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New Technique:

Application of biomonitoring and support vector machine in water quality assessment*

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Abstract: The behavior of schools of zebrafish (*Danio rerio*) was studied in acute toxicity environments. Behavioral features were extracted and a method for water quality assessment using support vector machine (SVM) was developed. The behavioral parameters of fish were recorded and analyzed during one hour in an environment of a 24-h half-lethal concentration (LC_{50}) of a pollutant. The data were used to develop a method to evaluate water quality, so as to give an early indication of toxicity. Four kinds of metal ions (Cu^{2+}, Hg^{2+}, Cr^{6+}, and Cd^{2+}) were used for toxicity testing. To enhance the efficiency and accuracy of assessment, a method combining SVM and a genetic algorithm (GA) was used. The results showed that the average prediction accuracy of the method was over 80% and the time cost was acceptable. The method gave satisfactory results for a variety of metal pollutants, demonstrating that this is an effective approach to the classification of water quality.

Key words: Water assessment, Behavioral feature parameter, Support vector machine (SVM), Genetic algorithm (GA), Water quality classification

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1 Introduction

Water security is a hot topic of concern in the development of human society. To ensure water security, methods for assessing water pollution have become a focus of study. Currently, there are two main methods for monitoring and evaluating water quality: first, physical and chemical analysis, and second, biological monitoring methods. Physical and chemical analysis is used to evaluate water quality by determining the existence and content of hazardous substances within the water directly using a variety of instruments. These methods are accurate and sensitive, but they are time-consuming and cannot be used continuously in situ. The principle of biological monitoring is to reveal changes in water quality and the presence of environmental pollution by identifying changes in the health status, physiological characteristics, and behavioral responses of individuals or populations of aquatic organisms, providing a basis for environmental quality monitoring and evaluation from a biological point of view. Biological measures of water quality may detect materials that analytical chemistry techniques cannot, because of inadequate detection limits or methodological limitations (van der Schalie *et al*., 2001). Biological methods have some advantages: (1) once a system is established, it can provide automatic alarms and can be used for long-time online monitoring of water quality; (2) the response of aquatic organisms to water quality is more sensitive and reliable; (3) biological methods are also useful for detecting mixed pollution; (4) they have a low cost and can easily be incorporated into a digital system.

In previous studies, many researchers proposed

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early warning methods using biological monitoring (Nogita *et al.*, 1988; Thomas *et al.*, 1996; Kane *et al.*, 2004). However, the feedback information was too limited for accurate evaluation. Some studies used neural network models to analyze water quality parameters and to evaluate the water quality directly (Palani *et al.*, 2008; Singh *et al.*, 2009). These methods cannot reveal water quality in time because they do not take advantage of indicator organisms in water quality monitoring.

Conventional assessment methods such as the gray-clustering method and the fuzzy math method do not solve the complex nonlinear relationships between assessment factors and water quality, and the assessment result is greatly affected by subjective factors of the assessing person. Support vector machine (SVM) is a small sample machine learning method based on statistical learning theory (SLT). It uses the structural risk minimization principle with good generalization ability. It can solve the problem that conventional methods face in assessing water quality and can overcome the defects of slow training speed, poor network generalization, and low learning accuracy in artificial neural networks (ANNs). As an important pattern recognition method, SVM is appropriate for water assessment, which is a typical pattern recognition issue. In this paper, a biological monitoring method was used to identify and classify water quality. The method involves analyzing the behavioral parameters of fish during an acute toxicity test that simulates the course of water pollution, followed by the use of SVM to assess the water quality.

2 Acute toxicity test

2.1 Materials and methods

The zebrafish (*Danio rerio*) is an important model organism in life science research and is the standard species used in water quality monitoring. Its genome is very similar to the human genome, so it is suitable for use as an early warning indicator. In our study, zebrafish were purchased from Ningbo Sanhe aquafarm, and were 2–3 cm in length, 0.2–0.3 g in weight, with good activity and bright body color. Newly selected fish first had to be domesticated to adapt to the living environment and laboratory conditions. We then chose the healthy fish for experiments. In the laboratory, the fish were acclimated for two weeks prior to the experiments. The water used for acclimation and experiments was dechlorinated tap water aerated for 48 h. During acclimation, the pH of the water was kept at 7.2±0.2 and the water temperature was maintained in the range of 22–24 °C. Fish were held under a photoperiod of 12 h of light and 12 h of darkness. There was a natural mortality rate of <1% and any anamorphic fish were not used.

Analytical reagents copper sulfate ($CuSO_4{\cdot}5H_2O$), cadmium chloride ($CdCl_2{\cdot}2.5H_2O$), potassium dichromate ($K_2Cr_2O_7$), and mercuric chloride ($HgCl_2$) were used to prepare test solutions.

2.2 Experimental system

The experimental equipment consisted of a tank, a charge-coupled device (CCD) camera (Microvision MV-VE120SC), a four-processor computer (Intel Core i5-740), an infusion bottle, a water pump, heating rods, thermometers, and a pH meter. The experimental tank (72 cm×12 cm×38 cm) (Fig. 1), was made of acrylic (the back and bottom were non-transparent). The tank was divided into three chambers by two fish baffles. There were many holes of uneven size in the baffles to allow water to flow through while preventing the movement of fish between chambers. Fish could swim freely in the middle chamber which was the main part of the experiment. There was a suction inlet and an overflow outlet in the right hand section of the tank forming a side filter to filter out fish waste and other residues. The water flowed from the left side to the right side and was pumped to the left side by an 8-W pump, to simulate

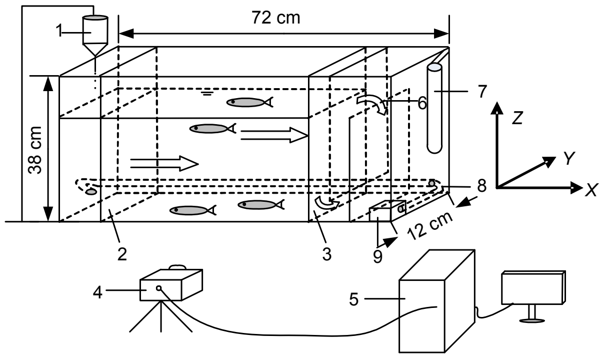

Fig. 1 Experimental system schematic diagram
1. Infusion bottle; 2, 3. Fish baffles; 4. Camera; 5. Computer; 6. Overflow outlet which determines the surface level of water; 7. Heating rod; 8. Water pipe from pump to left chamber of the tank; 9. Water pump

real water circulation. Simulating the real condition, the fish survive in the flowing water and the toxicant is infused into the whole tank through the flowing water. A thermometer, heating rods, and a pH meter were placed in the right hand chamber to prevent any influence on fish behavior.

2.3 Experimental preparation

Preliminary experiments were carried out to determine the 24-h half-lethal concentrations (LC_{50}) of four kinds of metal ions for zebrafish. The 24-h LC_{50} values of Cu^{2+}, Hg^{2+}, Cr^{6+}, and Cd^{2+} were 1.472, 0.292, 54.777, and 18.567 mg/L respectively, in our environment. The time required for a water cycle was 6 min, and was dependent on the power of the pump and the size of the tank. A volume of pollutant solution was made up according to the capacity of experimental tank. The velocity of the water flow from the infusion bottle was controlled to ensure the injection of the required dose of pollutant during each water cycle.

2.4 Quantification of school parameters using a vision system

The coordinate system (X, Y, Z) is shown in Fig. 1, where the origin is located at the lower left hand corner of the front side. The features that were used as behavioral indices were the coordinates of the center of gravity of the fish school on the X axis (CX) and the Z axis (CZ), and the spatial standard deviations in the X direction (SDX) and in the Z direction (SDZ) (Israeli-Weinstein and Kimmel, 1998). CX and CZ show the mean location of the school in the projection plane. SDX and SDZ are spatial standard deviations which measure the density of the fish school in two directions. The average swimming velocity (ASV) of the fish school is measured in multiples of body length per second (BL/s) (Xu *et al*., 2006a), reflecting the activity level of the school. When fish encounter toxicant, due to hypoxia or swimming weakness, most will rise to the water surface or sink to the bottom. So quantifying the numbers of fish on the surface and at the bottom can reflect the water quality to some degree. In addition, the body color of zebrafish will change gradually in some conditions such as metal pollution, so quantitative changes in body color can be used as another indirect index for water quality (Gerlai, 2003; Xu *et al*., 2006b). The quantification method of Xu *et al*. (2006b) was used.

2.5 Experimental procedure

Ten fish were used in each experiment. The fish were not fed during the day prior to the experiment. During the experiments, the water temperature was maintained at (23.5±0.5) °C, and pH varied between 6.9 and 7.2. The air bubbling system was removed from the tank. A reference monitoring phase of about 20 min was recorded before each experiment. During the toxicity testing phase of each experiment, the behavioral responses of fish were analyzed about one hour after the toxic solution was injected into the water. The behavioral responses were an acute stress reaction after injection of the solution. The computer captured the real-time video sequences from the camera and quantified the behavioral parameters described in Section 2.4. The data derived from the quantification were used to carry out real-time analysis, processing, and recording, and for developing the classification and early warning system.

3 Support vector machine (SVM)

3.1 Basic principle

SVM (Vapnik, 1995) is an emerging machine learning technology that is extensively used as a classification tool in a variety of areas. The theory is based on SLT. It maps input x into a high-dimensional feature space $\phi(x)$ (Fig. 2) by nonlinear transformation and constructs an optimal hyperplane to separate the two kinds of data points from two classes (Duan *et al*., 2003). It maximizes the distance of the separated points to find the optimal separating hyperplane. The nearest vectors from the hyperplane are called support vectors (Fig. 3).

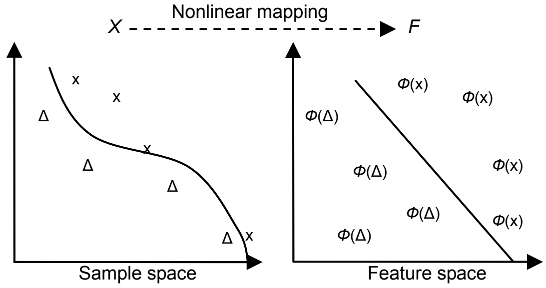

Fig. 2 Nonlinear mapping from the original data space *X* to the feature space *F*
x and Δ are two kinds of data points in the original data space. Φ(x) and Φ(Δ) are two kinds of data points in feature space

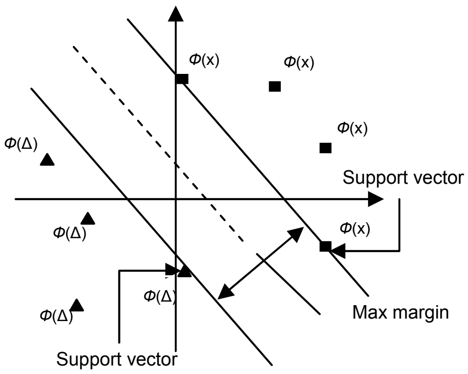

Fig. 3 Optimal separation plane that maximizes the distance from the members of each class to the plane
x and Δ are two kinds of data points in the original data space. Φ(x) and Φ(Δ) are two kinds of data points in feature space

Three main issues need to be considered when using SVM: feature selection, kernel function selection, and the penalty and inner parameters of kernel function selection.

3.2 Feature selection

The features that were used in SVM were CX, CZ, SDX, SDZ, ASV, number on top (NumTop), number low (NumLow), and intensity. NumTop and NumLow are the numbers of fish on the surface and at the bottom of the water, respectively. The color model HSI, common in computer vision applications, was used to analyze hue, saturation, and intensity. The intensity is the total amount of light passing through a particular area. In this study, intensity stands for the average body darkness of the fish school.

3.3 Kernel function selection

The kernel function $K(x, x_i)$ performs the nonlinear mapping between the input space and the feature space. The radial basis function (RBF) is most widely used among several kinds of kernel function and performs very well in most cases. It was used in this study because of its better ability to deal with the nonlinear relationship between the label set and the attribute set and because it has fewer parameters. The function formula is $K(x, x_i)=\exp(-\gamma\|x-x_i\|^2)$, $\gamma>0$, where $\|x-x_i\|$ is the Euclidean distance between input vector x_i and center x, and γ is a parameter specified by the user in advance.

3.4 Genetic algorithm (GA)

The penalty parameter C in SVM determines the trade-off between the fitting error minimization and the model complexity. The parameter γ determines the bandwidth of the RBF kernel (Wu *et al.*, 2007). For optimizing the two SVM parameters (C and γ) simultaneously, a heuristic algorithm, GA, was used. GA is a new global adaptive optimization algorithm which simulates biological evolution processes in computer systems (Davis, 1991). According to the Darwinian principle (Darwin, 1869) of "survival of the fittest," starting with a set of candidate solutions called a population, GA obtains a group of individuals better adapted to the environment after a series of iterative computations and makes the population evolve into better areas in the search space. So, after iteration, it finally converges to a group of individuals that are best adapted to the environment. It is the optimal solution. Because of its powerful abilities in space search and parallel processing, GA can efficiently handle a large search space, and thus is less likely to obtain a local optimal solution than other algorithms (Huang and Wang, 2006).

The main operations of GA which simulate biological evolution are selection, crossover, and mutation. Selection, based on the value of individual fitness, is proposed to select good individuals as parents. If the individuals selected as parents are high-quality species, the probability of propagating successful future generations will be higher. Crossover is a random mechanism for exchanging genes between two parents. The new individual combines the characteristics of its parents. Mutation, based on a certain probability, randomly changes the values of certain genes of each individual to provide opportunities for generating new individuals.

Selection, crossover, and mutation provide a good search path for a space search which enables the new generating individuals to develop in the direction of the optimal solution. In this paper, we compare GA with another commonly used optimization algorithm, particle swarm optimization (PSO).

3.5 Data preprocessing and classification

To prevent attributes with a wide range of values from dominating and to overcome difficulties with calculation, a training set and a test set were first normalized to the range of [0, 1]. The results showed that normalized data are easier to process. As it expedites the convergence of the training network and the training speed, accuracy is greatly improved.

Principal component analysis (PCA) was applied to reduce the dimensions of the dataset, giving information regarding the potential capability for separation of objects and providing principal component scores for SVM (Andre, 2003; Xie *et al.*, 2008). This method can effectively identify the most dominating component and structure in the data and eliminate redundancy, revealing the simple structure behind complicated data. After determining the kernel function and SVM parameters, the preprocessed data were used in training and testing. The overall flow chart is shown in Fig. 4.

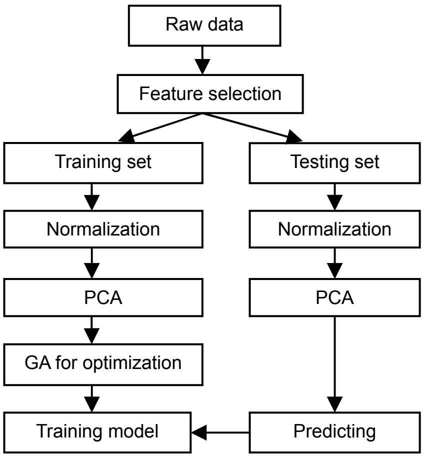

Fig. 4 Flow chart of training and predicting

4 Results and discussion

4.1 Result analysis

When the toxic substances empty into the water and the water quality changes, the fish will show a sensitive reaction such as gathering into a group and mad swimming. This stress reaction is an attempt to evade the toxicant and coincides with a rapid increase in swimming speed. When the toxicant takes effect, the fish swim erratically in all directions. The school's position coordinates fluctuate widely and the variance increases.

Fig. 5 shows the degree of activity of the fish during 900 s under normal conditions and during the subsequent 1000 s under toxic Cu^{2+} conditions. The data were acquired and saved to a database once per second. To eliminate noise and abnormal data points, the data used for analysis and display were the results of taking the average of every five points. In Fig. 5, the value of activity rises from around 2.0 BL/s to more than 2.5 BL/s when the water quality changes.

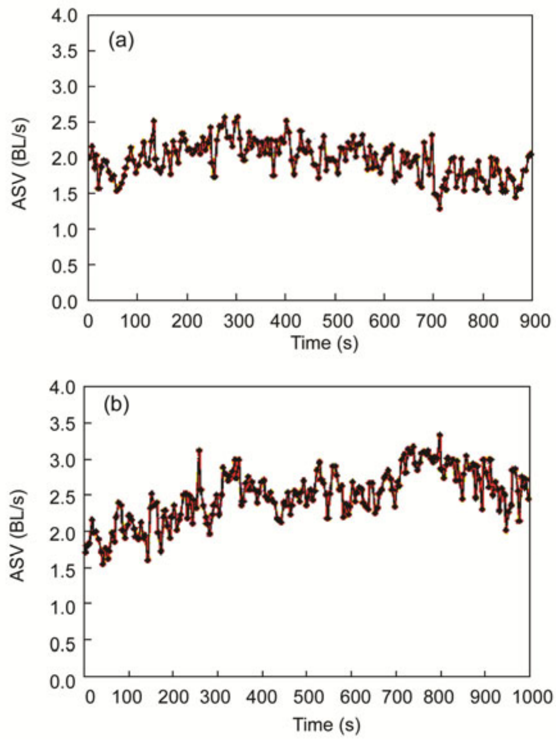

Fig. 5 Activity of the fish school in normal (a) and abnormal (b) conditions
ASV: average swimming velocity, measured in multiples of body length per second (BL/s)

Fig. 6 shows the coordinates and densities of two directions. It can be seen that SDX increases over time while CX, CZ, SDX, and SDZ have a greater fluctuation after adding copper sulfate solution, which demonstrates that a direct toxic effect leads to erratic swimming and jerky movement of the fish. Fifty points of each phase were taken randomly as the samples and the statistical analysis was performed using Minitab 15. The differences between the two groups were evaluated using the Mann-Whitney test. The significance test results are given in Table 1. The ASV in the abnormal condition was significantly higher (P<0.01, Mann-Whitney) than that in the normal condition, and there were also significant differences in CZ, SDX, and SDZ between normal and abnormal conditions.

Table 1 Statistical characteristics of samples in the Cu^{2+} experiment

Group	ASV (BL/s)	CX (pixel)	CZ (pixel)	SDX (pixel)	SDZ (pixel)
Normal	1.98±0.27	707.90±144.70	606.50±71.80	221.56±50.26	198.92±47.50
Abnormal	2.50±0.33**	655.70±197.40	679.50±90.20**	252.20±83.20*	164.50±73.70*

Data are expressed as mean±standard deviation (SD). ASV: average swimming velocity; CX: coordinate of the center of gravity on the *X* axis; CZ: coordinate of the center of gravity on the *Z* axis; SDX: spatial standard deviation in the *X* direction; SDZ: spatial standard deviation in the *Z* direction. Statistically significant difference from the normal group: * $P<0.05$, ** $P<0.01$ (Mann-Whitney)

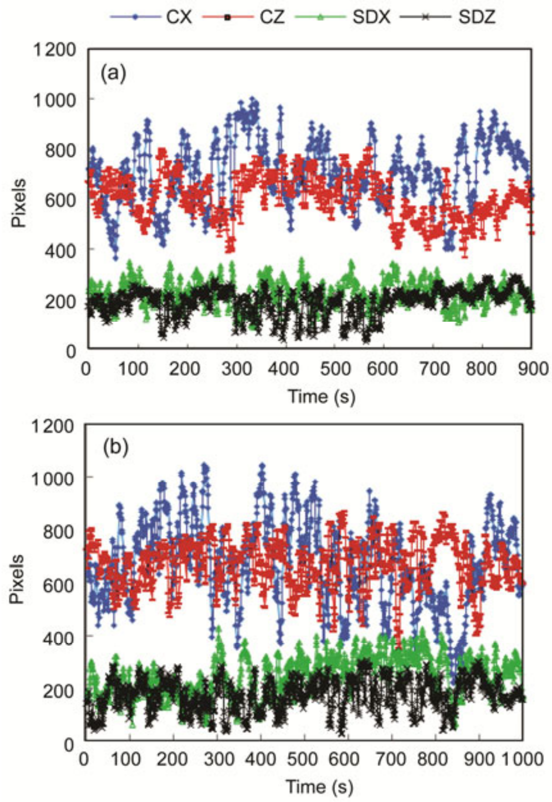

Fig. 6 Location parameters of the fish school in normal (a) and abnormal (b) conditions
CX: coordinate of the center of gravity on the *X* axis; CZ: coordinate of the center of gravity on the *Z* axis; SDX: spatial standard deviation in the *X* direction; SDZ: spatial standard deviation in the *Z* direction

4.2 Accuracy

The accuracies of training and testing are shown in Table 2. The total sample size was 4484 points, including 970 normal data points and 3514 abnormal data points. These data are from the acute Cu^{2+} toxicity experiment. The training set and testing set were each allocated 2242 points at random. The model was trained using the training set and was then used to predict the testing set. Because the training set and testing set data were selected randomly, this process was repeated ten times to check the consistency.

Table 2 Accuracies of training and testing

No.	Training accuracy (%)	Testing accuracy (%)
1	92.462	89.295
2	97.636	88.626
3	93.265	89.117
4	92.462	89.608
5	92.462	89.295
6	97.636	88.626
7	93.265	89.117
8	92.462	89.608
9	93.443	88.893
10	92.953	89.608

The testing accuracy was near 90%, so the model is suitable for use in an early warning system. However, the 10% false alarm rate is a problem. One acceptable solution is to delay the output of the current prediction result to allow the system to compare the current result with all outputs over a 5-s period. An alarm judgment is then made only when the five outputs have the same result.

4.3 Time cost

In this study, we use a computer with Intel Core i3 2.93 GHz central processing unit (CPU), 2 GB DDR3 1333 memory, and Windows XP operating system (OS).

Parameter optimization takes most of the time. The use of the PSO method for parameter optimization can achieve a recognition rate close to that of GA. However, it took (1489.97±81.64) s for PSO to finish parameter optimization in this experiment, compared with only (770.71±99.82) s using the GA method. So, the GA method is the better choice.

4.4 Some other experiments

Using the method described above, the results of experiments with other kinds of metal ions are shown in Table 3. The accuracies of training and testing with the Cu^{2+} experiment data were higher because the toxic effects of Cu^{2+} on zebrafish are stronger. The toxic effects of Hg^{2+} on zebrafish are weaker, leading to an inconspicuous change between the normal and abnormal conditions and a lower accuracy of classification. The toxic effects of Cr^{6+} and Cd^{2+} are intermediate between Cu^{2+} and Hg^{2+}. The results shown in Table 3 are consistent with the results of visual observations during the experiments. Best C and Best γ are the penalty and kernel parameters respectively, which give the highest accuracy.

Table 3 Results of comparing tests with different metal ions

Metal ion	Training accuracy (%)	Testing accuracy (%)	Best C	Best γ
Cu^{2+}	93.392	91.330	34.649	4.279
Hg^{2+}	82.583	79.722	9.274	8.731
Cr^{6+}	89.114	83.697	3.089	13.105
Cd^{2+}	88.438	88.120	7.773	5.212

The data shown above are results of single metal ion experiments. A model that can give an early warning for many kinds of metal pollutants would be more useful, so the collection of samples of each metal in large quantities is needed. In our research, samples of four kinds of metal ions including normal and abnormal conditions were combined. Samples selected at random from the combined sample set were used to train the model. The model was then used to predict the effects of samples of single metal ion, to examine the generalization ability of the model. The results are shown in Table 4. Our model can achieve very good results as long as the fish school is sensitive to some kinds of metal pollution. The effect of Hg^{2+} on the fish school was relatively slow, and was therefore more difficult to detect in a short time. However, in most cases, most heavy metal ions will have some effects on fish behavior and the model trained by our method can achieve satisfactory accuracy. So, the proposed method is a reasonable and feasible assessment method which can be used effectively for the classification of water quality.

Table 4 Testing accuracies of four kinds of metal ions

No.	Accuracy (%)			
	Cu^{2+}	Hg^{2+}	Cr^{6+}	Cd^{2+}
1	88.391	73.625	79.038	83.534
2	87.583	74.003	78.137	82.632
3	88.611	72.233	80.090	82.556
4	86.921	73.161	79.715	84.887
5	87.142	72.366	79.414	84.962

5 Conclusions

In this study, acute toxicity experiments using four kinds of metal ions were performed on zebrafish. The response of zebrafish to Cu^{2+} was strong, reflecting a rapid deterioration in water quality. With this method, changes in water quality can be detected with high sensitivity and reliability, suggesting that it is a good method for evaluating water quality.

The use of SVM combined with GA achieved satisfactory results and reduced the time needed to train the model. The model has a good generalization ability and a high classification accuracy, which is of great value.

Because the behaviors of fish vary at different temperatures and in response to the combined effects of different toxins, further studies are needed to consider the influences of composite pollution and water temperature on the behavior of fish.

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