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A Delaunay Triangle Network Based Model of Fish Shoaling Behavior for Water Quality Monitor

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Abstract

Fish actively form shoals, which is a behavior highly sensitive to experimental changes in environmental conditions. Here we evaluated the potential for using the shoaling behavior of red crucian carp as an early-warning biosensor system for assessing water quality. To reliably characterize shoaling behavior, we propose a novel method for determining the size of the shoal based on a Delaunay triangle network. We examined the effect of group size (two to 10 fish) in the shoaling paradigm and evaluated whether measurements of shoaling behavior could be used to assess water quality using test fish exposed to chemicals. The test chemicals were sodium hydroxide (NaOH), hydrochloric acid (HCl) and glyphosate, which are commonly used in agriculture or industry. There was a significant effect of group size on the shoaling behavior of unexposed fish. Furthermore, NaOH (20 mg/L), HCl (20 mg/L) and glyphosate at three concentrations (0.1 mg/L, 0.05 mg/L and 0.025 mg /L) significantly decreased shoaling behavior relative to controls. The average alarm time in response to a change in water quality was about 21 min. We conclude that the shoaling behavior of red crucian carp is a useful tool for monitoring water quality.

Keywords: Fish behavior; Shoaling behavior; Delaunay triangle network

Introduction

The increasing frequency of accidental spills into the aquatic environment has encouraged interest in real-time systems for assessment of water quality. Although a number of biochemical monitoring methods are available to evaluate water quality [1,2], they do not provide instantaneous warnings because they require a sequence of laboratory tests. Behavioral monitoring is viewed as a valuable low-cost biological warning system that can be rapidly applied in a variety of environmental conditions. However, it is difficult to quantitatively evaluate complex behavioral responses of test species to toxicants and to develop simple and reliable methods for assessing real-time risk on the basis of behavioral data.

To avoid some of the difficulties associated with complex and flexible behavioral patterns, investigators often use fish in behavioral assays following toxic chemical exposure because exposure-related behavioral alterations of fish can be considered as fixed action patterns [3,4]. Fish behaviors may be characterized on the basis of parameters derived from movement tracking, e.g., speed, acceleration, temporal trajectory, tail beat frequency and stop duration [5-9]. However, most of the existing monitoring systems rely on individual test fish [9,10-12]. This could reduce the robustness of the warning system, because individual differences among fish increase the uncertainty of the assay. Therefore, the behavioral characteristic of the responses of a group of fish may be more suitable for detection of toxins in the water [13].

Many species of fish tend to come together in shoals or schools (Figure 1a). A fish shoal can be identified as spatial aggregation of fish loosely attracted to the group but moving independently of each other with no mutual attraction between individuals. Shoaling is a complex social behavior used by fish to increase individual fitness, e.g., in foraging for food or avoiding prey [14-16]. Typically, a distance of between 0.6 and 2 body lengths from their nearest neighbors is maintained [17], although the size and speed of the shoal [18], the age of the fish [19], and the spacing within the shoal vary with species [20]. A change in water composition may be indicated by avoidance of an area by the shoal (Figure 1b); therefore, a change in the distribution of fish in a shoal could provide evidence of a change in water quality. Thus, the shoaling behaviors of fish might be suitable behavioral endpoints in sublethal toxicity assays and might serve as tools for environmental risk assessment and analysis of toxicological impacts.

This study proposes a simple method for detecting toxins in the water based on changes in the shoaling behavior of red crucian carp. Red crucian carp are widely distributed throughout China and show moderate swimming performance [21,22]. Here we conducted a series of exposure tests to investigate inhibition of shoaling behavior of fish in response to the toxins NaOH, HCl and glyphosate. Behavior was quantified by measuring the shape of the shoal obtained using the Delaunay triangle (DT) network, and from the number that escaped from the shoal. The paper concludes by offering a novel measure capable of characterizing shoaling behavior of fish. The results are sufficiently promising to support further research into this area.

Materials and Methods

Test subjects

Red crucian carp (*Carassius auratus*) 5-7 cm in length were used in our experiments. Red crucian carp, belonging to the family Cyprinidae (Telestei), is widely distributed on the Eurasian continent (Chen and Huang 1982). Red crucian carp were selected because they provided better contrast between the fish and the background for image processing. A total of 600 adult red crucian carp were obtained from

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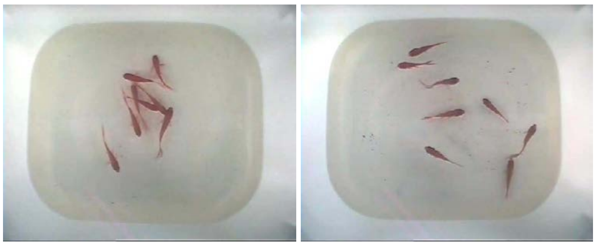

Figure 1: A group of red crucian carp swimming in the tank. (a) Control conditions; (b) Treated with 0.25 g/L NaOH.

a pet supplier in Hang Zhou, China and allowed to acclimate to the laboratory environment for 1 week. They were maintained in groups of 40-50 in 60-L aquaria containing filtered dechlorinated fresh water at room temperature (25°C ± 5°C). Crucian carp are cold-water fish and were not sensitive to this variation in temperature. Fish were fed with one artificial tropical-fish meal in 3 days. All experimental procedures were approved by the Zhejiang University of Technology Institutional Animal Care and Use Committee.

Experimental apparatus

The experimental platform consisted of a computer, a CMOS camera (Imavision MER-200-20UC(-L); Daheng Science and Technology, Beijing, China; 15 frames/s), an experimental tank, two 20 L water channels and two water pumps for switching between water circulation systems (Figure 2). The experimental tank was divided into three chambers by two glass baffles. A number of small uniform holes in the lower half of the inflow baffle allowed gentle water flow, eliminating turbulent flow from the pump, which had a strong influence on the swimming behavior of fish. Fish could swim normally in the middle chamber (nearly 6 L of water; 31 cm × 24 cm × 8 cm high). To record all fish in the experimental tank, we set the camera 45 cm above the water surface of the tank. An overflow outlet of adjustable height was situated in the side of the outflow section of the tank. The water was pumped into the inflow chamber by an 11-23W pump (flow rate; 2.5 L/min) and flowed through to the outflow chamber. Water circulation system could be switched between control and exposed conditions by valves in the inflow pipe, allowing the toxicant to be infused into the water flow of the tank as needed. Water circulation system included two tanks that contained unexposed water and the test solution, respectively. The water temperature was kept at 22°C ± 5°C. Illumination (12:12 h light:dark) was controlled automatically to prevent external influences to fish behavior.

Experimental design and statistical analysis

We examined whether the red crucian carp exhibited abnormal behaviors in the presence of three compounds: NaOH (20 mg/L), HCl (20 mg/L) and glyphosate (0.025 mg/L, 0.05 mg/L and 0.1 mg/L). Glyphosate is widely used in agriculture as an effective broad-spectrum herbicide. Exposure of fish to glyphosate is known to cause abnormal behaviors [23,24]. NaOH is a strong base used in many industries, particularly in the manufacture of pulp and paper, textiles and soaps. The pH values of water treated with HCl and NaOH were approximately 5.5 and 8.5, respectively. Exposure to NaOH has been reported to affect the swimming behavior of fish [25]. All chemicals were purchased from LiuXia Chemical Industries Co. Ltd., Hang Zhou, and China. For each trial, the test fish were randomly selected in the aquarium and were pre-exposed to water without the test substance in a glass tank for 30 min to acclimate to the environment through the test tank. Then the test fish were exposed to contaminated water for 60 minutes (exposed period) in the same test tank that containing the test solution. The test solution was pumped into the test tank by a roller pump. Fish behavior in a trial was video-recorded for 90 minutes including pre-exposed and exposed periods.

The true positive rate (TPR) was defined as TPR=#true positives/(#true positives+#false positives), where a true positive was the trial that the model yields an alarm to indicate the water quality have changed, and fish is indeed exposed to the water containing a test substance. A false positive was a trial that the model releases an alarm, but fish is exposed to uncontaminated water. The false positive rate (FPR) was defined as FPR=#false positives/(#true positives+#false positives). The values of behavioral parameters were statistically compared between the control (unexposed) periods and the exposure periods of the trials. The time-course of the values was also examined. Data were analyzed using one-way analysis of variance (ANOVA; factor: treatment or dose), followed by Duncan's test or *t*-tests to determine significant differences among the values for different treatment conditions; *t*-tests were used to analyze differences between values in the control and the treatment conditions. Data are presented as means ± SEM; $p<0.05$ was considered significant. The raw videos were analyzed using custom software written in C^{++} using the OpenCV library and all statistical analyses were performed using Matlab.

Model of fish shoaling behavior

We studied the trajectories of a group of crucian carp and observed that they formed a condensed shoal in clean water but left the shoal during the exposure treatment. As shown in Figures 3a and 3b, we used a circle (CDT) determined by a DT network to measure the entire shoal of fish. The DT network that also known as Voronoi diagram is a important data structure in computational geometry. It is the dual structure of the Voronoi diagram in 2-D plane. It satisfies the empty circle property, that is, for each edge in a DT network, we can find a circle passes through the edge's endpoints without enclosing other points. In our model, fish will be connected into triangles in the DT network to characterize the dynamical pattern of shoaling fish.

The CDT was defined by two parameters: a center point and a shoaling radius. The center point was calculated from the DT network constructed using the centroid of each fish. The shoaling radius describes the degree of aggregation of the fish. We computed the average shoaling radius (ASR) in water without the test substance to determine the CDT. Fish was not considered as inside the CDT as the centroid of the fish appeared outside it. Therefore, shoaling behavior was considered to have disappeared when the number of fish (NOC) outside the CDT with radius ASR reached a certain threshold. The threshold can be determined as an average NOC in 12 trials under untreated water, each trial lasts for 5 mins.

To determine the CDT in each frame, we first calculated each target fish and then the centroid of each fish according to its individual contour. Real-time images were captured at 15 frames/s using a camera fixed above the fish tanks. The captured images were divided into one of three channels with thresholds set according to the color of the fish ($90<r<150$, $60<g<120$ or $60<b<110$). Since the colors of red crucian carp were simple and bright and obviously different from the background, fish were detected as foreground blobs in the image using a segmentation method [26]. The contour of each fish was identified by applying an edge-detection algorithm on the thresholded image. Thus, the fish centroid $f(x', y')$ was obtained by:

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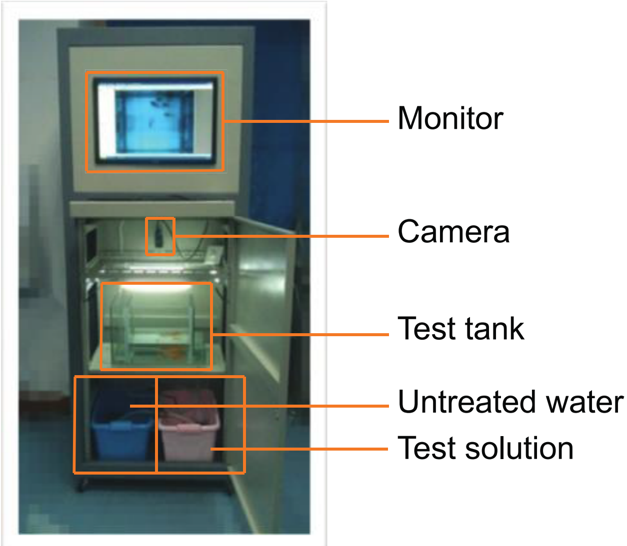
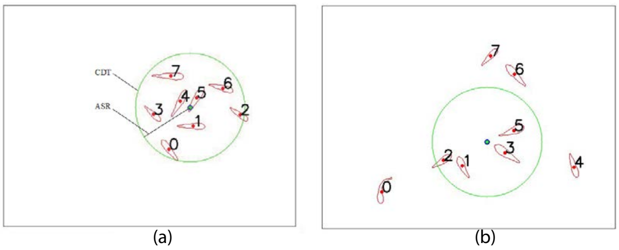


Figure 2: The experimental apparatus.



(a) Fish tend to swim as a shoal in untreated water. The size of the shoal is described by a circle.
(b) The shoaling behavior of fish is disturbed under exposure conditions. The two shoaling radiuses shown are equal to the ASR computed in untreated water. The NOC is 0 in (a) but 6 in (b).

Figure 3: Method of measurement of the shoal.

$$x^x = \frac{\sum_i^N x_i}{n}, y^x = \frac{\sum_i^N y_i}{n}$$

Where (x_i, y_i) for $i = 1, 2, \ldots, n$ are the contour points of the fish target.

We then calculated a group center of the fish from the individual centroids of all fish based on the DT network (Figure 4a-4c). The centroids of the fish correspond to the vertices in the DT network. One of the essential conditions in a DT network is that the circumcircle of any triangle in the triangulation contains no point in its interior, which leads to a unique division of the whole network [27]. Let $F=\{f_1, f_2, ..f_N\}$ is the centroids of individual fish. We build the DT network of a group of fish for every image frame as follows [28]:

1) Choose the bottom-right point f_i of F;

2) Let f_{-1} and f_{-2} be two points sufficiently far away such that F is contained in the triangle $f_i f_{-1} f_{-2}$;

3) Initialize T as the triangulation consisting of the single triangle $f_i f_{-1} f_{-2}$;

4) Insert $f_j \in F - T$ into T as follows:

Find a triangle $f_r f_s f_t \in T$ containing f_j. If f_j lies in the interior of the triangle $f_r f_s f_t$, then edges from f_j to the three vertices of $f_r f_s f_t$ are added so that $f_r f_s f_t$ is split into three triangles. If f_j lies on an edge (e.g. $f_r f_s$) of $f_r f_s f_t$, an edge from f_j to f_t should be added; in addition, an edge from f_j to p_l should also be added, where $p_l f_r f_s$ is also a triangle. So, the two triangles $f_r f_s f_t$ and $p_l f_r f_s$ with the common edge $f_r f_s$ are split into four triangles. If f_j lies outside the triangle $f_r f_s f_t$, the shortest edge to f_j is found from the three sides of the triangle $f_r f_s f_t$; Let $f_r f_s$ is this shortest edge, then two edges $f_j f_r$ and $f_j f_s$ are added;

5) According to the local optimization algorithm as described in [27] to update all the triangles generated above;

6) Repeat step 4) and 5) until all points are inserted;

7) Delete f_{-1} and f_{-2} and those edges associated with these two points.

Having built all triangles based on fish positions, we could get the centers of all triangles in the DT network. Therefore, the center point of the shoal G (x, y) was calculated as follows:

$$x = \frac{\left(\sum_{i=1}^N a_i x_i^x\right)/3}{M}, y = \frac{\left(\sum_{i=1}^N a_i y_i^x\right)/3}{M}$$

Where (x_i', y_i') represents the coordinates of each fish centroid; N is the total number of fish; M is the number of triangles in the DT network; and a_i is the number of triangles connected to the fish. The value of a_i increases as fish density increases. As shown in Figure 4d, the center of a group of fish varied during swimming. The trajectories of the center were obtained from each frame image.

Results

To determine whether the DT network can be used to model fish shoaling behavior, we first needed to determine the group size and the ASR in water without the test substance. In these experiments, the ASR increased as the number of fish increased. However, there were no significant differences in the area per fish between groups of 4, 6 and 8-fish (p>0.01). In addition, the 4, 6 and 8-fish shoal displayed a smaller ASR vs. the other groups (Figure 5a). In this study, we chose eight fish as the group size to evaluate the shoaling behavior of fish. The ASR of eight fish was 6 cm. Therefore, we assessed the water quality according to changes in the number of fish that lay outside the circle with radius 6 cm. In addition, the ratio of areas between the CDT and the test tank did not significantly changed (p>0.05) when the area of the test tank become larger (Figure 5b).

We assessed the effects of NaOH, HCl and glyphosate on the shoaling behavior of red crucial carp (Figure 6a-6c). NaOH (20 mg/L) and HCl (20 mg/L) significantly decreased shoaling behavior relative to controls ($F(1, 8)=15.93$, $p<0.01$ and $F(1, 8)=18.71$, $p<0.01$, respectively). The shoaling behavior also decreased significantly relative to the controls in fish exposed to glyphosate at different concentrations (0.1 mg/L: $F(1,18)=215.15$, $p<0.01$; 0.05 mg/L: $F(1,18)=118.16$, $p<0.01$; 0.025 mg/L: $F(1,18)=96.48$, $p<0.01$). Furthermore, the NOC values of fish exposed to glyphosate were more than three times greater than in the controls. There were significant differences in NOC among the three concentrations of glyphosate ($F(2,27)=93.67$, $p<0.01$); NOC increased with increasing concentration of glyphosate. There were no significant differences in the shoaling behaviors of the control treatments ($F(4,27)=0.18$, $p>0.5$).

We examined the shoaling behavior of test fish in consecutive time periods after exposure to glyphosate at different concentrations. There

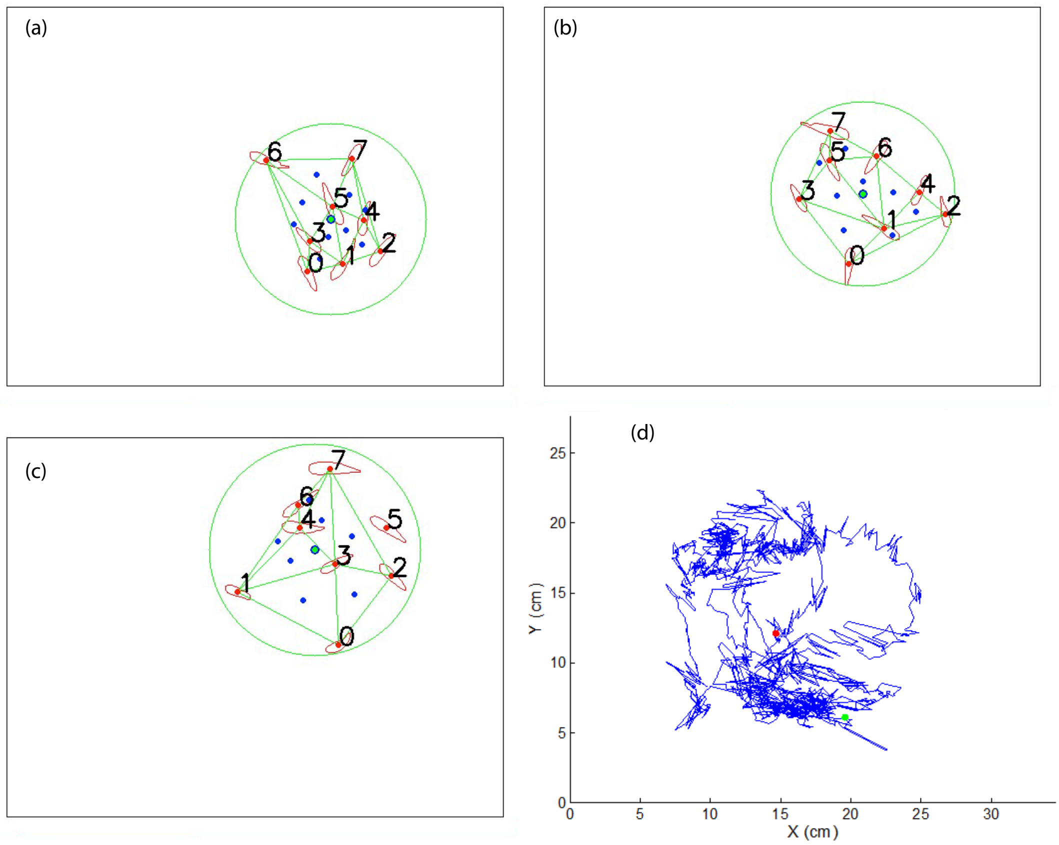


Figure 4: The shoaling fish based on the DT network in untreated water. The red points that were computed from the contour points (red line) of individual fish represent the centroids of individual fish. The triangles that determined by red points are created according to the DT network algorithm. The blue points are the centers of the triangles. The green point represents the center of a group of fish calculated from the centers of the triangles (blue points). The circle with the center point (green point) is used to model the shoal of fish. The DT network is changed from (a) to (b) then to (c) after 10 sec respectively. (d) Two-dimensional trajectories (x-y coordinates) of the center of a group of fish over 5 min. The red dots are the starting points and the green dots are the end points of the trajectories.

were significant differences in the shoaling behavior of the exposed fish between 0 and 60 min exposure relative to controls (Figure 7). The NOC gradually increased after about 25 min of exposure. In addition, higher concentrations of glyphosate were associated with higher NOC. The changes of NOC after exposure were higher at 0.1 mg/L and 0.05 mg/L glyphosate relative to 0.025 mg/L.

To evaluate the robustness of our model for the shoaling behavior of fish, we identified the relationship between NOC and ASR. NOC decreased as ASR increased. However, the shoaling behavior was significantly diminished in exposed relative to control conditions at all ASR values ranging from 2.5 to 7.5 cm. These results indicate that test fish normally swim as a shoal and stay in close proximity to each other in uncontaminated water and that shoaling behavior is disturbed during exposure conditions.

By comparing shoaling behavior in untreated water with that in the presence of chemicals, we might obtain early warning signals of water quality deterioration. To assess the quality of water based on shoaling behavior, we computed the average NOC in 5 minutes over 12 trials under untreated water. An alarm was released if the NOC was greater than a nominal threshold in consecutive 10 seconds. The alarm time was determined by the time the test solution began to be pumped into the test tank and the time at the triggering of the alarm. As shown in Table 1, the true positive rate (TPR, see Materials and Methods) and false positive rate (FPR, see Materials and Methods) using glyphosate are varied at various threshold settings. The threshold of the NOC was determined to result in more true positives and fewer false positives. We found that when the value 3.0 was selected as the threshold NOC, the TPR was 100%, the FPR was about 0%, and the average alarm time was about 21 min after exposure. We carried out another 30 trials to achieve performance using NaOH and HCl, respectively. Eight test fish were selected for observation and each trial lasted for 30 minutes. The performance was 80% in NaOH and 93.33% in HCl. These results indicate that the proposed model of the shoaling behavior of crucian carp is a useful tool for monitoring water quality.

Discussion

Although the behavior of test fish varied between individuals, the shoaling behavior of test fish was stable during the control exposure to fresh water. Test fish typically maintained a distance of nearly one body length from their nearest neighbors. Shoal cohesion is reflected in a correspondence between the speeds and headings of test fish with those of their nearest neighbors [29,30]. We found the effect of group-size of test fish on the shoaling paradigm. Smaller groups (2 and 3 fish) or

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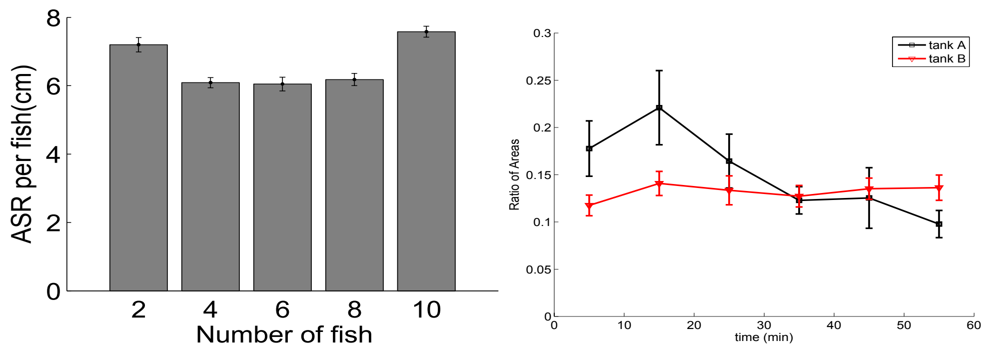
NOC(#)	1.6	1.8	2	2.2	2.4	2.6	2.8	3	3.2	3.4	3.5
FPR (%)	50	33.33	26.67	20	13.33	6.67	3.33	0	0	0	0
TPR (%)	100	100	100	100	100	100	100	100	93.33	93.33	86.67
AAT (min)	8.5	9.5	10	11	11.33	12.83	16.83	21.83	25	28.67	29.33

Table 1: True positive rate (TPR), false positive rate (FPR) and average alarm time (AAT) at each threshold of NOC.



(a) Fish number (2-10 fish) on ASR per fish under control conditions. Each value was obtained from 10 trials and each trial lasted for 30 minutes.

(b) The size of test tank on shoal. The area of tank B is twice the tank A. Y-axis represents the ratio of areas between the CDT and the test tank. Data are presented as means ± SEM.

Figure 5: Effect of parameters on shoal behaviors.

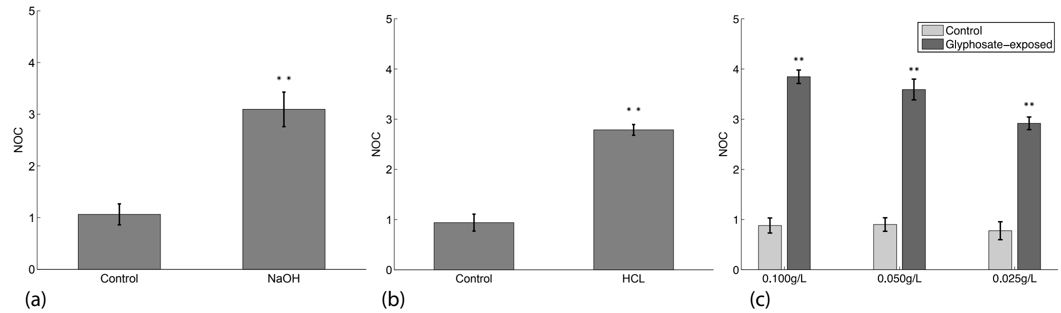


Figure 6: NOC of test fish exposed to three chemicals. NOCs are computed during control conditions and during exposure to (a) NaOH, (b) HCl, and (c) glyphosate at three different concentrations. Eight fish were used in each trial and each trial lasted for 60 minutes. Data are presented as means ± SEM; **p<0.01.

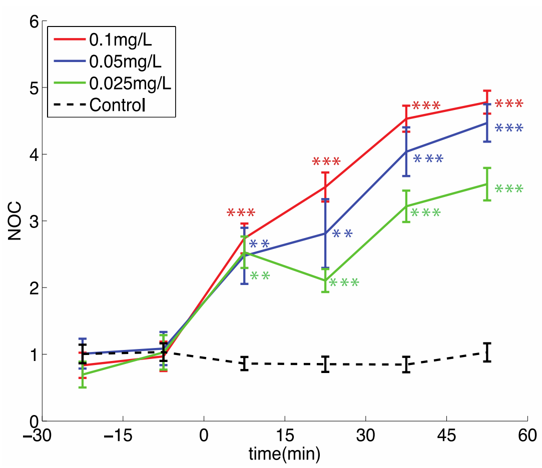


Figure 7: Time-course of NOC during 60 min exposure to glyphosate. The data correspond to those shown in Figure 6c. Black dashed line represents data obtained in the corresponding control conditions. Data are presented as means ± SEM; ***p<0.001, **p<0.01and *p<0.05.

larger groups (above 10 fish) did not easily form a shoal. On the other hand, appropriate groups (4-8 fish) displayed similar shoaling tendency, which is similar to other recent data on zebra fish [31]. It is possible that the effect of group-size of test fish on the shoaling can be changed as the test tank became larger. Further work could be conducted using different size of test tank.

Several fish escaped from the circle defined by the ASR criterion after the exposure. Both NaOH and glyphosate induced increased NOC after about 15 min exposure. We observed that test fish swam together to form a shoal in clean water but, after exposure, they increased their swimming velocity and the heads of several test fish bumped against the wall; apparently in an attempt to avoid water containing chemicals. This suggests that when fish perceive a contaminant as noxious, they respond by avoiding the area containing the chemical, and by decreased shoaling behavior. These results are consistent with existing data on the effects of chemicals on fish shoaling. For example, alcohol and nicotine were both found to exert significant effects on shoaling behavior of zebrafish and modestly reduced shoal cohesion [32]. The psychotropic drugs lysergic acid diethylamide and 3,4-methlenedioxymethamphetamine reduced shoaling (assessed by increased inter-fish distance) and proximity (time spent together) among zebrafish [31]. Shoaling behavior of zebrafish was significantly inhibited (as measured by nearest-neighbor distance) at high concentrations of ethanol (1.0%) [33]. Furthermore, shoaling behavior of test fish was inhibited by exposure to high pH. Generally, the pH values of fresh waters lie in the range 6-8. In this study, the pH values of the water treated with HCl and NaOH were 5.5 and 8.5, respectively, and we observed that, in both cases, the NOC was above the threshold after about 20 min exposure.

According to a recent result, the safe exposure concentrations were 0.0252 mg/L, 0.0259 mg/L and 0.0260 mg/L for *C. idellus*, *H. molitrix* and *C. auratus* (crucian carp) respectively, and the median lethal concentration (LC50) of glyphosate on crucian carp was 0.2599 mg/L after 96 h exposure [34]. In our study, we found that the behaviors of test fish characterized by fast swimming in different directions and an attempt to avoid the toxicant between 0 and 30 min of exposure. After 12 hours of exposure, the response from more than half of the test fish to tactile stimuli ceased completely. Consistent with previous result [34], our results indicate that glyphosate in such low concentrations was harmful to crucian carp survival.

Nearest-neighbor distance (NND) is the most common measurement of group cohesion [35] and the mean NNDs for all individuals is often employed. However, fish in a moving shoal constantly change positions within the shoal, adjusting their distance from other members of the shoal, and some may leave the shoal. Thus it is difficult to accurately capture data for individual fish. In addition, the NND only represents the condition of the shoal at a single point in time, or averages it over several time points. However, the NND may fluctuate widely during the course of shoaling [36]. Thus, the single average NND ignores the fluctuating structure inherent in the shoal behavior. In our model, the dynamic center of mass of individuals represents changes in the structure of the shoal. We used a circle with its center at the center of mass to represent the size of the shoal. Furthermore, each fish centroid was weighted by a coefficient equal to the number of triangles connected to the target fish. A greater density of fish results in more triangles and a greater weighting. This reduces the effect of outlier fish that leave the group and later rejoin it, and the mass point is more accurately determined. Several different definitions have been used in the literature to specify the sizes and shapes of shoals [37-40]. The shape of a shoal may depend on the number of fish in the shoal or correlate with its polarization. Further work should be conducted using shapes other than the simple circle used in our model to measure the size of the shoal (e.g., the convex hull).

Conclusions

This study introduces a novel method for measuring the shoaling behavior of fish. The method was used to detect toxicological responses of red crucian carp to sublethal concentrations of NaOH, HCl and glyphosate. In our model, the dynamic shoaling behavior of a group of fish was characterized by the center point and the fixed ASR of a circle. Use of a DT network more reliably estimates the center point and size of the group than averaging the positions of each fish. Greater fish density results in more triangles reducing the effect of outlier fish. Further work is needed to examine whether the behavioral features of shoaling of other species of fish are similar to those of red crucian carp. Furthermore, in our trials we limited the depth of the water and the size of test tank. Further work could be conducted using multiple recorders with multi-angle views to obtain three-dimensional data concerning the response of the fish to toxins. Exposure tests using a mixture of test chemicals over longer monitoring periods would enhance the generality of the proposed method.

Acknowledgments

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