Ecotoxicology and Environmental Safety 111 (2015) 185–191

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

# Water quality monitoring using abnormal tail-beat frequency of crucian carp

Gang Xiao [a], Min Feng [a], Zhenbo Cheng [a,*], Meirong Zhao [b], Jiafa Mao [a], Luke Mirowski [c]

[a] Department of Computer Science and Technology, Zhejiang University of Technology, Hangzhou, China
[b] College of Environmental and Resource Sciences, Zhejiang University of Technology, Hangzhou, China
[c] School of Computing & Information Systems, University of Tasmania, Australia

ARTICLE INFO

*Article history:*
Received 6 June 2014
Received in revised form
23 September 2014
Accepted 25 September 2014
Available online 28 October 2014

*Keywords:*
Water toxicology
Fish
Behavior
Movement
Tail-beat frequency
Wall-hitting rate

ABSTRACT

Fish are rapidly becoming favored as convenient sentinels for behavioral assays of toxic chemical exposure. Tail-beat frequency (TBF) of fish is highly correlated with swimming speed, which has been used to detect toxicants. Here we examined the effect on TBF of exposure to two chemicals, and evaluated the ability of this novel behavioral parameter to accurately monitor water quality. To further refine our approach, the Wall-hitting rate (WHR) was used to characterize behavioral avoidance after exposure. Overall, exposure to test chemicals at different levels induced significant increase in both behavioral parameters of the red crucian carp during 1-h exposure periods. Furthermore, the TBF achieved better performance as an indicator when it was calculated in cases where the fish hit the tank wall. Collectively, this study demonstrates the capacity of the TBF of fish to assess water quality in a reliable manner.


## 1. Introduction

With increasingly rapid economic and agricultural development in China, pollution caused by industrial and agricultural spills into water supplies has become common in recent years (Wang et al., 2008). In particular, there has been an increasing number of spill accidents into the aquatic environment involving toxic chemicals, such as cyanide compounds, phenol, and several pesticides (Liu et al., 2005; Soldán et al., 2001). Polluted water may have serious direct and indirect impacts on human health (Wu et al., 1999). Although a number of biochemical monitoring methods are available to evaluate water quality (Lavado et al., 2006; Owen et al., 1979), these do not provide instantaneous warnings because they require a sequence of costly laboratory tests. Therefore, it is highly desirable to provide a complementary monitoring system that is able to respond quickly and at low cost.

Behavior has been regarded as an effective indicator for linking the physiology and ecology of an organism in its environment (Chon et al., 2009; Little et al., 2001). Behaviors may selectively and continuously adapt in response to direct interaction with physical and chemical aspects of the environment. Thus, behavior has been used to discern and evaluate the effects of exposure to environmental stressors (Baldwin et al., 1994; Balk et al., 1996; Gruber et al., 1994; Kane et al., 2004; Kim et al., 2011). Behavioral monitoring in toxicology provides well-defined endpoints that are practical to measure and to understand in relation to environmental factors that cause variation in the response; use of such indirect monitoring is referred to as surrogacy. In nature, behavioral surrogacies are sensitive to a range of contaminants and have been observed in a range of species (e.g., carp and prawns) to provide continuous information on in situ environmental changes (Chon et al., 2009; Scott and Sloman, 2004; Scott et al., 2003; Sloman and Armstrong, 2002; Sloman et al., 2003; Zhou and Weis, 1998). Consequently, behavioral monitoring is viewed as a valuable biological early warning system that can be applied in variety of environmental conditions.

Fish have been considered as ideal surrogates in behavioral assays of toxic chemical exposure (Kane et al., 2005) because exposure-related behavioral alterations of fish behavior can be distinguished as fixed action patterns. Investigators often characterize the behaviors of fish on the basis of parameters derived from movement tracking, e.g., speed, acceleration, temporal trajectory, and stop duration (Chon et al., 2009; Kuklina et al., 2013). Cairns et al. indicated changes of water quality by studying movement patterns and breathing patterns of fish, and developed a systematic approach to water quality management (Cairns et al., 1970). Fukuda et al. (2010) proposed that the entropy of behavioral parameters could characterize the toxic response of the fish, and Thida et al. (2009) described an approach for automatic clustering

* Corresponding author.
*E-mail address:* czb@zjut.edu.cn (Z. Cheng).

http://dx.doi.org/10.1016/j.ecoenv.2014.09.028
0147-6513/

of fish swimming patterns and abnormality detection using a spectral clustering method. Ma et al. (2010) proposed a real-time water quality monitoring scheme, which was based on judging time-series motion trajectories of live fish.

For fish species, the tail plays a crucial role in propelling and guiding the animal during swimming (Handegard et al., 2009; Motani, 2002). The relationship between a fish's tail beating and the fish's swimming performance has attracted broad attention for a long time (Bainbridge, 1958; Fu et al., 2013a; Hunter and Zweifel, 1971; Steinhausen et al., 2005). The tail beat of fish contains a highly structured and predictable sequence of activities and tail beat frequency (TBF) has therefore been used to estimate the active metabolic rate of fish (Ohlberger et al., 2007). In addition, the swimming speed of fish, which is also considered to be a good behavioral indicator of toxic chemical exposure (MacLatchy and Robinson, 2009), is highly related to the frequency and amplitude of the tail beat (Bainbridge, 1958; Hunter and Zweifel, 1971). In particular, it was proposed that microcystins and malachite green induced toxic effects that could be detected by the signal of TBF (Zhi-dong et al., 2011). These observations suggest that the TBF might be a suitable behavioral characteristic for detection of the responses of fish to toxins in the water. However, to date, there has been limited analysis of this possibility.

This study proposes a simple method for detecting the responses of red crucian carp to toxins in water, based on TBF. The long-tailed red crucian carp is widespread throughout China and shows moderate swimming performance (Johnston and Goldspink, 1973; Pang et al., 2011). Here we conducted a series of exposure tests to determine the responses of the fish to the toxins sodium hydroxide (NaOH, 20 mg/L) and glyphosate (0.05 mg/L, 0.1 mg/L and 0.2 mg/L). Behavior of the fish was quantified by measuring TBF and wall-hitting rate (frequency of collision using the head with the wall of the tank), both which are quantitative evaluations of escape behavior (Budick and O′Malley, 2000; Webb, 1979). This paper concludes by offering a novel behavioral parameter capable of characterizing responses of fish to water-borne toxins. The results are sufficiently promising to support further research into this area.

## 2. Materials and methods

### 2.1. Test subjects

Crucian carp (*Carassius auratus*), belonging to the family Cyprinidae (Telestei), is widely distributed on the Eurasian continent (Chen and Huang, 1982). Red crucian carp, of 5–7 cm body length and 2–3 cm tail length, were considered suitable for image recognition in this study because of the marked difference between their color and that of the water. A total of 140 adult red crucian carp were obtained from a pet supplier in Hang Zhou, China. Fish were acclimated to the laboratory environment for one week and housed in a 60-L aquarium containing filtered de-chlorinated water maintained at room temperature (25 °C ± 5 °C) and in pH value at 7 ± 1. Illumination was provided by ceiling-encircled fluorescent lights on a 14-h cycle (on: 8:00 h, off: 22:00 h). All fish used in the experiment were fed artificial tropical fish food once per 3 days. All experimental procedures were approved by the Zhejiang University of Technology Institutional Animal Care and Use Committee.

### 2.2. Measurement of tail-beat frequency

The images were first converted to gray scale and inverted because the fish was darker than the surrounding area. A background image was made by averaging 45 frames in the first 3 s, and this was subtracted from each frame. The fish were then detected by thresholding with an empirically-determined constant value, and the contour of each fish was identified by applying an edge-detection algorithm on the thresholded image (Fig. 1). Next, we identified the centroid, tail point and head point of the fish in the raw images (Fig. 2a). The centroid was obtained from the average coordinates of the points on the contour. Since the frontal area of red crucian carp is greater than the area of the tail, the centroid is located further from points on the tail than from points around the frontal region (Chen et al., 2009). Therefore, we defined the tail point of fish as the farthest point from the centroid and the head point as the farthest point on a line between the tail point and the centroid extended in the opposite direction. As shown in Fig. 2a, we computed the tail-beat amplitude as the perpendicular distance from the tail point to the centerline drawn between the tail point and the centroid in the unflexed tail. The angle of the tail beat ($\theta$) was computed as the maximum acute angle between the centerline and the line between by the centroid and the tail point. A tail beat was defined as movement between $\theta = 0°$ in one frame and $\theta = 0°$ in the next frame, provided $\theta$ was $> 5°$ between the two frames. The TBF was then computed as the number of tail beats per minute.

### 2.3. Wall-hitting rate

To determine whether a fish hit the wall of the tank, the water contour was first detected as the outer boundary between the tank and the horizontal surface of the water. The boundary circle was then designated as a zone three pixels wide inside the water contour. A wall hit was defined as the head point of the fish reached the boundary circle (Fig. 2b). A wall-hitting event was set to 1 if more than three hits occurred during 1 s; otherwise it was

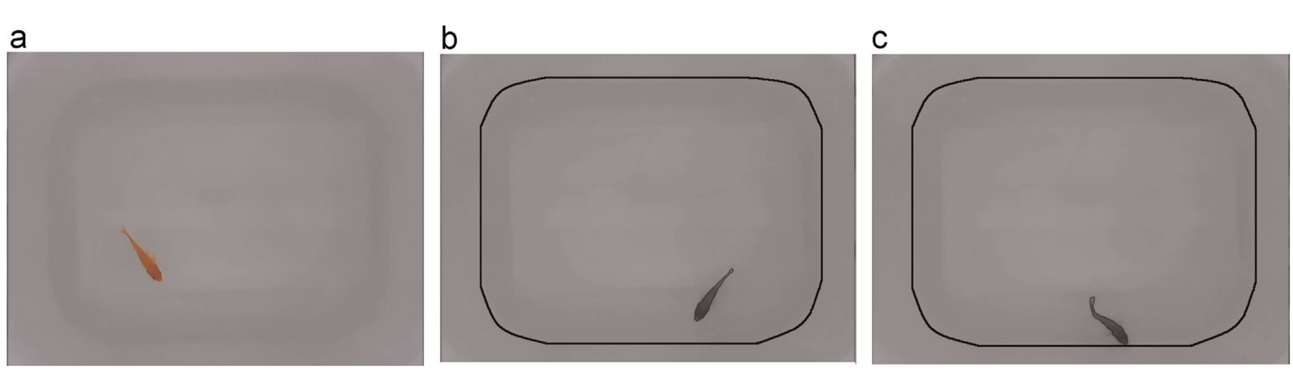


**Fig. 1.** Test fish in the experimental tank. (a) Original image. (b) Fish under unexposed condition (gray image). The fish has a moderate swimming activity with a low tail beat frequency, preferring to swim near the wall of the tank. (c) Fish under exposed condition (gray image). The fish strikes against the wall using its head, apparently attempting to escape from the area exposed to the contaminant, and accompanied by violent tail beating.