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AN INTERACTIVE VIDEO-COMPUTER TRACKING SYSTEM FOR QUANTIFICATION OF LOCOMOTOR BEHAVIOR

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Abstract—We have developed a flexible, moderately priced, behavioral analysis system which has been used to determine the response of salmonids to certain olfactory stimulants. The system, which we call ITS for interactive computer-video tracking system, consists of a 128K Apple IIe computer with software, a video camera and videocassette recorder, and a special-effects generator. Experiments are video taped and then, during playback, the special effects generator is used to simultaneously display the video image and the graphics output of the computer on a monitor. The user tracks the animal of interest using an electronic pen, and the position of that animal in the test chamber, in the form of x-y coordinates, is determined by the computer at user-defined time intervals. When tracking is complete, a plot of the track of the animal is printed within the outline of the test chamber. The following data can also be calculated: swimming velocity, distance from a predetermined point in the chamber (for example, olfactory stimulant source), and time spent in a given area. These variables can be calculated over any chosen time periods and/or for the entire experiment. ITS has numerous advantages over commercially available devices that perform similar tasks. First, it is relatively inexpensive, especially if one already owns video equipment and a computer. Second, it can analyze many types of experiments that can be stored on video tape, including field observations or manipulations. Third, because it is not automated, it is easy to track multiple objects, even if their tracks cross or are not easily located against a low-contrast background. Finally, because whole images do not have to be digitized, and data collection intervals can be adjusted by the user, it is possible to analyze very long experiments with a microcomputer. In this paper we describe ITS and then we demonstrate how we have used it to demonstrate that changes in ambient pH alter the behavioral response of juvenile Atlantic salmon to olfactory stimuli.

Key Words—computers, video, tracking, locomotion, fish behavior, acid precipitation, olfaction.

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INTRODUCTION

One of the most difficult challenges encountered when investigating animal behavior is developing a way to express data quantitatively. In recent years, behavioral analysis of an animal's reaction to environmental or toxicological manipulation has become much more quantitative, due in large part to technological advances in remote monitoring and manipulation of data by computers. Several commercially available systems now enable researchers to track both aquatic and terrestrial animals and to determine behavioral parameters such as distance traveled, time spent in particular areas, and even the number of social interactions (Crawley et al., 1982; Miller et al., 1982; Kaufman, 1983). These modern laboratory tools have enabled investigators to analyze and manipulate behavioral data in increasingly more effective and efficient ways and thus perform experiments that previously would have been too tedious or complex to undertake.

While these systems have proven to be quite useful, they have three major disadvantages for the average investigator. First, they are expensive, costing on the order of $10,000–80,000. Second, the investigator is often separated from the raw data. Many subtle, but important, behavioral responses might not be noticed by the computer. Finally, sophisticated systems are often not flexible enought to allow for widespread applications, such as field work. In the field the areas in which behaviors are being monitored are often not standarized and have varied backgrounds, which would confound most automated tracking systems. What is needed is an inexpensive and flexible, yet sophisticated, technique that allows for close investigator interaction with the data from the initial stage to plotting of the final results.

We have developed a system that uses a standard video camera and video cassette recorder (VCR) to record the movements of an animal, and an Apple IIe computer, with peripherals, to digitize these tracks. We have written software to perform data analysis and statistical calculations, store data in appropriate files, and, if desired, plot the final values. The only unique piece of hardware in the system is a special-effects generator that allows the user to display a video image along with the computer's graphics output on a single monitor. The user can then use the computer-generated targeting crosshairs to track an animal and, thereby, create a digitized record of the animal's movements.

The experimental techniques and methodology described herein have been applied to an investigation of the effect of pH on the olfactory-related behavior of fish. However, this system can be, and has been, used for a wide variety of experiments. A number of different types of quantitative analyses can be performed with it if one simply modifies the controlling software.

METHODS AND MATERIALS

The interactive tracking system (ITS) consists of three parts: (1) recording components; (2) a video–computer interface and digitizer; and (3) integrated data analysis software.

The recording module consists of the behavioral chamber, video recording components, and video integration hardware. The behavioral chamber we used was the Y-maze shown in Figure 1 (Greer and Kasolsoski, 1978; Royce-Malmgren, 1985). The video recording equipment included a color video camera and video recorder (Figure 1). The video integrating hardware enabled us to overlay, on the recording of the fishes' behavior, data pertinent to the experiment, which in this case included pH, temperature, date, and time (Figure 1). The video recording components of the ITS are all portable, enabling data collection in the field and analysis in the lab.

The computer components of the ITS consist of an Apple IIe 128K computer equipped with an Apple digitizer tablet, dual disc drives, internal clock, *x*/*y* plotter, and dot matrix printer (Figure 2A). Coupled to these is the video integration hardware that allows for simultaneous projection of the output of the video recorder and computer graphics onto the display monitor (Figure 2B,C). The user tracks one animal at a time using the targeting crosshairs controlled by the electronic pen of the graphics tablet. The computer samples the *x*–*y* coordinates at user-determined time intervals and writes these to data files on disc for later analysis.

The software consists of 25 interactive programs, all under the control of a master menu program. The master menu program leads the user through the tracking, data analysis, and plotting programs using a question and answer format. A complete analysis of a 2-hr experiment involving 720 *x*–*y* coordinate locations (one every 10 sec) takes approximately 30 min.

Components

Video Equipment. The color camera we used was a moderately low light model with high sensitivity and resolution (Hitachi model 8A). It has a 12 : 1, 50- to 200-mm telephoto zoom lens with macro capabilities and a minimum focal range of 8.4 mm. The video recorder was a Cannon VR-20. It uses a four-head system with separate recording and playback circuits for low-distortion recording and playback of high-resolution images at variable speeds. A high-resolution Sanyo monitor was used during the experimental recording phase. During the playback and subsequent data digitization phase, we used either an Amdek model I, 13-inch color monitor or a 25-inch high-resolution RCA RGB monitor (model 2526). Recording speed was super long play (SLP) for 6 hr of data collection per video tape (Scotch Color Plus T-120).

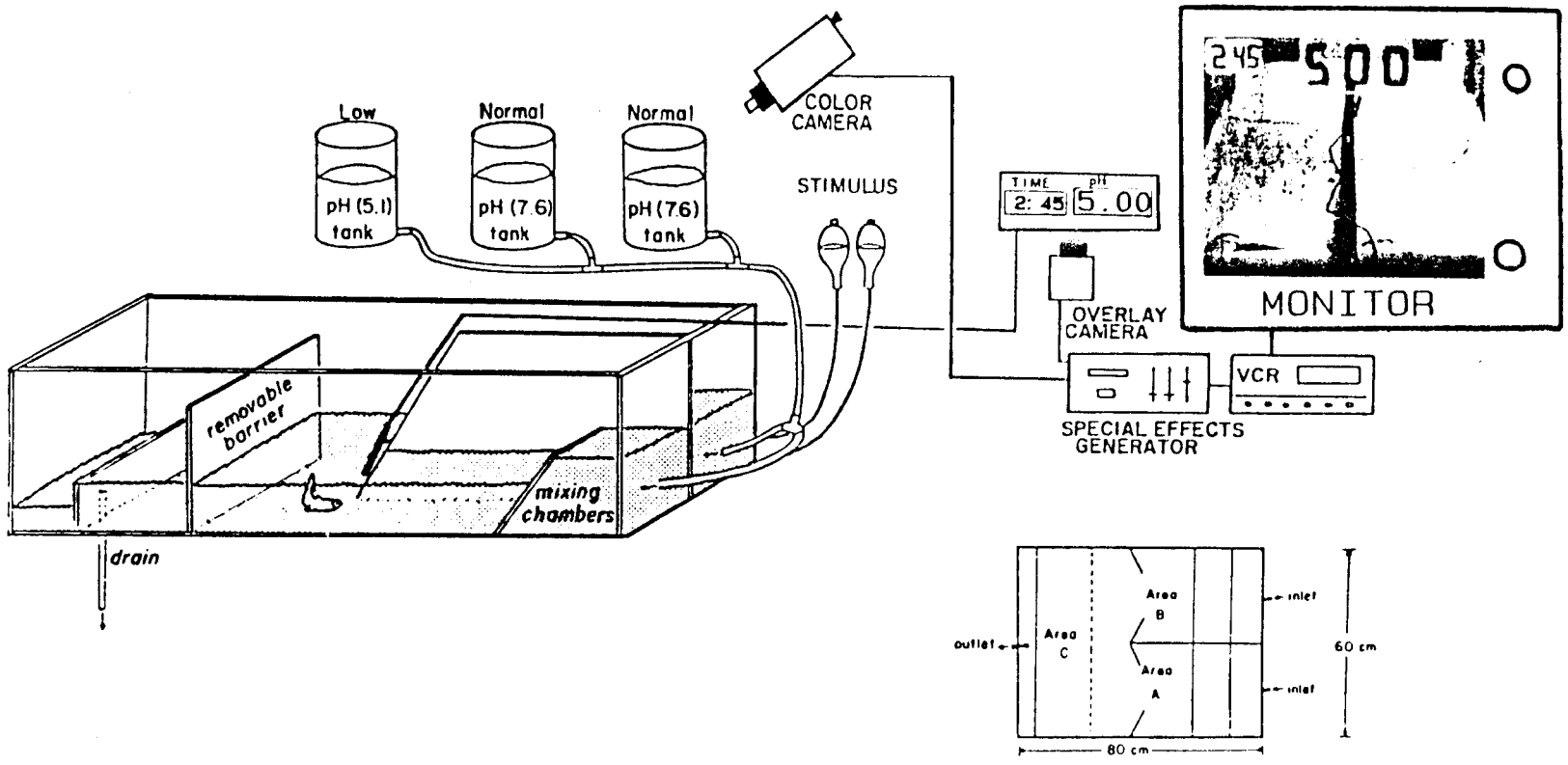
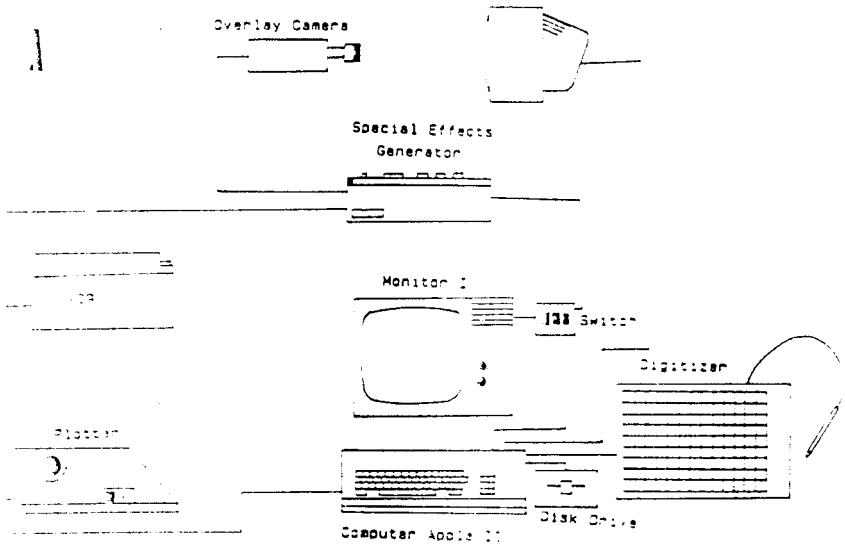
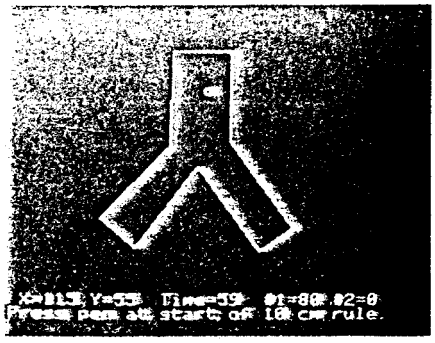


FIG. 1. Test chamber. Fish are introduced into area C behind the removable barrier. Dechlorinated, aerated tap water from overhead storage tanks flows by gravity through the chamber at a rate of 3 liters/min. Following an acclimation period, the removable screen is lifted to start the experiment. During control experiments, no olfactory stimulus is added. During the test experiments, stimulus is added to area A or B from the stimulus reservoirs. The concentration of stimulus falls off as the water from area A and B mix in area C. This creates a concentration gradient from the stimulus input to the opposite side of the tank. This gradient has been confirmed with dyes with fish present. A color video camera mounted above the chamber is used to record the experiment. The video image of the test chamber and a video display of the pH, temperature, and time throughout the experiment is combined using the special-effects generator, recorded by the VCR, and displayed on the monitor. The photograph on the monitor is a view of the experimental chamber from an actual experiment, showing the digitized display of pH and time overlayed on the video image. Two fish are visible in the lower left corner of the test chamber in area A and another in the lower left corner of the right channel in area

The wire in the middle of the chamber comes from the pH elect





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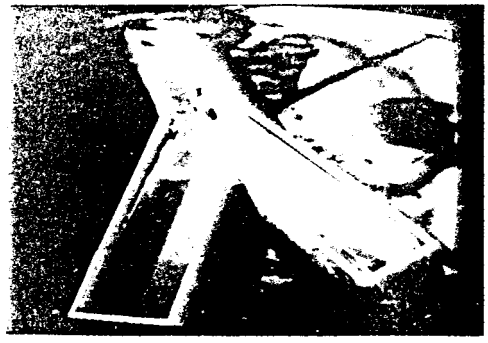

C

FIG. 2. Interactive computer-video tracking system (ITS). (A) Schematic showing equipment for data analysis. Experiments are video taped, and this record along with the graphics output of the computer is combined for analysis using a special-effects generator to create a composite video of the two signals. (B) Computer generated graphics output consisting of flashing cursor and a test chamber outline. The text at the bottom includes x-y coordinates, time in seconds, and additional key system levels. The user is being told to place the pen at the start of the 10-cm calibration rule and press the button on the tablet. (C) Photo of an actual screen during analysis. Computer generated flashing cursor (presently over a fish near the top of the chamber) is used to track individual fish, and x-y coordinates from the digitizer table are sampled at user determined time intervals, and then transferred to the data files on the disc.

Our video equipment is more sophisticated than necessary for most purposes. A simple black and white camera, VCR, and monitor can be purchased for as little as $700. It is likely, however, that most investigators will have suitable video components available to them, which can be adapted for use with this ITS. This eliminates the need to purchase any new video equipment, other than the special-effects generator.

The most unique piece of hardware in the ITS is the Ambico special effects generator that comes equipped with a special monochrome camera sensitive to the red/orange range of the visual spectrum. This unit costs approximately $700. It allows us to perform two important procedures: (1) overlay output of instruments such as a pH meter, clock, and thermometer on the original video signal, and (2) combine the graphics output of the computer (Figure 2B) with the prerecorded video images of the experiment to yield a composite image for data analysis (Figure 2C).

Computer and Associated Equipment. An Apple IIe with 128K memory was utilized for track digitization, data analysis, and presentation of results. Two disc drives were used, one for the controlling software, and the other for storage of data and/or results. A RAM (random access memory) disc was utilized to store temporary work files for rapid access. Two output devices were used, a Panasonic dot-matrix printer and a Hewlett-Packard x–y plotter. An Apple digitizer tablet ($800) with electronic pen was used to control the on-screen targeting crosshairs. The computer determined the x–y coordinates at user-defined time intervals as determined by an onboard clock. The precision of the digitizer tablet was much greater than needed and a Koala pad ($125) or a light pen ($150–300) was used when the high precision of the tablet was not required.

Software. All controlling programs for data acquisition and analysis were written by one of the authors (C.R.M.). The integrated data acquisition consists of three discrete programs, all controlled from a master menu program (Figure 3). These include: (1) the experiment protocol program, (2) the chamber outline and distance calibration program, and (3) the track digitization program.

The experiment protocol program records information such as data; technicians initials; number, species, and life stage of animals involved; type of experiment; physical parameters (including water temperature, pH, water hardness, and calcium concentration); video tape code name and number; tape counter at the beginning and end of the experiment; and other pertinent information (Figure 3B). Analysis information for track digitization, such as video playback speed, sample interval, experimental length, and time periods to be analyzed are then recorded. These data are stored in two files, an INFO/ on data disc and PRMTRS file on RAM disc.

The master menu program then passes the user to the chamber outline and distance calibration program (Figure 3C). The user is asked to use the electronic pen to outline the test chamber, using the video-overlay system and to designate

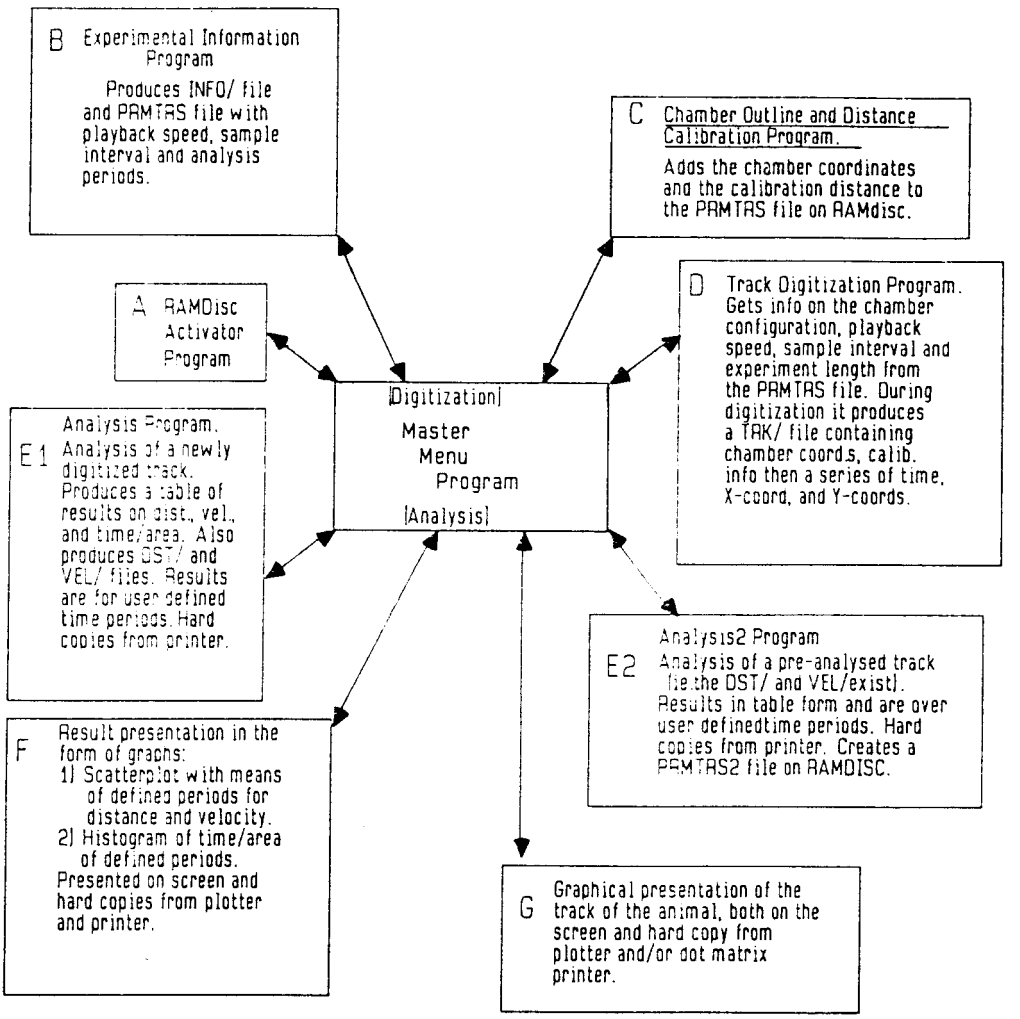

FIG. 3. ITS software block diagram. (A) The RAMdisc Activator Program first determines the availability of memory and correct system configurations. If adequate, it installs a 64K electronic disc drive (RAMdisc). (B) The Experimental Protocol Program documents the experimental and analysis parameters, then saves these to both RAMdisc and data disc. (C) Using the video overlay system, the chamber outline and calibration distance is recorded and stored on RAMdisc. (D) The Track Digitization Program, using information stored in the PRMTRS file on RAMdisc, will perform the actual track digitization with the user controlling the electronic pen. At the end of tracking, the TRK/ file is written to RAMdisc and data disc. (E1) The Analysis Program uses the TRK/ file on RAMdisc and the analysis parameters in the PRMTRS file to analyse the track. (E2) The Analysis2 Program can reanalyze a dignitzed track over new (or different) time periods. (F) The results determined in E1 or E2 can be presented graphically using the Results Plotting Program. (G) The Track Display Program can show all or any part of a digitized track on the monitor screen and hard copies can be produced on either the printer or plotter.

the ends of a 10-cm calibration rule placed in the test chamber. This information is added to the PRMTRS file on RAMdisc.

The user is then passed to the track digitization program by the master menu program (Figure 3D). Using information from the PRMTRS file, the tracking program outlines the test chamber and supplies targeting crosshairs overlayed on the video image of the experiment. The user starts the video tape and collects data simply by keeping the targeting crosshairs over the image of the fish for the duration of the experiment. Time and x–y coordinates are displayed at the bottom of the screen during data acquisition and stored in RAM (not on RAMdisc). At the end of track digitization, the user enters the stimulus source(s) position(s). The calibaration distance and the stimulus source(s) location(s), along with the time and x–y coordinates, are now written to a TRK/ file on both data disc and RAMdisc.

There are two types of analysis programs. The first (Figure 3E1) is for a newly digitized track that will create distance (DST/) and velocity (VEL/) files. The second, the reanalysis program (Figure 3E2), analyses a track for which the DST/ and VEL/ files already exist. Following analysis (either program), tabular results are displayed on the screen.

Above the table is a summary of the analysis and tracking parameters, including (1) the code name of the track analyzed and files produced and/or read, and (2) the tracking information, such as VCR playback speed, sample interval, and experiment length. A sample of such a table is shown in Figure 7. Following analysis, the data from the table can be graphically presented (Figure 7B–E). Distance and velocity are presented as a scatterplot of all data with means (± SEM) of each period analyzed (Figure 7D,E). Time per area data appear as a histogram with separate clusters for each analysis period (Figure 7C). Finally, the actual track of the fish can be plotted over any time period(s) (Figure 7B). Hard copies of all tables, graphs, and tracks can be produced on the dot-matrix printer, and the graphs and tracks can be produced on the x–y plotter.

Analysis of Sample Populations and Statistics. Data from individual animals can be combined to form a sample population of animals responding to a given situation. For example, in our experiments, data recorded for individual fish identified by color-coded tags was stored in data files on floppy discs. Distance and velocity files for individual fish which had undergone similar experimental procedures were then combined, and composite files were created which contained the mean and variance at each sample interval. These represent the response of the sample population to the stimulus. Analysis of the results included a chi-square test to determine homogeneity of variance of replicate means. Two-way ANOVAs were used to determine if activity levels varied between treatments for individual fish.

The pH throughout each experiment was determnined from the video tape

record and transcribed into pH data files for graphic presentation along with the behavioral data (see Figure 4, bottom).

Sample Experiment

To illustrate how the system functions, a sample experiment will be outlined from its beginning to the final data presentation. Our objective is to determine the effect of pH on the response of juvenile salmonids to olfactory stimuli.

Protocol.

1. The test chamber receives water (3 liters/min) from overhead reservoirs (Figure 1), and an olfactory stimulus, in this case L-glutamine, is added to one of the inputs (chosen randomly).

2. Four fish are placed in the common area behind a barrier for a 15-min acclimation period.

3. The video camera is placed overlooking the test chamber and the pH electrode, automatic temperature compensation (ATC) probe, and pH meter are all calibrated with the probe and electrode in place at the center of the chamber. Output of the pH meter, including calibration and temperature, are overlayed on the video record of the experiment (Figure 1).

4. The experiment commences with the removal of the barrier (time = 0 sec).

5. A pH of 7.6 is maintained in the chamber for 30 min.

6. At 1800 sec (30 min), a pH change is initiated using a mixture of 50% H_2SO_4 and 50% HNO_3 (both 0.5 M). It takes 15 min to change from pH 7.6 to pH 5.1. A pH of 5.1 is held for the second 30 min.

7. At 4500 sec (75 min), the second pH change is initiated, returning the pH from 5.1 back to the original level of 7.6, over a 15-min period. A pH of 7.6 is maintained for the final 30 min.

All fish are identified with color-coded tags. At the beginning of the video tape, the experiment is given a code number, which is all the analysis technician will know about the experiment (for double-blind analysis). Pertinent data, such as stimulus type, stimulus concentration, and stimulus source location are recorded verbally at the end of the tape and in a separate notebook containing the key to the experiment's code number.

Track Digitization and Analysis of Data. Following entry of experimental information and analysis parameters, a series of calibrations begins. The first is the chamber outline procedure. The user is required to press the digitizer pen down at the corner boundaries of the chamber whose outline is then drawn on the screen and the user is asked if it is correct. If so, the distance calibration procedure follows. In this, the user is required to press the pen down at opposite ends of a 10-cm rule present in the test chamber. Again, the user is asked if

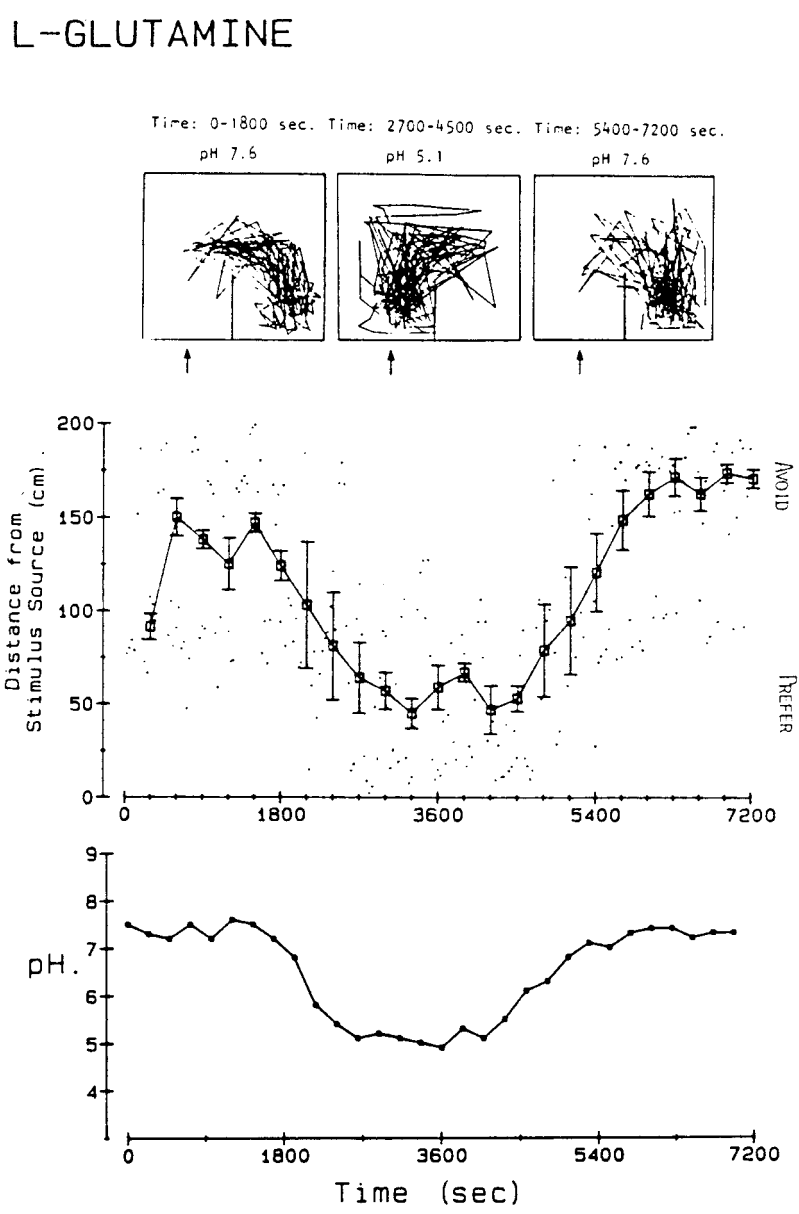

FIG. 4 Modulation of the behavioral response of twenty salmon to L-glutamine by changes in pH. Each animal was tracked for the entire experiment and mean distances (cm) from the stimulus source was calculated (see text). Top: The track of a fish during three different time periods. The arrows indicate the area receiving the L-glutamine (10^{-4} M). At pH 7.6 the fish spent most of its time away from the source of L-glutamine, but as the pH was lowered (see bottom plot), the fish's response changed to an attraction to L-glutamine. The effect was reversible with a return to pH 7.6. Middle: The mean distance from the stimulus source was determined every 300 sec for the twenty fish (squares, ± SEM). The dots represent means determined over every 30 sec, although sampled at 10-sec intervals. The closer the fish are to the stimulus, the more they prefer it. As the pH changes from pH 7.6 to pH 5.1, the avoidance changes to a neutral reaction and finally to an preference. Bottom: Record of the pH during the course of the experiment. Data was read off the video tape and entered into the computer by hand.

this is correct. All this information is placed at the beginning of a digitized track data file that will eventually contain the time, x coordinates, and y coordinates of the animal. Following this, the user begins the playback of the experimental video tape and simply tracks the animal using the digitizer pen to keep the targeting crosshairs on the image of the fish (Figure 2C). The track digitizing program accesses an onboard digital clock for time information and the digitizer tablet for the x–y coordinates of the targeting crosshairs. This information is written to RAM. The digitizer table continuously provides the computer with x and y coordinates during the tracking, but only x and y coordinates at user-defined sample intervals are recorded. This conserves memory and allows considerable flexibility in terms of track resolution. Following track digitization, the stimulus parameters are requested from the user. These include stimulus source(s) location(s), stimulus type and concentration. The user is then given the option of digitizing another track immediately or analyzing the one in memory. If the choice is the latter, the computer confirms the user's choice of time periods to be analyzed and then asks the user to wait while the analysis is conducted. Depending on the length of the experiment and the sampling rate, the analysis can take from less than 1 min for a short 30-min experiment with a data sampling rate of once every 20 sec, to 35 min for long experiments (2–5 hr) with high sampling rates (more than one sample per second). At the end of data analysis, a table summarizing the results appears on the screen. The user can then plot the results graphically. Hard copies of all or part of the data with graph can be produced by the printer and/or plotter (Figures 4–7). Following this, the track of the animal over user-specified time periods is drawn on the screen, with hard copies available (Figure 7).

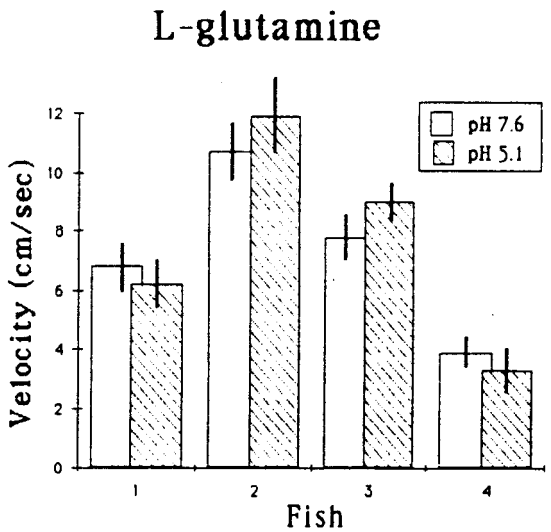

FIG. 5 Mean velocities (± SEM) of four individual fish at different pH levels with L-glutamine (10^{-4} M) present. The velocities were determined by sampling data at 10-sec intervals over 30-min periods of stable pH and then averaging these data.

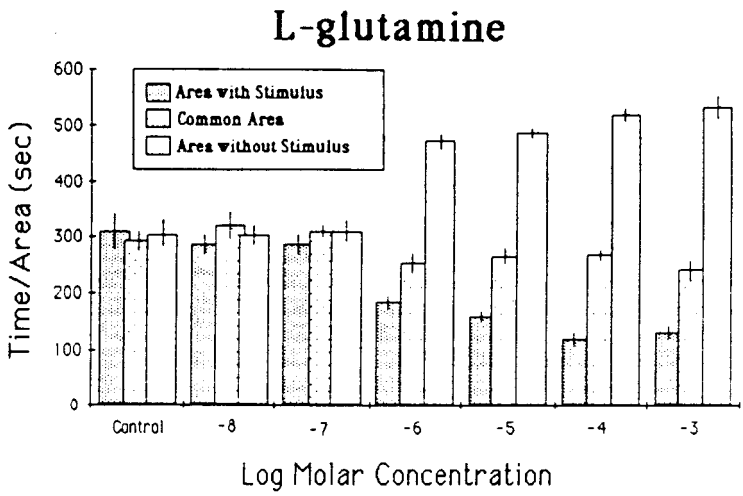

FIG. 6. Mean time (± SEM) spent per area at different concentrations of L-glutamine (N = 8 fish/concentration). The control periods did not have any stimulus present. Clearly, the avoidance of Atlantic salmon parr to L-glutamine is dose-dependent.

RESULTS

The ITS described in this manuscript was used to demonstrate that changes in acidity of water in our test chamber modified the behavioral response of juvenile Atlantic salmon (*Salmo salar*) to olfactory stimuli. We tracked each animal, in each experiment, and calculated its mean velocity, distance from the stimulus source, and time per area, every 10 sec. The data from 20 animals was compiled (15,000 data points), statistical determinations were performed by the computer, and the results plotted (Figure 4). The distance of a fish from the stimulus and time per area were used as an indication of avoidance or attraction to a given substance.

When L-glutamine (10^{-4} M) was perfused into one area of the test chamber (see Figure 1), fish spent most of their time in the area of the chamber farthest away from the stimulus (Figure 4, top), indicating an avoidance response at pH 7.6. This can be illustrated either by plotting the track of an individual fish (Figure 4, top), or by averaging the distance from the stimulus source over every 30 sec for all 20 fish (Figure 4, middle plot). The tracks at the top of Figure 4 also show that it is possible to view any portion of the experiment desired. The middle plot demonstrates that one can plot all, or part, of the data. The plot of the pH at the bottom of Figure 4 was obtained using the pH data as recorded on the original video tape of the experiment.

It is also possible to perform a number of other calculations with our software. For instance, we have determined the velocity of the animals over specific periods during control experiments (Table 1) and stimulus experiments (Figure 5). These data indicate that the acidic pH levels in these experiments do not

A. Data Table

Track file: TRK/TAC-4 GRL1
Number of periods examined: 2
# of entries in data file: 180

Viewing speed: 10X
Experiment duration: 1800 sec
Distance file: DST/TAC-4 GRL1

Period	Start time	End time	Mean Dist(cm)	SD	Mean Vel(cm/sec)	SD	Time (sec)/area A*	B	C
1	0	900	282.	61.5	1.64	.561	320	240	340
2	910	1800	180.	53.5	1.10	.155	650	130	120
Experimental totals:			231.	125.	1.37	1.26	970	370	460

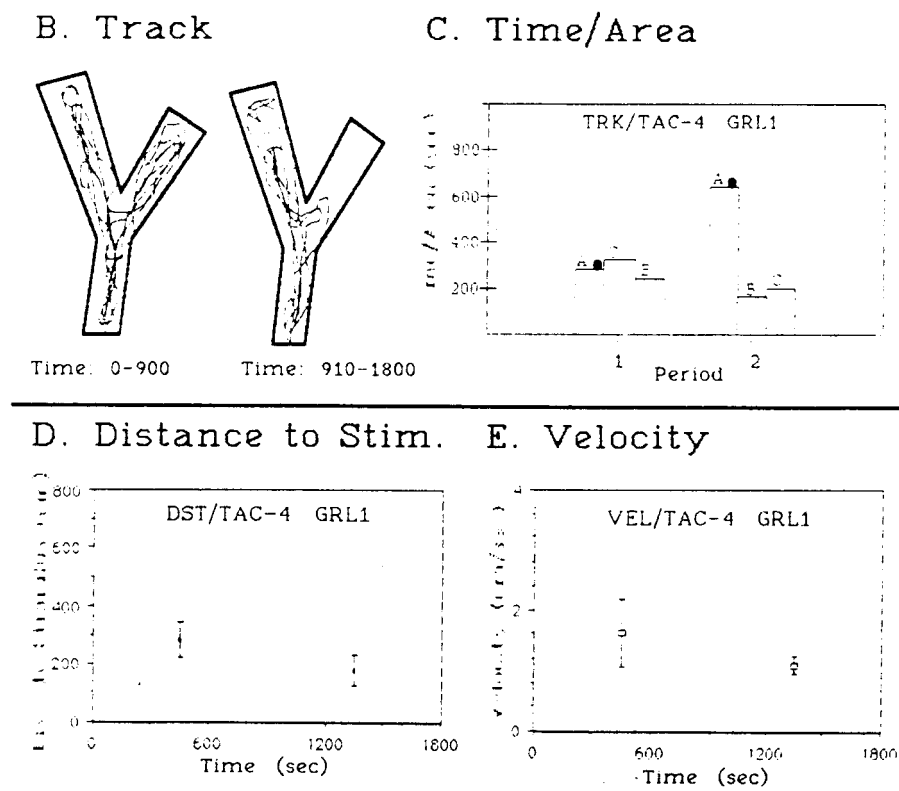

FIG. 7. Hard copy of results generated on a dot-matrix printer. An adult Atlantic salmon was tracked in a Y-maze during a two-part experiment: first a 15-min control period (no stimuli), followed by a 15-min experimental period with taurocholic acid (10^{-4} M) added to area A. (A) Results data table includes analysis parameters and pertinent file names and summary of results arranged by time periods. (B) Track of the fish over the two periods of the experiment (control, 0–900 sec; experimental, 910–1800 sec). Clearly the random movement of the fish during the control period changed to preference of the area containing taurocholic acid during the experimental period. (C) Histogram of time per area for the two time periods indicating a preference for the area with the stimulus only when the stimulus was present. (D) Distance scatterplot including mean (± SEM) of the two periods, again illustrating the preference for the area with stimuli only when it was present. (E) Scatterplot of velocity, including mean (± SEM) of the two periods indicating a fairly constant level of activity between the control and experimental periods.

TABLE 1. MEAN VELOCITY (cm/sec) OF FISH AT DIFFERENT pH LEVELS DURING SINGLE 2-HOUR CONTROL EXPERIMENT[a]

	Fish			
pH	1	2	3	4
7.6	9.14 ± 0.78[b]	7.23 ± 0.70	6.70 ± 0.69	10.37 ± 0.87
5.1	8.59 ± 0.90	6.93 ± 0.66	5.81 ± 0.76	10.75 ± 0.65
7.6	8.31 ± 0.79	6.38 ± 0.66	5.71 ± 0.65	9.45 ± 0.79

[a] These were control experiments in which there was no stimulus present. Means were obtained from data sampled at 10-sec intervals (see text for experimental protocol).
[b] Mean ± standard error mean.

effect the general activity of the animal. In Figure 4 (top), the tracks of the fish show that activity levels stay fairly constant between pH levels. Statistically (ANOVA), there is a significant ($P \leq 0.05$) difference in velocities among different fish, while there is not a significant ($P \geq 0.1$) difference in the velocity of the same fish at different pH levels. We have plotted a dose–response curve illustrating that the response of these salmon to a particular odor is dose-dependent (Figure 6). It is also possible to a do vector analysis over any given time period to determine an animal's orientation and/or directionality. These calculations illustrate that once the digitized tracks of all the animals are stored in appropriate data files, one can rapidly manipulate the data in a variety of different ways, depending on need.

DISCUSSION

The behavioral modification experiments described here, both in the laboratory and in the field, shown the high potential of ITS for quantitative analysis of behavior. There are similar systems on the market which track animals automatically. However, we chose not to fully automate the system for five reasons. First, we did not want to miss subtle, yet important, information that may not be recognized by a fully automated system. Second, it is not necessary to have large differences in contrast between object to be tracked and the background. Often, especially in the field, conditions were not suitable for a system that automatically tracks dark objects against a light background or vice versa. Third, although one has to repeat the analysis for each animal if there is a group of animals present, there is no problem with paths of the animals crossing. Fourth, we wanted sufficient flexibility to be able to analyze any suitable videotaped behavioral experiment. Many researchers are turning to video as a means of recording behavioral experiments. Our system (ITS) can be used to

analyze any appropriate experiments that can be recorded on video tape. Thus, a large number of researchers with varied interests can simply video tape their experiment and bring the video tape to the video–computer station in the laboratory for analysis. Finally, this arrangement allows ITS to be used for analyzing field data. We have already used it successfully to study the role of pheromones in horseshoe crab (*Limulus polyphemus*) mating behavior and the response of adult Atlantic salmon to olfactory stimuli (Figure 7).

The ITS system allows us to store data from six complete 2-hr experiments, sampled at 1-sec intervals, on a single disc, whereas other video digitizers can only store 3 min of video picture frames per disc (Kaufmann, 1983). This is because a single video frame can contain up to 52,000 bits of data and at the normal rate of 60 video frames per second, 3 min of video generates 936,000 bits of data that, when stored, will fill a data disc. While these systems need all this information in order to track a given subject automatically, our system saves memory by having a technician track the animal. The memory thus saved can then be used to store the data from a number of complete experiments in less space.

The analysis program(s) in our system can handle large amounts of data at a moderately high speed and make statistical determinations which previously would have required many man hours. This short turn-around time is beneficial because, after viewing results from one run, the investigator could rapidly modify the experimental protocol to more effectively investigate the behavior.

While there are several comparable systems available commercially or described in the literature, they tend to be much more expensive. The system we have described would cost approximately $5000 for the total assembly. However, most modern laboratories are already equipped with a microcomputer and video equipment. Therefore, the only item required would be the special-effects generator ($700) and the software. Further development of data analysis software should give rise to a wide application package that may lead to routine quantification and standardization of sensitive behavioral bioassays in the environmental monitoring field.

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