

Orientation Through Chemo-Reception in Fishes

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INCREASING NUMBERS of research reports continue to stress and document the role of the chemical senses in fish behavior. The behavioral patterns in which chemical stimulation has been shown to be important or even indispensable already cover virtually all phases of the life history of these animals. The capability to chemically discriminate, frequently at inconceivably low thresholds, prey, predator, food, nest, young, individuals of the same or other species, sex, water quality, and other conditions has been well-documented for a large number of species. Although olfaction is by far the more sensitive of the chemical senses and is required for the perception of the stimuli involved in most of the behavioral patterns mentioned, it has been demonstrated recently that, in some instances, the sense of taste can be the sole sensory modality necessary (ref. 1). There is ample evidence that olfaction is indispensable for the successful homing of anadromous fish, at least during their upstream migration (refs. 2 and 3).

It will not be necessary here to rehearse the dramatic and well-known spawning migrations of the Pacific and Atlantic salmons and their land-locked cousins. The ability of the individual fish of these species to return

for spawning, after prolonged absence in the ocean or a lake, to the specific sites of their birth has been well-documented, and a number of experiments have demonstrated that anosmic animals are unable to perform this feat. Particularly, compared to controls, they are unable to make the correct choice of direction as they reach a fork of the river in the course of their upstream migration.

Under experimental conditions, chemical stimulation frequently affects the locomotor behavior of the animal and may result in so-called random movements often referred to as alarm or appetitive responses. The term "so-called" is used intentionally in that the designation "random" has been based on the gross appearance of these movements when visually inspected. However, more precise analyses of the parameters of many such locomotor patterns in some sharks and teleosts have demonstrated that, at least in the species studied, the movements are not random but have rather rigidly organized characteristics (ref. 4). On the other hand, many of the behavioral patterns elicited by olfactory stimulation comprise directed movements which are oriented either in relation to the source of the odor or to other environmental cues.

The descriptive but extensive documenta-

tion of the widespread significance of the chemical senses, particularly of olfaction, stands in great contrast to our ignorance about almost all the underlying physiological mechanisms. As to the peripheral transduction of the olfactory cell membrane, theories abound, and although promising progress is being made, there is no acceptable explanation for the phenomenon of odor perception. The central and possibly peripheral decoding systems which, in many species, allow for the discrimination of thousands of odorous substances, isolatedly and in mixtures, is as yet not understood. As to the mechanisms of orientation through olfaction in fishes, the topic of this paper, we are not much better informed.

In most behavioral responses to olfactory stimulation, cited earlier, directed movements occur, such as the approach by the predator to the odor source of prey and the upstream spawning migration of the salmon. There are many other similar responses, all of which are abolished or greatly modified in anosmic animals. The main problem these directed movements pose—a problem not limited to fishes—is the difficulty to define the orientation mechanisms involved since the molecular concentration gradient of an odor source does not contain vectors that can be used as directional cues by an animal surrounded by the field. Thus we must look for other means of orientation in these instances.

In the course of an earlier study on orientation behavior in some species of shark and a teleost, it was established that in multiple-choice situations none of the animals tested was able to locate the source of odor of a preferred prey when this was released from one of 16 compartments placed radially along the periphery of a cylindrical tank. From each compartment water flowed through an open gate toward an overflow located in the central open area of the tank. However,

when the rate of flow of water from the compartment releasing the odor was increased slightly, the animals located the source of the odor quickly and without error although increase of flow without odor stimulation did not affect the animals' locomotor patterns. It may be concluded, therefore, that the role of odor perception in a concentration gradient is limited to that of a sign stimulus which, in the experiments in question, releases positive rheotaxis (ref. 4). It is likely that in different circumstances the same sign stimulus may release response to other directional cues. This mechanism would seem particularly appropriate to explain the role of olfaction in the orientation of salmon and other species in the course of their upstream spawning migration. The fish within the odor gradient released by the home ground would remain positively rheotactic as long as the specific odor substances were present. When presented with the crucially important choice of direction at a fork of the river, only the "correct" branch of the fork would contain the olfactory sign stimulus to maintain the rheotactic response. Entry into the "wrong" branch would eliminate the stimulus, abolish rheotaxis, and cause the animal to be brought back to the fork. The problem of orientation in a gradient does not arise. Behavior at river forks of migrating, normal, and anosmic salmon seems to be consistent with the proposed mechanism.

In nature, odor substances are not likely ever to be distributed in a smooth concentration gradient. Flow characteristics, differences of temperature, turbidity, density, viscosity, radiation, the positions of affluents, bottom contours, to mention just a few, produce discontinuities in many or all of the physical and chemical characteristics of a body of water. Therefore, a uniform spatial distribution of substances, including odorous substances, is unlikely. Even for rivers, the

existence of discontinuities in the characteristics of the water mass is well-known. In lakes this condition is, of course, still more pronounced. If it is considered, furthermore, that many biologically significant odors are released by spatially and temporally discrete sources, it becomes clear that a swimming fish will intermittently, probably very frequently, encounter and pass through odor concentration gradients whose boundaries are determined by the animal's thresholds for the substances involved and by the physical and chemical factors that affect the spatial distribution of the odorous substances. At many of these encounters, the animal will have to respond promptly and adaptively with directed locomotor responses. Once more, the problem as to the nature of the mechanism of orientation is before us. Various kineses and taxes, classified and discussed by Kühn (ref. 5), Fraenkel and Gunn (ref. 6), Kennedy (ref. 7), and others, particularly in respect to invertebrates, have been considered as possible mechanisms by investigators of this problem. However, the theoretical and experimental results are inconclusive and little agreement has been reached so far.

In the light of recent studies in our laboratory on the locomotor behavior of fishes, it seems probable that the difficulties arise from the limitations of conventional methods of observation and analysis which have been applied to the problem in the past. It seems obvious that an understanding of the mechanisms underlying oriented responses to environmental stimuli must be dependent above all on our ability to record these responses adequately. However, none of the methods used in the past seemed adequate to monitor locomotor behavior of fish during extended periods and with the accuracy and detail required for an analysis in depth of the locomotor patterns and their parameters. This rationale was the incentive for the design and

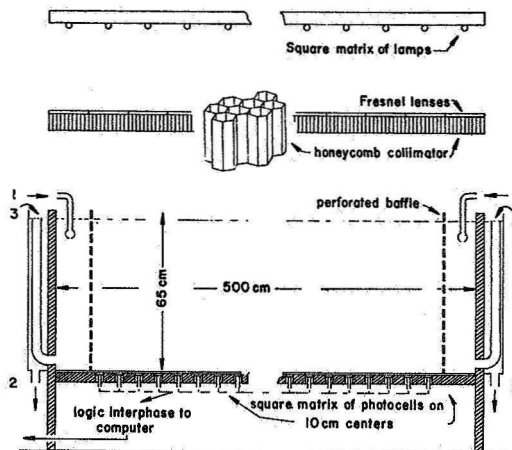


FIGURE 1. Schematic cross section of the monitor tank.

development in our laboratory of a system for the acquisition and processing of accurate data describing the locomotor behavior of fish in a spacious experimental environment appropriately controlled. The system was described in some detail earlier (refs. 8 to 10). Recently the technique allowed us¹ to obtain information that may contribute to the solution of the problem of orientation to chemical cues in fishes. It seems useful, therefore, to quickly outline the basic approach developed in the technique in question.

The movements of a single fish are monitored by means of a square matrix of 1936 photoconductive cells, on 10-cm centers, embedded in the floor of a tank measuring $5.0 \times 5.0 \times 0.5$ m (fig. 1). A collimated light field illuminates the cells uniformly from a suspended ceiling above the tank at a color temperature of 2300 to 2400° K. Interception of the light by the fish sharply increases the electrical resistance of the photo-

¹ TIMMS, A. M.; AND KLEEREKOPER, H.: The Locomotor Response of Male *Ictalurus punctatus*, the Channel Catfish, to a Pheromone Released by the Ripe Female of the Species. Unpublished, 1970.

cell affected, an event which forms the input for an electronic logic interface with an on-line computer (fig. 2). The latter is programmed to establish the address of the photocell in the x,y matrix and the time of the event. These raw data are recorded on magnetic tape for subsequent analysis by the same or a larger computer but can also be processed in the course of the experiment by the computer on line which puts out on a teletypewriter, event by event, information on the velocity, absolute orientation in the tank, change of direction in radians, distance covered, and left- or right "handedness." The locomotor patterns can be plotted and are continuously displayed on a cathode ray tube. The linear resolution for the smallest target (3 mm) which can affect the resistance of a photocell in the conditions described is 10 cm. For a fish 30 cm long, the approximate minimum size generally used in our work, the angular resolution is not less than 4° . The effects of consecutive targets on a same photocell are discarded by the computer programs so that minor, repetitive movements of parts of the body, which do not produce locomotion, do not enter into the computations. By appropriate programming the information can be fragmented temporally and spatially so that behavior in a specific area of the tank (for example, that affected by a source of chemical stimulation) may be compared with that in other areas.

The manner in which water is supplied and odors or other chemical stimulants are introduced is important. Perforated panels, placed vertically along the four walls of the tank, form 20-cm-wide, peripheral channels. Water enters any one of these through a large number of small holes in a horizontal supply pipe and is admitted to the main body of the tank through the perforations of the panel; it then traverses the tank, commonly at a velocity of 1.6 cm/min, and leaves

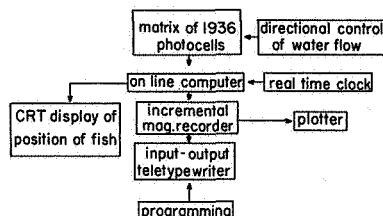


FIGURE 2. Flow sheet of data-acquisition and processing.

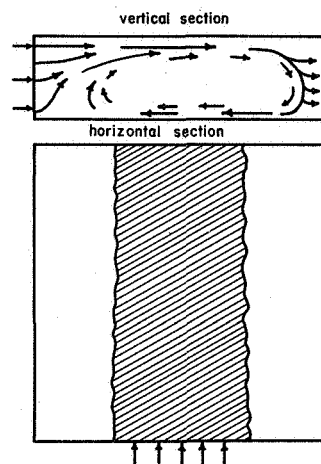


FIGURE 3. Sketch of flow pattern in the monitor tank.

through the perforations of the panel of the opposite channel where it discharges through an overflow pipe. By dividing the supply channel into three noncommunicating compartments of equal size and admixing a source of chemical stimulation in the central compartment, a "curtain" of the chemical stimulus is formed which, according to three-dimensional conductivity, temperature profile, and dye flow measurements, maintains its lateral boundaries with a high degree of stability and little diffusion until it reaches the vicinity of the discharge panel where it spreads out laterally. Vertically the flow pattern is more complex; its hydrodynamical and diffusion profiles are being studied in

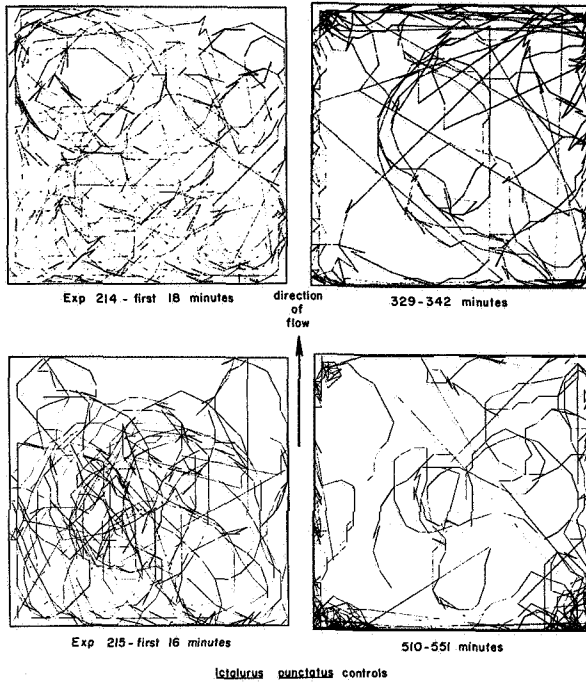


FIGURE 4. Locomotor patterns of *Ictalurus punctatus* prior to release of the female's pheromone.

detail in collaboration with fluid mechanics specialists. However, for the purposes of this paper, it may suffice to outline the flow pattern at the hand of figure 3 which is based on calculations by D. J. Norton, Department of Aerospace Engineering, Texas A&M University.

With this equipment we made some observations, referred to earlier, on the following phenomenon. In the breeding season, ripe males of the species *Ictalurus punctatus*, the channel or spotted catfish, are strongly attracted by the odor of mature females around which they quickly aggregate. This behavior is well-known to fishermen of the Mississippi who easily catch large numbers of the fish by placing mature females in a cage in the river. Recently we monitored this rather spectacular locomotor response by means of the technique just described.² Following a 12-hr monitoring period of a ripe male in the ex-

perimental tank, water from a container holding a ripe female was admixed with the supply in the middle compartment of one of the channels. Figure 4 represents plots of the locomotor patterns of two males prior to the release of the female pheromone. The left-hand plots correspond to the initial periods of the experiments; those to the right were made 311 and 494 min later, respectively.

Figure 5 illustrates the response of the male to the female odor; the left-hand plots cover periods of, respectively, 18 and 30 min immediately following the admission of the odor into the central compartment of the supply channel. The response is illustrated in greater temporal detail in figure 6. As soon as the fish entered the odor field (which in ex-

² TIMMS, A. M.; AND KLEEREKOPER, H.: The Locomotor Response of Male *Ictalurus punctatus*, the Channel Catfish, to a Pheromone Released by the Ripe Female of the Species. Unpublished, 1970.

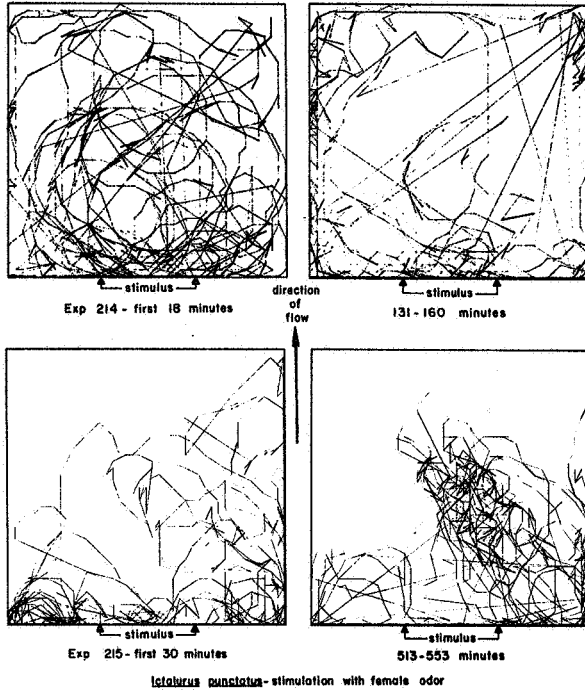
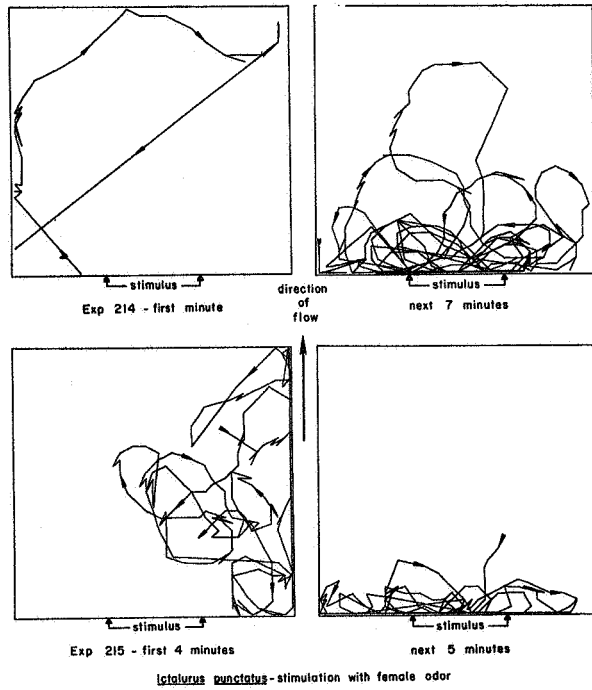


FIGURE 5. Response of a male *Ictalurus* to the female's pheromone.

FIGURE 6. Part of same response as in figure 5 but resolved into shorter periods.



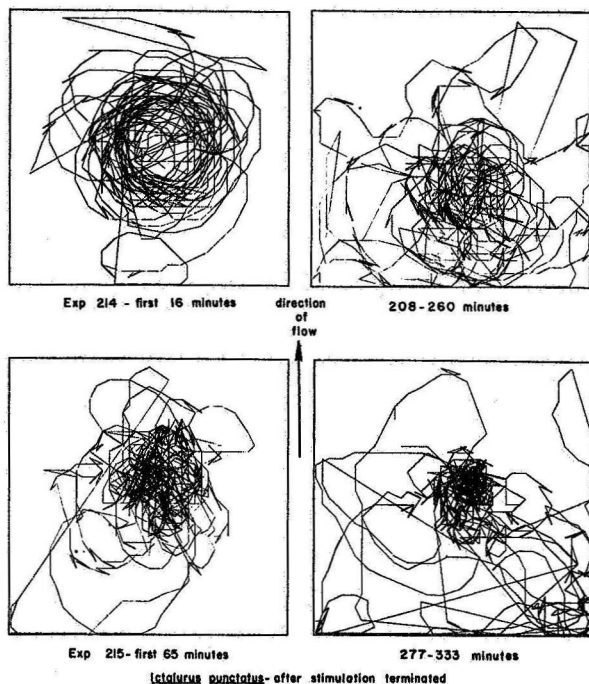


FIGURE 7. After interruption of the odor supply, the male follows the remaining odor patch in the tank.

periments 214 and 215 required 1 and 4 min, respectively), they restricted their movements mostly to the immediate vicinity of that section of the wall through which the stimulus entered the tank, rather than extending their locomotion to the much larger area of the tank affected by the female's odor. The resulting locomotor patterns are those of the right-hand plots of figure 5, which correspond to periods of from 2 to 7, and 5 to 9 min after the start of the chemical stimulation. These patterns consist mostly of forth and back movements parallel to and along the whole length of the section releasing the odor; on the plots this pattern is represented by a single, heavy line drawn by the pen of the plotter as it retraces repeatedly the same trajectory.

The orientation mechanism that most readily accounts for the behavior described is that of tropotaxis by which the animal simul-

taneously compares the intensity of the odor in the two olfactory sacs and turns toward the side of the strongest stimulus. Since the perforated wall does not allow the fish to turn, locomotion remains closely parallel to the wall until the stimulus is lost, that is, at either extremity of the odor-releasing section of the channel. The animal then moves away from the wall, subsequently becomes "trapped" again in the odor field, and the process is repeated.

Strong support for this interpretation is provided by the behavior of the fish following the interruption of the female odor supply (fig. 7). The male immediately leaves the area along the wall and proceeds in continuous, almost perfect circles at some distance away from the wall but within the pathway of odorous water that moves from the central compartment of the supply channel, across the tank, toward the opposite wall of the

tank. As soon as the odor supply is cut off, "normal" instead of odorous water leaves this compartment pushing the mass of odorous water, released previously, ahead of it. As long as the stimulus strength is above threshold for this behavior, the fish remains entrapped in this odor "blot" as it moves across the tank. The perfect circus movement now displayed by the fish is entirely based on chemical cues and is difficult to explain by any mechanism but tropotaxis.

It has been stated repeatedly, but without experimental support, that tropotaxis, particularly chemotropotaxis, could only operate if the paired sensory organs were widely separated. "How widely" is the question that must be answered. In general, the capabilities of sensory organs and their central correlates have been consistently underestimated and the disbelief in a sensitivity that would enable closely placed, olfactory sacs to discriminate odor quality and quantity bilaterally seems to have precluded serious inquiry into tropotaxis as an orientation mechanism. In this connection it is particularly interesting that several authors (refs. 11 to 13) have reported the presence of free nerve endings of the trigeminal system in the olfactory epithelium of fishes. In some other vertebrates these trigeminal fibers have been shown to be odor-sensitive (ref. 14), and it is suspected that, in varying degrees, all odorous substances stimulate the trigeminal as well. Recently Schneider and Schmidt (ref. 15) found that, in man, the source of those odors for which the trigeminal endings are particularly sensitive can be located more accurately than that of odors which do not stimulate the trigeminal or do so to a lesser degree. They attribute the capability of source localization to the trigeminal neural pathway which, in contrast to the olfactory system, has a unilateral central connection so that inputs from left and right nostrils are processed independently in the

central nervous system. Thus differences in time and/or concentration of stimulation between the lateral sense organs may be used for the localization of the source of an odor in a manner analagous to the mechanism of sound localization. A finding of particular interest is the long-lasting response of the animals to the pheromone odor. This is in direct contradiction to the often-stated "fast" adaptation of olfactory systems generally. Adaptation is either non-existent in *Ictalurus punctatus* or is prevented by intermittent exposure of the sensory epithelium to the odor. We do not know this yet, but the prolonged behavioral response exhibited by these fish should caution us against dismissing olfaction as a possible long-range orientation mechanism.

A tropotactic orientation system does not necessarily have to result in circular movements as displayed by *Ictalurus punctatus*. A few years ago, we observed that in *Ginglymostoma cirratum*, the nurse shark, the pathways leading to an odor source very frequently were part of a logarithmic spiral. Similar observations were made and tested mathematically in other species³ (fig. 8). In such animals the two lateral olfactory organs may be part of a target-seeking mechanism similar to that of the theoretical model developed by F. Brouwer⁴ in which the vehicle describes a logarithmic spiral pathway in approach to the target.

It may be mentioned, incidentally, that the circular movement described by *Ictalurus punctatus* is consistently dextrous. Whether or not this is related to the observed prevalence of right-handedness (predominance of right-hand turns) in the normal locomotion of these animals remains to be ascertained.

³ KLEEREKOPER, H.; ANDERSON, V. M.; AND TIMMS, A. M.: Logarithmic Spiral Components in Locomotor Patterns of Fish. Unpublished, 1970.

⁴ BROUWER, F.: Stewart-Darmer Corp., Unpublished.

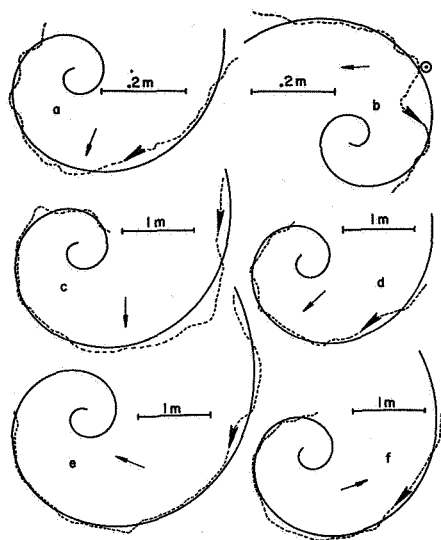


FIGURE 8. Pathways of *Ginglymostoma cirratum* describing logarithmic spirals.

It has been pointed out (ref. 16) that discontinuities in the water mass of a river system would make it difficult, if not impossible, for a fish in upstream spawning migration to be guided by the olfactory perception of spawning ground odors that may locally disappear at such discontinuities. If, however, chemotaxis becomes operative on these occasions, this difficulty should disappear since the fish would be able to orient so as to remain within the odor field which, in turn, would sustain positive rheotaxis. It would seem that these two orientation mechanisms should be entirely sufficient to maintain the migrant on its upstream course.

However, not all observed oriented movements can be explained by the two mechanisms discussed so far. For some time now we have been engaged in an analysis of locomotor responses by 30-cm-long goldfish to non-biological chemical agents such as ions of heavy metals and sodium chloride at very low, sublethal concentrations (maximally 25

$\mu\text{g}/1$ metal ion), using the methods already mentioned with minor modifications. Again all the locomotor parameters within and outside the chemically affected areas of the monitor tank were measured, compared, and the differences tested for statistical significance. A report on this work is forthcoming.⁵ Suffice it to state here that the fish, on entering the water mass containing CuCl_2 (with NaCl of the same molar concentration as the control), modifies only one of the many locomotor characteristics analyzed. There is a significant positive deviation in the headings of the movements in response to the copper ions against the direction of the flow of the water.

DISCUSSION

QUESTION: How do these results compare with those reported in your paper in *Nature*?

KLEEREKOPER: We have made additional observations under different conditions, and the results presented in *Nature* still stand. We have further evidence that in spite of an apparently irregular locomotor pattern, approximate equality in cumulated left- and right-hand turns is maintained. In other words, the animal moves about but seems to be able to keep track of its deviation from the straight line of progression and to compensate for these by turns in the opposite direction. This is neither a random phenomenon nor does it result from the presence of the walls. Angle compensation occurs also in stagnant water and is, therefore, not an effect of water current.

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⁵ KLEEREKOPER, H.; WESTLAKE, G. F.; MATIS, J.; AND GENSLER, P.: An Analysis of the Orientation of Goldfish (*Carassius auratus*) in an Open Field in Response to a Shallow Gradient of a Sublethal Concentration of Copper. Unpublished.

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