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Behavioral response of tilapia (*Oreochromis niloticus*) to acute ammonia stress monitored by computer vision*

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Abstract: The behavioral responses of a tilapia (*Oreochromis niloticus*) school to low (0.13 mg/L), moderate (0.79 mg/L) and high (2.65 mg/L) levels of unionized ammonia (UIA) concentration were monitored using a computer vision system. The swimming activity and geometrical parameters such as location of the gravity center and distribution of the fish school were calculated continuously. These behavioral parameters of tilapia school responded sensitively to moderate and high UIA concentration. Under high UIA concentration the fish activity showed a significant increase ($P<0.05$), exhibiting an avoidance reaction to high ammonia condition, and then decreased gradually. Under moderate and high UIA concentration the school's vertical location had significantly large fluctuation ($P<0.05$) with the school moving up to the water surface then down to the bottom of the aquarium alternately and tending to crowd together. After several hours' exposure to high UIA level, the school finally stayed at the aquarium bottom. These observations indicate that alterations in fish behavior under acute stress can provide important information useful in predicting the stress.

Key words: Ammonia stress, Tilapia, Computer vision, Aquaculture
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INTRODUCTION

Fish behavior under culture conditions holds important information for aquaculturist (McFarlane *et al*., 2004; Kristiansen *et al*., 2004). Most physiological, environmental changes and handling process can induce variations in fish behavior (Israeli and Kimmel, 1996; Israeli-Weinstein and Kimmel, 1998; Petrell and Ang, 2001; Almazán-Rueda *et al*., 2004). Methods of monitoring and quantifying the behavioral response have become potential alternatives for assessing stress, disease, water pollution and toxic material in water (Nogita *et al*., 1988; Kane *et al*., 2004).

In intensive rearing systems, particularly recirculating aquaculture systems, ammonia concentrations may reach high levels. In water, total ammonia-nitrogen (TAN) exists in two forms: ionized form NH_4^+, and unionized form NH_3 (UIA) which is far more toxic than NH_4^+ (Lawson, 1995). Water pH levels have profound influence on the proportion of UIA, with an increase of one pH unit generally causing tenfold increase in the proportion of UIA (Albert, 1973). Addition of baking soda to a system to increase its alkalinity can inadvertently increase UIA concentration resulting in catastrophic effect within a short time (Timmons *et al*., 2002). Early detection of acute stress conditions can reduce production losses.

Growth of tilapia, routinely measured in chronic ammonia toxicity experiments (Hargreaves and Kucuk, 2001; El-Shafaia *et al*., 2004) was not a sensitive indicator of toxicity. Behavioral parameters can be measured continuously in-situ, and are sensitive to

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responses by fish to acute stress condition (Beitinger, 1990). The objective of this study was to monitor the behavioral alterations of a tilapias school in response to different UIA concentration using a computer vision system.

MATERIALS AND METHODES

Fish and experimental system

Tilapias (*Oreochromis niloticus*), (75.2±12.1) g (mean weight±*SD*) were assigned into experimental aquaria with 15 fish per aquarium (50 fish/m^3) to acclimatize them for 3 weeks prior to the experiment. Water quality parameters were: temperature (23 to 27 °C), oxygen level (6.5 to 7.5 mg/L), TAN (below 1.0 mg/L) and pH (6.5 to 7.3). The photoperiod was 12 h light and 12 h dark. The fish were fed a commercial diet at 3% bodyweight/day. Glass aquarium (115 cm×60 cm×70 cm, 58 cm water depth) with a transparent side was divided into two compartments by a vertical partition wall. The bigger one (90 cm long) was for rearing and the smaller one (25 cm long) for filtering, aeration and heating. The water in the small compartments flowed to the rearing compartments by means of an aquarium pump at the rate of approximately 7 L/min. Water in the rearing compartment returned by gravity to the small compartment for filtering and heating through a hole in the partition wall. Water was replenished with heated, dechlorinated tap water at a flow rate of 15 L/h.

Computer vision system

Fish images were acquired by color video cameras (Honeywell, GC-655N) connected to a Pentium IV computer equipped with a frame grabber (Microview, MVPCI-700). The light intensity at the water surface was about 300 lx illuminated by overhead fluorescent lamps. Each frame was digitized with a resolution of 768 pixels×576 pixels. Frames were captured using the modified ATVC version 5.0 software package (All_Time Imaging System Corp. Hangzhou, China). The experimental facilities were separated to prevent disturbances to fish. The hardware and coordinate system is shown in Fig.1, in the *X* direction between 0 and 500 pixels, in the *Z* direction between the bottom of the compartment (0 pixels) and the water surface (489 pixels).

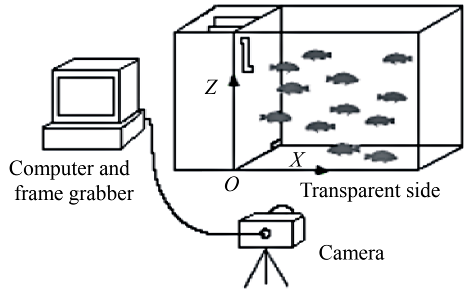


Fig.1 Schematic map of the experimental system

Behavioral parameters calculations

Quantified fish school behavioral parameters included swimming activity and distribution of the school. Swimming activity was calculated using the method of Xu *et al*.(2006). In short, frame sequences captured at special time interval were subtracted in pairs after image segmentation and extraction. By labelling components caused by fish movement in difference frame, the projected area caused by the movement of every fish in the capture interval was calculated; this projected area was divided by the projected area of every fish in the later frame to get body length moving distance of each fish, and further obtained the body length (BL) speed. The average speed of all fish can respond well to the fish school activity. The distribution parameters were calculated using the method of Israeli and Kimmel (1996) with the coordinates of the gravity center of the fish school *CX*, *CZ* indicating the mean location of the school in *Z* and *X* directions, and the spatial standard deviations of fish coordinates in *X* and *Z* directions *SDX*, *SDZ* indicating average dimension or density of the school. These indices were measured in pixels.

Experimental procedures

All fish were fasted the day prior to experiments. During the experiment water replenishment was halted and water temperature was maintained at (24.9±0.3) °C. The experiment began at 8:30 am. Ammonia stress was induced by adding ammonium chloride to each aquarium to achieve TAN concentrations of 2.0, 12.0, 40.0 mg/L. At the beginning the pH was maintained at 6.9±0.1 for about 120 min to act as a reference and corresponding UIA concentrations of 0.008, 0.048, 0.159 mg/L respectively. Sodium hydroxide solution was added gradually into the water to increase the pH to 8.1±0.1 during a period of about 20 min, accordingly UIA concentrations increased to 0.13 (low), 0.79 (moderate), 2.65 mg/L (high) level. pH was measured by a pH meter (Hanna Instruments, HI9024); TAN concentration was measured by Nesslerization; UIA was calculated as a function of TAN, temperature, and pH (Albert, 1973). UIA remained at the high level for two days. The ammonia and pH level declined slowly due to some ammonia evaporation and nitrification. The water parameters were measured and adjusted by adding ammonium chloride and sodium hydroxide solution twice daily.

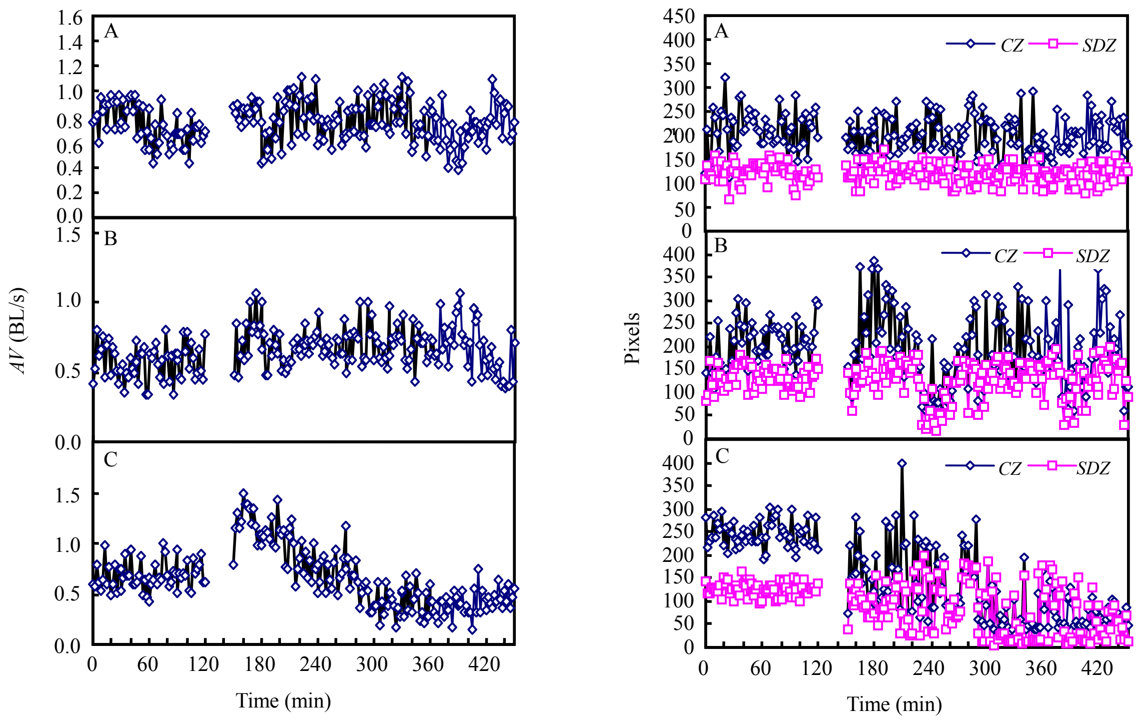
The first monitoring period (120 min) was recorded for reference when pH was 6.9; the second (300 min) began at 10 min after pH increased to 8.1. During the monitoring period 11 frames were captured once every 2 min. The fish swimming speeds at 10 intervals between 11 frames were averaged and the result *AV* was taken to represent the activity of the school. The geometrical parameters of the school *CX*, *CZ*, *SDX* and *SDZ* were the average value calculated from the 11 frames.

All trials were conducted in triplicate. *F*-test was used to test the heterogeneity of variance before and after the UIA treatment. Analysis of variance (ANOVA) was used for determining the temporal effects under the same UIA level. To test for the difference among means under different UIA level at the same time period, Tukey's test was used.

RESULTS AND DISCUSSION

AV, *CX*, *CZ*, *SDX* and *SDZ* under normoxic condition (three periods of an hour respectively in morning, noon, afternoon) had no significant changes.

The typical variations of school activity *AV*, geometrical parameters of school *CZ* and *SDZ* at three UIA concentration levels are shown in Fig.2. Under low UIA concentration level the fish usually occupied the whole tank and were observed to swim actively and randomly in all directions; the activity and distribution parameters had no large fluctuation. The moderate UIA level condition elicited readily observable changes in fish school location and density in vertical direction; *CZ* and *SDZ* had significantly large fluctuation ($P<0.05$). After a period of exposure, *SDZ* decreased with the fish being more crowed together



(a) (b)

Fig.2 The alteration of school behavioral parameters (*AV* (a) and *CZ*, *SDZ* (b)) under different UIA concentration. All parameters are given out every 2 min. A: Under low UIA level; B: Under moderate level; C: Under high level

between times. High UIA level condition elicited a marked increase in school activity at the first hour ($P<0.05$) exhibiting an avoidance reaction (trying to flee from high UIA condition). The vertical distributions became wavering, *CZ* and *SDZ* had obvious larger fluctuation ($P<0.05$), and later showed concurrent decrease when the swimming activity decreased. The fish gradually dived to the bottom of the aquarium, and stayed there, finally 36% died.

CZ and *SDZ* had more sensitive responses to UIA level changes than swimming activity, frequency distribution of *CZ* at different location and times under three UIA levels are shown in Fig.3. The locations of *CZ* were divided into three ranges: low (0~100 pixels), moderate (100~200 pixels) and high (200~300 pixels). Under normal and low UIA levels *CZ* were all in the moderate and high location ranges; under moderate UIA level *CZ* had significant increase ($P<0.05$) in low location range, time had no significant effect on the frequency distributions of *CZ*; under high UIA level more *CZ* were distributed in the low location range, time had significant effect on the frequency distributions of *CZ*, and in the 5th hour almost all *CZ* were in low location range. The results of *SDZ* under different levels and time periods were the same as those of *CZ*. *CX* and *SDX* had no obvious characteristic change during the whole course. Under high UIA level exposure, darkened skin color of fish was observed in 2 h.

At the second day, under low and moderate UIA level the behavior had no significant difference with that of the first day, while under high UIA level the fish school had significant small distribution ($P<0.05$) and the location was also significantly low ($P<0.05$).

CONCLUSION

The behavioral parameters responded sensitively to the increase in UIA level. The fish schools under normal conditions swim actively and distribute evenly. The abrupt increase in swimming activity, acute fluctuation of school: staying at the bottom or ascending to the water surface, obvious decrease in distribution and darkening in body color are typical stress signals of fish to high UIA concentration. These methods of monitoring behavioral parameters have the potential to be developed for detecting stress early in aquaculture systems and therefore decrease production losses.

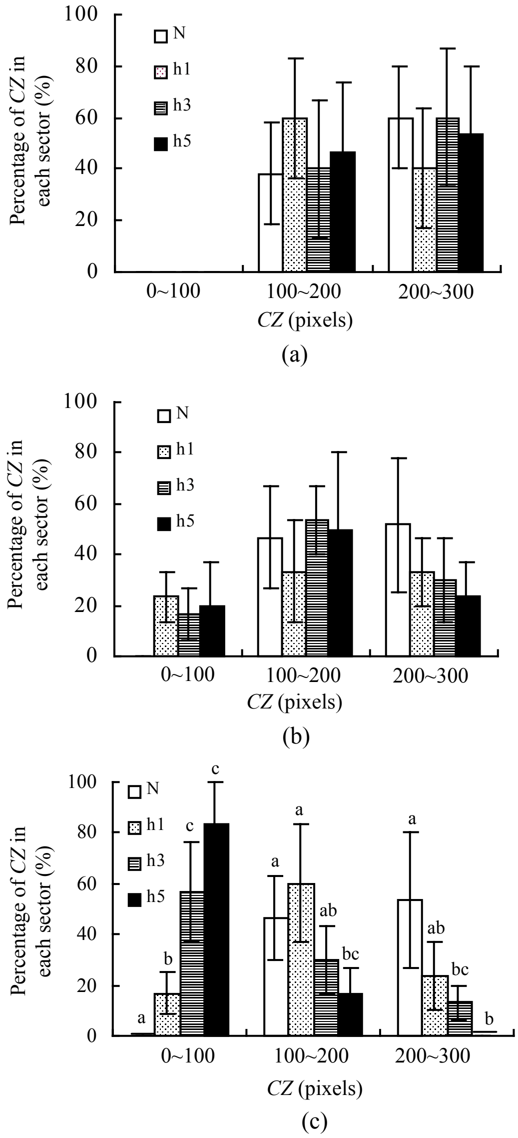


Fig.3 Frequency distribution of school location *CZ* at different time under different UIA level (a) under low UIA level; (b) under moderate UIA level; (c) under high UIA level. N: normal level period before treatment; h1: the first hour; h3: the 3rd hour; h5: the 5th hour. Bars with different letters are significantly different from each other (*P*<0.05)

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