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Avoidance Behavior and Swimming Activity of Fish to Detect pH Changes

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Usually, the initial response of an animal to an environmental perturbation is changing its behavior. With fish, this may hold an alteration in swimming activity or reactions like avoidance or attraction. The usefulness of fish behavior to detect the changes in chemical water quality was recognized more than 70 years ago (Shelford and Allee 1913). Since that time, many laboratory studies have been performed on the behavioral reactions of aquatic organisms to pollutants, including those resulting from pH changes (Wells 1915; Jones 1948; Hogland 1961; Bishai 1962).
However, still there is no conclusive evidence that fish behavior offers an adequate tool to detect chemical pollution. In this study, the use of R-value for swimming activity and D^2-value for avoidance behavior of fish as early warning methods to indicate the development of toxic condition is discussed based on experimental data on pH effects.

MATERIALS AND METHODS

As test fish, Japanese fat minnow (Phoxinus lagowski Dybowski) was used. The fish (5 to 7 cm in length, weighing 1.2 to 2.8 g) were collected from a small river in Yamanashi prefecture and acclimated to the laboratory conditions for at least one month prior to testing.
All experiments were carried out under conditions of natural photoperiod in aerated dechlorinated tap water, (average water quality: alkalinity; 32 ppm as $CaCO_3$, hardness; 38 ppm as $CaCO_3$, pH; 7.4, conductivity; 105 x 10^{-6} mho/cm, 20+ 1°C). To avoid disturbances by the experimenter the test system was shielded by a curtain and boards during the test. The experimental system used is of the up-flow shallow gradient type as shown in Figure 1. The system consists of a test chamber and 6 small cells that can receive different pollutant concentrations from a toxicant delivery system. From each cell, the water flows through a flow-controlling plate (1200 openings, ϕ = 2.0 mm) into the test chamber and, subsequently, is discharged from 12 orifices at each side of

the chamber. The total flow rate was 6 L/min (1 L/min/cell), resulting in an up-flow velocity ranging from 0.26 cm/s at the middle of test chamber to 0.66 cm/s directly above the flow controlling bottom plate. The system was operated by adding a test concentration via cell 1, 50% of that concentration via cell 2, whereas the remaining 4 cells were supplied with tap water only. The pH changes were induced by the addition of H_2SO_4 or NaOH, and pH was measured by glass-electrode methods. It was found that a stable concentration gradient was formed within 6 to 8 min after initiating the pH change and disappeared within almost the same period after stopping it.

The activity of fish was determined as the number of light beam interruptions according to Cairns et al. (1970) and counted by a timer counter at 5- or 30-min intervals. Six pairs of photo-electric switches (Omron E3S-1D) were placed just beneath the orifices and the light beams were adjusted to 3.5 cm height from the flow controlling bottom plate. In the experiment, only fish were used showing an average activity of 100-300 counts/h during 3 days. After acclimatization to the test conditions for 3 days and recording the fish swimming activity for another 3 days to obtain the reference values, pH of the water was changed in the middle (1300 to 1400 h) of the next day.
The activity of fish varies with time and shows a circadic rhythm. Furthermore, the activity is relatively high in the central part of the test chamber and low at both ends in the daytime, whereas during the night period the activity is decreased and equally distributed. Therefore, to detect the changes in overall swimming activity, R_k-value was used which is the ratio of the average activity recorded in the 6 zones per time interval (k) during the control period to the total activity in corresponding time intervals during the test period.

$$R_k = \frac{\sum_{i=1}^{6} N_{i,n,k}}{\sum_{i=1}^{6} \sum_{j}^{n-1} N_{i,j,k}/n-j}$$

where

N= number of counts
i= number of cells
j= the day on which counting started
n= the day of pH change
k= time interval

For the detection of the distortion from the normal distribution pattern of fish, the magnitude of deviation ($D^2{}_k$-value) in the activity rate of each zone per time interval (k) was calculated.

$$D^2_k = \sum_{i=1}^{6} \frac{(P_{i,j,k} - E_{i,j\wedge(n-1),k})^2}{E_{i,j\wedge(n-1),k}}$$

$$\text{where:} \quad P_{i,j,k} = \frac{N_{i,j,k}}{\sum_{i=1}^{6} N_{i,j,k}} \times 100$$

$$E_{i,j\wedge(n-1),k} = \sum_{j}^{n-1} P_{i,j,k}/n-j$$

As shown in equation, $P_{i,j,k}$ is the ratio of the amount of activity in the detector of cell i to the total amount of activity in the test chamber per time interval k on the jth day. $E_{i,j\wedge(n-1),k}$ is the average activity rate in the cell i per time interval k in the control period ranging from the jth day to the (n-1)th day. D^2_k-value corresponding time interval k was considered as a response to the changes in pH.

RESULT AND DISCUSSION

The variation in the amount of activity takes place only immediately after the inflow of acidified water because fish avoid it and move to safer water zone. The influence of pH changes on the variation in overall swimming activity (R-value) is shown in Figure 2. After acidification of the water, the R-value generally increased within 30 min and returned to its initial value within about the same period after ceasing the introduction of acidified water. At pH 1, R-value reduced to zero within 30 min because of the death of the fish, whereas no significant deviations in R-value were found at pH level 6 to 9. Decrease to pH 2 to 5 resulted in a fluctuation in R-value ranging from 0.5 to 2.0. It should be noted, however, that such a variation also may occur under non-stressed conditions (Nakamura, in press). Therefore, the R-value in this system cannot be considered as a reliable and sensitive index for detecting unfavorable conditions of water. Only in case all or most of the test animals in the aquarium are killed, R-value (R = 0)may be used as an indication for toxic conditions of the water.

Figure 3 shows the distribution pattern of the fish in the test chamber before, during, and after introducing water of pH 3 into cells 1 and 2. During the pre-exposure period, the highest activity was observed in the center of the test chamber.

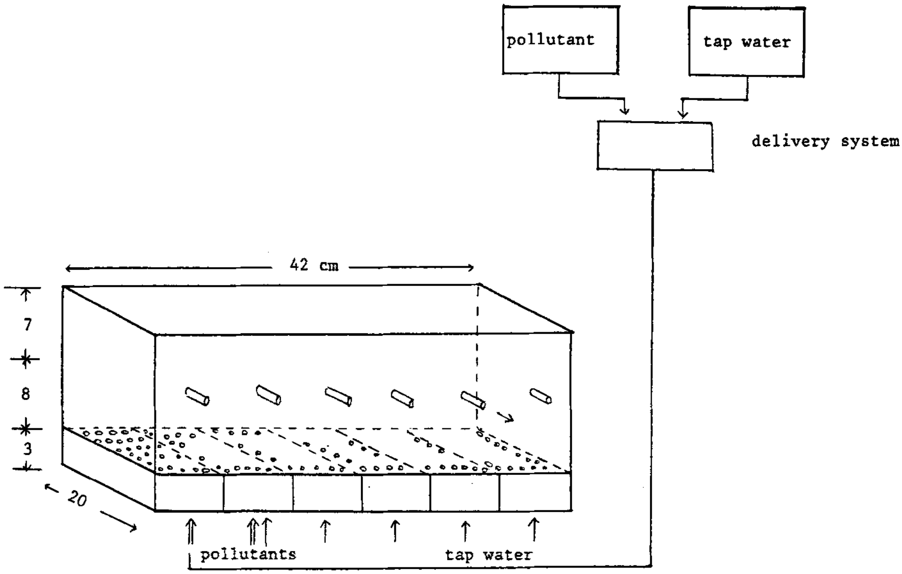

Figure 1. Test aquarium used in this study. Photoelectric switches were placed just beneath the orifices.

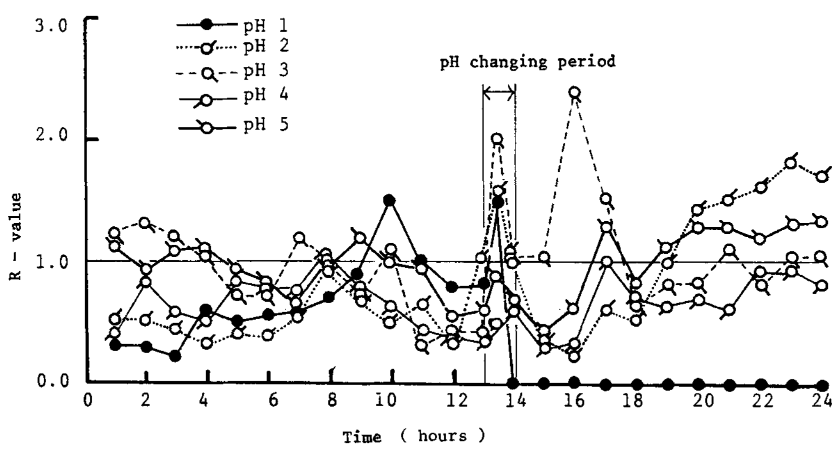

Figure 2. Variation in R-value at 30 min-intervals. Since points and lines become complicated, data other than pH changing period are plotted at 1-h intervals.

Immediately after decreasing the pH level, the fish showed avoidance behavior and their swimming region shifted to the zone of pH 6 to 7. During the exposure period, the amount of activity clearly decreased. In the post-exposure, both the distribution pattern and the amount of activity returned to the original state within 1 to 2 hrs.

Similar results were obtained when water with pH levels of 1, 2 or 4 was introduced. At pH 5, only a slight distortion in

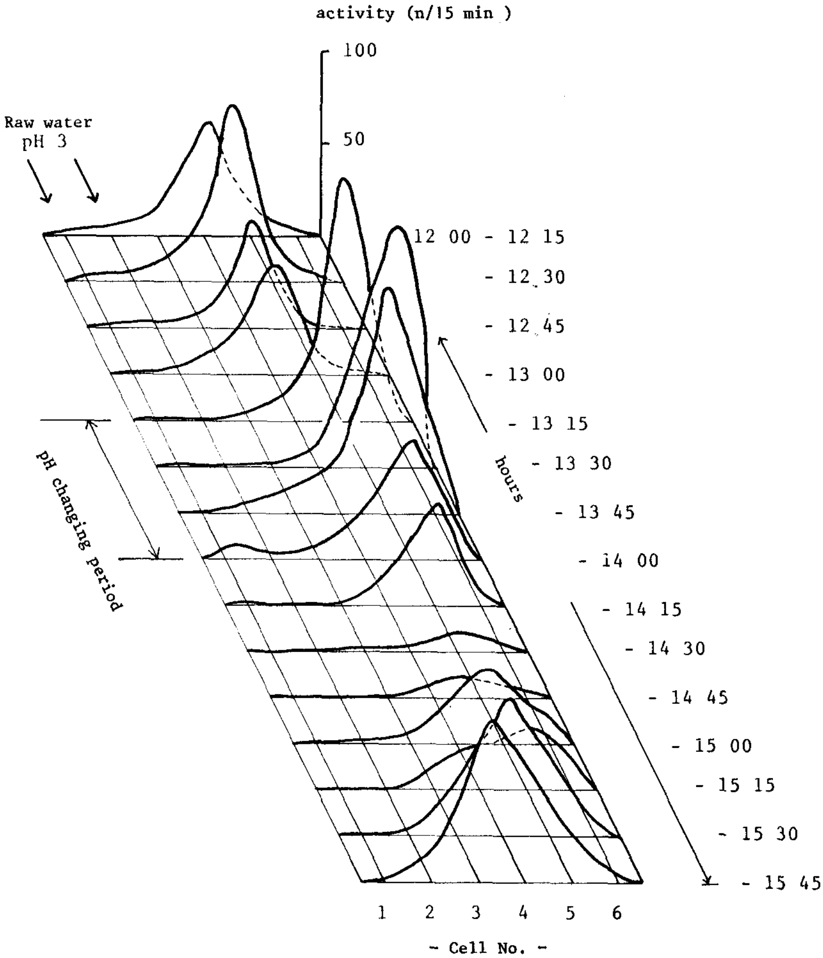

Figure 3. Distortion in the distribution of swimming zone caused by the inflow of acidified water (pH 3).

distribution was observed. No effects were found at pH levels 6 to 10.

In Figure 4, the influence of pH changed on the variation in the distribution pattern (D^2-value) is presented. D^2-values reflect well the distortion in distribution. Under normal conditions, the value $D^2<100$ was obtained. Acidification of the water resulted in an increase in D^2-value, varying from 200 to 1600 depending on the H^+ ion concentration. The extent and the rate of increase was in the order of pH 2, 3 > 4 > 5. Also, in the recovery period, concentration-effect relationship was observed; the lower the pH level, the longer time was required for returning to the normal distribution. Only at pH 1, all test animals died resulting in $P_{i,j,k} = 0$, $D^2 = 100$. No deviation in D^2-value could be found at pH levels 6 to 9. Therefore, detection limit by the use of D^2-value will be in the vicinity of pH 5. This detection limit corresponds to 72 to 96-h TLm value for Japanese fat minnow (Nakamura 1986).

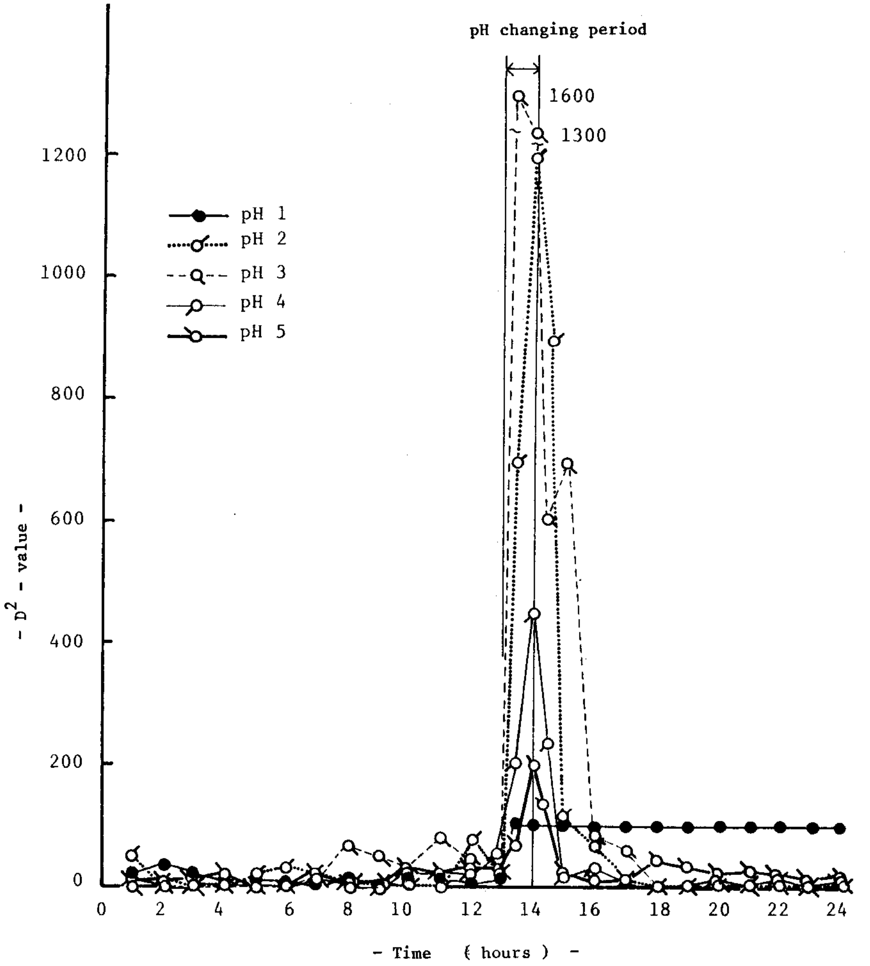

Figure 4. The variation of D^2-value at 30-min intervals.

Johnson and others (1977) found that trouts avoid water zone having pH = 4 to 4.5. Laughlin and others (1978) found that crabs began to show avoidance behavior at pH = 4.6 or less. Ishio (1960) reported that the action of fish to avoid H^+ ion was in accordance with Weber-Fechener's law and 50% avoidance concentration of roaches was $[H^+] = 1.4 \times 10^{-5}$ mol/L. Accordingly, although the differences due to the kind of animals or type of experimental methods have to be taken into consideration, the H^+ ion avoidance concentrations of Japanese fat minnow detected by the variation of D^2-value are in agreement with pH avoidance effect level mentioned in literature.

From these results, it seems that D^2-value will be a good index to detect the avoidance response as well as the preference response.
$D^2 > 150$ to 250 may be used as an indication of avoidance-

preference response though suitable value will depend upon the determination time interval. D^2 = 100 can be used to indicate the death of test animal. Although the degree of toxicity and the avoidance response level are not always correlated (Hara 1983), the avoidance preference test proved to be sensitive in detecting many substances such as agricultural chemicals (Wildish 1977; Hitaka 1984), heavy metals (Sprague 1964), residual chlorine (Cherry 1982). Therefore, D^2-value may be used more effectively as an early warning index if it is used at the same time with other indices such as respiration activity, rheotaxis of fish and so on.

In conclusion, R-value is considered as less effective in detecting the inflow of acidified water. However, R = 0 may be used as an index to suggest the inflow of extremely toxic water. D^2-value is recommended to determine avoidance-preference behavior of fish as a result of unfavorable water conditions. D^2>150 to 250 may be used as an indication of avoidance-preference response, whereas D^2 = 100 may be used to indicate the death of the test animal.

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