschel) of the order Atheriniformes (*6*).

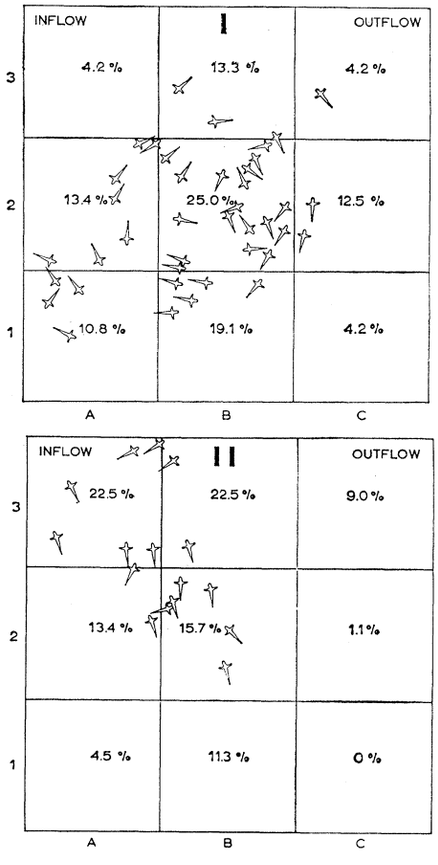

Fig. 1. Schematic representations of photographs, showing location and orientation of the fish in the projected grid pattern: before introduction of the methanol extract of top smelt (I) and 45 seconds after introduction (II). The percentage within each quadrat refers to the total number of fish for all photographs during that portion of the test. Fewer fish are shown in II because many were aggregated near the inflow pipe at the corner of the tank; they were either obscured by turbulence or beyond the photographic field.

It was therefore interesting when Skinner *et al.* (*7*) reported an unequivocal alarm reaction from another atherinid, the top smelt, a schooling, open-water species. Their methods differed from those of previous workers in that a methanol or ether extract of skin or of whole fish was used, rather than a water extract of minced skin. As a first step toward extension of this approach to other noncyprinifom fishes, we attempted to duplicate their results with top smelt.

Several hundred juvenile-to-adult top smelt (10 to 20 cm in total length) collected in Mission Bay, California, were placed in an outdoor concrete tank (2.4 by 2.7 m, 4.1 m^3) (*8*). One month later, after the initial mortality, the 100 fish remaining were used for the experiments. There was a constant inflow (12 liter/sec) of filtered sea water; the tank was shielded above, and observers were hidden from the fish by a black plastic blind. Methanol extracts were prepared by the method of Skinner *et al.*: 20 to 30 living adult fish were placed in a glass jar and covered with methanol; after 24 hours of extraction at 0°C, the methanol was removed in a rotary evaporator and the resultant product was diluted with distilled water to a volume of 3 liters.

Extracts were introduced into the tank by way of the inflow pipe at a rate of 0.75 liter/min for 4 minutes. Results were recorded by a still camera placed over the center of the tank; only the central 75 percent of the tank was in the photographic field. Three pictures were taken during a 1-minute pretest control period, just before introduction of the test material. After introduction, four photographs were taken during the 1st minute; four during the 3rd minute. The negatives were projected on a grid pattern, and the number of fish in each quadrat was counted. Three experiments were run with methanol extracts of top smelt; another, with a similar methanol extract of frozen edible shrimp, with which the fish had been fed. For one control experiment we used a little methanol in distilled water.

Typical results appear in Fig. 1. In each trial the fish were clumped around the inflow pipe within a few seconds of introduction of the conspecific extract. By the 3rd minute they showed a strong tendency to aggregate along the flowpath of water between the inflow and outflow pipes. The objective validity of these observations was tested by comparative analysis of the data for the control period and the test periods by the Kolmogorov-Smirnov method (a nonparametric method that tests the significance of the difference between two independent samples) (*9*). In this instance the independent samples were the frequency distributions of the photographed fish by quadrat.

None of the pretest controls differed from each other at the 0.1 level. The tests with the methanol control were not significantly different from the preintroduction period ($P > .1$). All the tests with top-smelt-extract differed from the controls ($P < .01$). The test with shrimp extract was different from the control ($P < .01$), yielding essentially the same result as tests with the top-smelt extract. Our experiments (Fig. 2) thus show that a methanol extract of top smelt, like that of shrimp, attracts top smelt.

Hemmings (*10*) has shown that in the roach *Rutilus rutilus*, Cyprinidae,

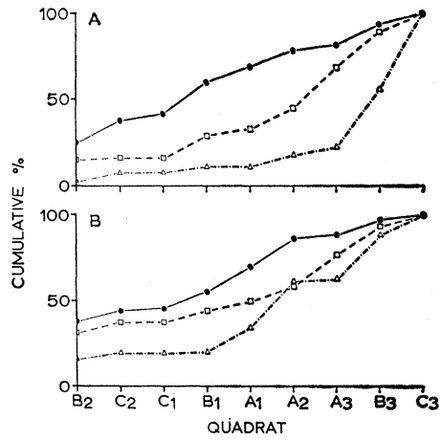

Fig. 2. Number of fish per quadrat, presented as cumulative percentage: solid circles reflect the pretest control period; squares, the 1st minute after introduction of the stimulus; triangles, the 3rd minute. A is a test with methanol extract of top smelt; B is a test with methanol extract of shrimp. Higher values at the left indicate clumping in the center of the tank; sharply increasing values at the right indicate clumping at the stimulus end of the tank.

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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25 September 1967

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