วารสารวิทยาศาสตร์บูรพา ปีที่ 26 (ฉบับที่ 3) กันยายน – ธันวาคม พ.ศ. 2564
BURAPHA SCIENCE JOURNAL Volume 26 (No.3) September – December 2021 บทความวิจัย

การศึกษาความเร็วการว่ายน้ำของปลานิลโดยใช้เทคนิคคอมพิวเตอร์วิทัศน์

Tilapia Swimming Velocity Study Using Computer Vision Technique

รุ่งพฤทธิ์ จงเจริญสุข และ วราห์ เทพาหุดี*

Roongparit Jongjaraunsuk and Wara Taparhudee*

ภาควิชาเพาะเลี้ยงสัตว์น้ำ คณะประมง มหาวิทยาลัยเกษตรศาสตร์

Department of Aquaculture, Faculty of Fisheries, Kasetsart University

Received : 19 November 2020

Revised : 16 December 2020

Accepted : 18 December 2020

บทคัดย่อ

การศึกษาในครั้งนี้แบ่งออกเป็น 2 การทดลอง คือ 1. ผลของการใช้จำนวนเฟรมต่อวินาทีที่แตกต่างกันต่อข้อมูลที่ได้รับ และ 2. ผลของการใช้ฟอร์มาลีนความเข้มข้นที่แตกต่างกันต่อความเร็วการว่ายน้ำของปลานิล โดยทั้ง 2 การทดลองใช้ปลานิลขนาด 0.5-1 กรัม จำนวน 3 ตัว/ชุดการทอลอง (3 ซ้ำ) ผลการทดลองที่ 1 จากการใช้จำนวนเฟรมต่อวินาที (Frame per second; FPS)ในการประมวลผล 4 ระดับคือ 1 3 5 และ 10 FPS พบว่าผลของความเร็วในการว่ายน้ำของปลาที่ได้ (Fish swimming velocity; FSV) ไม่มีความแตกต่างกันทางสถิติ ($P > 0.05$) ในทุกชุดการทดลอง และชุดความเร็วการว่ายน้ำที่ได้ (V-set) และจำนวนเฟรมที่ได้สำหรับการใช้ในการประมวลผลความเร็ว 1 ชุด (Frame/V-set) ของการใช้ 1 และ 3 FPS ไม่มีความแตกต่างกันทางสถิติ ($P > 0.05$) ดังนั้นจึงเลือกใช้ 3 FPS ในการทดลองที่ 2 เนื่องจากมีระยะเวลาในการประมวลผลรวดเร็วกว่าการใช้ 1 FPS 3 เท่า ในการทดลองที่ 2 ความเร็วในการว่ายน้ำเฉลี่ยของลูกปลานิลใน 3 ชุดการทดลอง มีความแตกต่างกันทางสถิติ ($P < 0.05$) โดยชุดการทดลองที่ 1 (ไม่มีการใช้ฟอร์มาลีน) ปลามีความเร็วเฉลี่ยสูงที่สุดคือ 0.038±0.005 เมตร/วินาที ในขณะที่ ชุดการทดลองที่ 2 (ใช้ฟอร์มาลีน 300 mg/L (37 เปอร์เซ็นต์ ฟอร์มาลดีไฮด์)) ค่าความเร็วเฉลี่ยในการว่ายน้ำที่ได้ไม่แตกต่างกับทั้งชุดการทดลองที่ 1 และ 3 (600 mg/L) โดยมีค่าเฉลี่ยคือ 0.029±0.025 เมตร/วินาที และ ชุดการทดลองที่ 3 ค่าความเร็วเฉลี่ยที่ได้ มีค่าน้อยกว่าชุดการทดลองที่ 1 แต่ไม่แตกต่างทางสถิติ ($P > 0.05$) กับชุดการทดลองที่ 2 คือ 0.019±0.015 เมตร/วินาที ตามลำดับ จากผลการศึกษาในครั้งนี้แสดงให้เห็นว่าสามารถนำเทคนิคคอมพิวเตอร์วิทัศน์มาประยุกต์ใช้กับการศึกษาพฤติกรรมของสัตว์น้ำอื่น ๆ ต่อไปได้ ซึ่งจุดเด่นคือสามารถแสดงผลได้อย่างต่อเนื่อง และไม่รบกวนสัตว์น้ำ

คำสำคัญ : ปลานิล ; ความเร็วการว่ายน้ำ ; คอมพิวเตอร์วิทัศน์

Abstract

This study was divided into 2 experiments: 1) the effect of different numbers of frames per second on the received data, and 2) the effect of different formalin concentrations on tilapia swimming velocity. Three fish, each weighing between 0.5 and 1 g were used for each experiment (3 replicates). The first experiment applied four frame rates of 1, 3, 5 and 10 frames per second (FPS) to determine fish swimming velocity (FSV). Results showed no significant differences among treatments ($P > 0.05$). The swimming velocity set (V-set) and number of frames obtained for use at one processing speed (Frame/V-set) using 1 FPS and 3 FPS were not statistically different ($P > 0.05$). Hence, the 3 FPS rate was chosen for the second experiment and three levels of formalin concentrations were applied at 0, 300 and 600 mg/L to compare FSV differences. Results showed that average FSV in the three trials were statistically different ($P < 0.05$). Average FSV of the first treatment (no formalin applied) was highest at 0.038 ± 0.005 m/s, the second treatment (300 mg/L of formalin (37% formaldehyde, Sigma-Aldrich ®) was 0.029 ± 0.025 m/s, and not significantly different ($P > 0.05$) from the FSV results of the first and third treatment (600 mg/L) and the third treatment was 0.019±0.015 m/s, that less than the first treatment but not significantly different ($P > 0.05$) with the second treatment respectively. The results of this study show that the computer vision techniques can be applied to study behavior of other aquatic animals. The advantages are obtaining accurate result and can continuously monitor without disturbing the aquatic animals.

Keywords : tilapia ; swimming velocity ; computer vision

*Corresponding author. E-mail : ffiswrt@ku.ac.th

Introduction

Physiological and environmental condition changes instigate variations in aquatic swimming velocity and behavior. (McFarlane *et al*., 2004; Kristiansen *et al*., 2004). Methods for continuously monitoring swimming velocity or behavioral responses of aquatic animals are significant ways to assess stress, disease and water quality parameters. If swimming velocity or behavioral changes are detected quickly, the aquaculturist can solve problems that arise immediately to reduce production losses (Xu *et al*., 2016).

Acute stress in fish may result in adverse physiological and behavioral manifestations. Changes in plasma catecholamines, corticosteroids, glucose and cortisol are accepted as physiological indicators of acute stress in aquatic animals or fish (Barton & Iwama, 1991; Maxime *et al*., 1995; Vijayan *et al*., 1997).

Traditional methods for stress measurement use drilling and blood sampling; however, these disturbances can rapidly increase hormonal stress parameters and also require an experienced technician. Therefore, many researchers have investigated ways to detect stress in fish by continuous in situ non-invasive and non-contact measurement methods (Shezifi *et al*., 1997; Israeli-Weinstein & Kimmel, 1998).

Ventilatory frequency (VF) can be used as a visual tool to evaluate fish behavior. The VF study must not interfere with the normal behavior of the fish. VF studies are able to identify stress conditions in aquatic animals but cannot indicate the level of stress. Barreto & Volpato (2004) studied the degree of ventilation stress in tilapia and found that this changed rapidly under confinement but did not reflect the severity of the stimulus. Xu *et al.* (2016) studied tilapia behavioral responses to oxygen levels using computer vision. Results showed that tilapia were fast swimming with high ventilation rate during the first stage of hypoxia; these parameters both reduced under abnormal conditions but stress levels could not be quantified.

The use of computer vision techniques may allow qualification of the stress levels of aquatic animals as an automated, non-invasive and cost-effective method to record behavioral parameters that have already been applied to assess the toxicity of chemicals on behavioral changes (Nogita *et al.*, 1988; Baganz *et al.*, 1998; Handy *et al*., 1999).

Tilapia were used in this study because they are economically important. Aquacultural production of Nile tilapia (*Oreochromis niloticus*) has substantially increased over the past decades and this fish species is now ranked among the most cultured in the world (FAO, 2016; Wang & Lu, 2016). In Thailand, tilapia ranks among the main cultured fish species with production of approximately 337,500 metric tons per year (Global-Aquaculture, 2016).

Toxic chemicals as pesticides and insecticides cause changes in fish behavior (Sabra & El-Sayed, 2015). Pesticides are used to control or remove pests such as algae or aquatic plants that cause poisoning or affect water quality, while insecticides are used to control insects by killing or preventing them from engaging in unwanted destructive behaviors. Pesticide and insecticide usage in agricultural or aquaculture to control pests or parasites is extremely toxic to non-target organisms like fish and affects fish health through the impairment of metabolism, sometimes leading to mortality (Shankar *et al*., 2013). In addition to pesticides insecticides, useful chemicals are also commonly used in aquaculture. Formalin is a highly effective chemical used for disease management in many fish species, replacing malachite green (Buchmann & Kristensson, 2003). Formalin is easily metabolized by aquatic organisms and has low potential for bioaccumulation (Picón-Camacho *et al*., 2012). The main disadvantage of using formalin in aquaculture is its high toxicity to fish in cases of excessive use. Tancredo *et al*. (2019) reported that formalin at 100 mg/L did not cause mortality or change fish behavior over a 1 h period, while at over 200 mg/L the fish died within 24 h. In this study, the tilapia were required to change their swimming behavior within 60 minutes and two levels of formalin at 300 and 600 mg/L were chosen.

Normally, change in tilapia swimming motion is caused by the fish's stress response process. There are two types of fish stress responses as physiological stress response and adaptation to stress to restore homeostasis (General Adaptation Syndrome; G.A.S.). Three levels of physiological stress responses have been defined. Primary response occurs within the nervous system and adrenal glands. This results in the production of hormones in response to stress. Secondary response occurs as the result of hormonal changes in the primary response, including changes in hematology, such as numbers of white blood cells and glucose levels (Mazeaud *et al*., 1977 & Barton, 2002). Tertiary response is observable as changes in fish growth, swimming performance or swimming velocity and aggression. Adaptation to stress can be divided into the three phases of alarm reaction, resistance and exhaustion (Barton, 2002).

A Tracker software program (V.5.0.6) was applied in this study. This open-source software was developed for video analysis (Douglas, 2019) and uses a computer to store the data. The modeling software marks the role of the object in each video frame derived from a video camera recording. The motion of the object is analyzed, to show outcomes such as position, speed, acceleration and time using the appropriate number of frames per second (FPS). Here, the effect of number of frames per second or frame rate on normal fish swimming velocity (FSV) was established using computer vision, and the effect of different formalin concentrations on change in FSV of tilapia was also determined.

Methods

Fish samples and study site

This experiment was conducted under laboratory conditions at the Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand. Fish initial weight was 0.5-1.0 g/ fish and 500 fish were obtained from the Kamphaeng Saen Fisheries Research Station, Faculty of Fisheries, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom and transported to the laboratory in plastic bags. The fish were acclimatized for one week in two 1,000 L rectangular tanks (250 fish/tank) with a flow-through system. Three air stones were placed in each tank to control water quality within the appropriate range as dissolved oxygen (DO), water temperature, pH, total ammonia-nitrogen (TAN), and nitrite-nitrogen (NO_2-N) at > 3 mg/L, 25-32 °C, 7.5-8.5, < 1 mg/L and < 1 mg/L, respectively (Azaza *et al*., 2008; Kolding *et al*., 2008; Tran-Duy *et al*., 2012). The fish were fed with floating pellets containing at least 35% protein twice a day at 8:00 am and 5:00 pm by hand until satiation. After the acclimatization period, three fish were randomly selected and placed in each of four experimental glass aquarium tanks, width 25 cm x length 51 cm x height 31 cm and 80% full of water. One air stone was added to each tank to improve air supply. Sediment suction was performed and 40% of the water volume in each tank was exchanged every day after completing feeding at 5:00 pm. All the fish were rested for 3 days before commencing the experiments.

Experimental design

1. Effect of number of frames per second (FPS) or frame rate on normal fish swimming velocity (FSV) using computer vision.

A completely randomized design (CRD) was performed. The experiment was divided into four treatments with three replicates as follows:

Treatment 1 (control), application of 1 frame/second (1 FPS).

Treatment 2, application of 3 frames/second (3 FPS).

Treatment 3, application of 5 frames/second (5 FPS).

Treatment 4, application of 10 frames/second (10 FPS).

2. Effect of different formalin concentrations on FSV using computer vision technique.

The second experiment was conducted after completion of experiment 1. A completely randomized design (CRD) was also performed. The second experiment was divided into three treatments with three replicates as follows:

Treatment 1 (control), no application of formalin.

Treatment 2, formalin at a concentration of 300 mg/L (37% formaldehyde, Sigma-Aldrich ®).

Treatment 3, formalin at a concentration of 600 mg/L (37% formaldehyde, Sigma-Aldrich ®).

Formalin at 300 and 600 mg/L was added to glass aquariums of known water volume.

Image processing

In both experiments, the camera used was a CCTV Kenpro Model KP-TVI8004HI. Image size was 1,024 x 764 pixels and 24 images per second were recorded using an Acer Aspire E 15 computer (Windows 10 Pro, AMD FX-9800P Radeon R7, 12 GB COMPUTE CORE 4G + 8G, 2.70 GHz, memory (RAM) 8 GB, System type 64-bit operating system) for data analysis as shown in *Figure 1*.

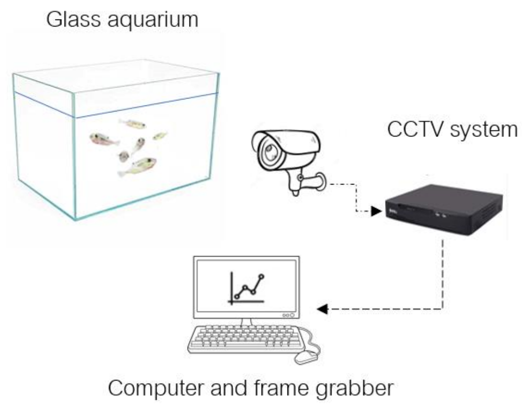

Figure 1 Schematic diagram of the experimental system.

The process began by selecting the video, then an axis was set and locked. Scale calibration was performed using a known length of the glass tank; 0.01 m. The coordinate axes were set at the center of the video to create a point mass, with the object chosen to capture movement speed (the fish body). The search button was pressed to capture the velocity the fish (*Figure 2*). The program analyzed the velocity of the fish by measuring the distance that the object moved in each frame against the time recorded for each frame.

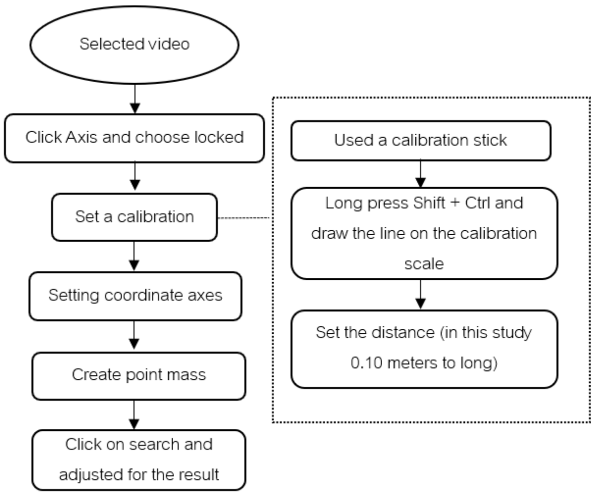

Figure 2 Animation process using the Tracker program.

Data analysis

Data were analyzed for average fish swimming velocity (FSV), velocity set (V-set) and the number of frames obtained for processing the velocity of 1 set (Frames/V-set). Details were as follows:

FSV analysis from the animation image using Tracker software was calculated by Equation 1.

$$FSV_{average} = \frac{\mathbf{v_1+v_2+v_3+\cdots+v_n}}{\mathbf{N}} \quad (1)$$

where N is the number of images used for analysis,

and $V_{1\text{-}n}$ is calculated from Equation 2.

$$V = \frac{\mathbf{S}}{\mathbf{T}} \quad (2)$$

Where V is the velocity of the fish (m/s), S is the displacement or distance between fish image capture and the last point (m), and T is the period from the beginning of the capture until the end of the recording at image (s).

Where each S is calculated for the resulting frame as shown in Equation 3

$S_1 = F_1+F_2+F_3+\ldots+ F_n$ (3)

$S_2 = F_1+F_2+F_3+\ldots+ F_n$

$S_3 = F_1+F_2+F_3+\ldots+ F_n$

…

…

S_n

Where F_1-F_n is the velocity achieved in each frame, with the number of frames per process following the method of (Xu *et al.*, 2006), i.e., each S must have a minimum of 10 frames or F_1-F_{10} per S.

In the first experiment, 5,000 frames were processed. The experimental data i.e. FSV, V-set and Frame/V-set were analyzed using one-way analysis of variance (one-way ANOVA) and the mean differences of each experiment were compared by Duncan's multiple range test at $P < 0.05$. All analyses were performed using SPSS Statistics version 24. In the second experiment, the best result of FPS from the first experiment was applied to investigate FSV using the same statistical analysis and software.

Results

1. Effect of number of frames per second (FPS) or frame rate on normal fish swimming velocity (FSV) using computer vision.

The experimental results showed no statistical differences ($P > 0.05$) of FSV among treatments. Average FSV of treatment 1(1 FPS), 2(3 FPS), 3(5 FPS) and 4 (10 FPS) were 0.041 ± 0.019 m/s, 0.036 ± 0.017 m/s, 0.036 ± 0.017 m/s and 0.027 ± 0.015 m/s, respectively.

The number of velocity sets (V-sets) that were processed at over 5,000 frames showed statistical difference ($P < 0.05$) between treatments. Treatment 1 had the highest V-set at 21.33 ± 4.0 sets, followed by treatments 2 (14.33 ± 0.58 sets), 3 (8.67 ± 1.58 sets) and 4 (6.00 ± 1.00 sets).

The number of frames obtained for one V-set (frames/V-set) was statistically different ($P < 0.05$) between treatments, with treatment 1 having the highest number of frames at 102.64 ± 67.92 frames/set but it did not differ from treatment 2 at 62.57±26.58 frames/set. Treatments 3 and 4 were 40.37±24.88 and 22.20±9.97 frames/V-set which were statistically different from treatment 1 but not statistically different from treatment 2.

An operation time (5,000 frames), the first treatment used the most computer time of 83.33 minutes, with processing time for treatments 2, 3 and 4 as 27.77, 16.66 and 8.33 minutes, respectively as shown in *Table 1*, *Figure 3* and *Figure 4*. The fish swimming directions are shown in *Figure 5*.

Table 1 Fish swimming velocity (FSV), V-set, number of frames/set and operation time under different FPS using Tracker software.

Treatment	FSV (m/s)	V-set (set)	Frames/V-set (Frame)	Operation time (min)
1	$0.041{\pm}0.019^{a}$	$21.33{\pm}4.04^{a}$	$102.64{\pm}67.92^{a}$	83.33
2	$0.036{\pm}0.017^{a}$	$14.33{\pm}0.58^{b}$	$62.57{\pm}26.58^{ab}$	27.77
3	$0.036{\pm}0.017^{a}$	$8.67{\pm}1.58^{c}$	$40.37{\pm}24.88^{b}$	16.66
4	$0.027{\pm}0.015^{a}$	$6.00{\pm}1.00^{c}$	$22.20{\pm}9.97^{b}$	8.33

**Note:* Mean±standard deviation with different superscript letters in the same column were significantly different ($P < 0.05$).

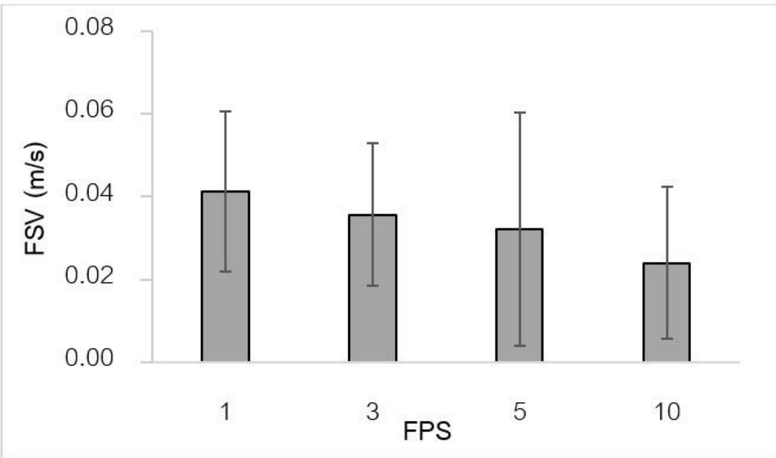

Figure 3 Fish swimming velocity at different frame speeds.

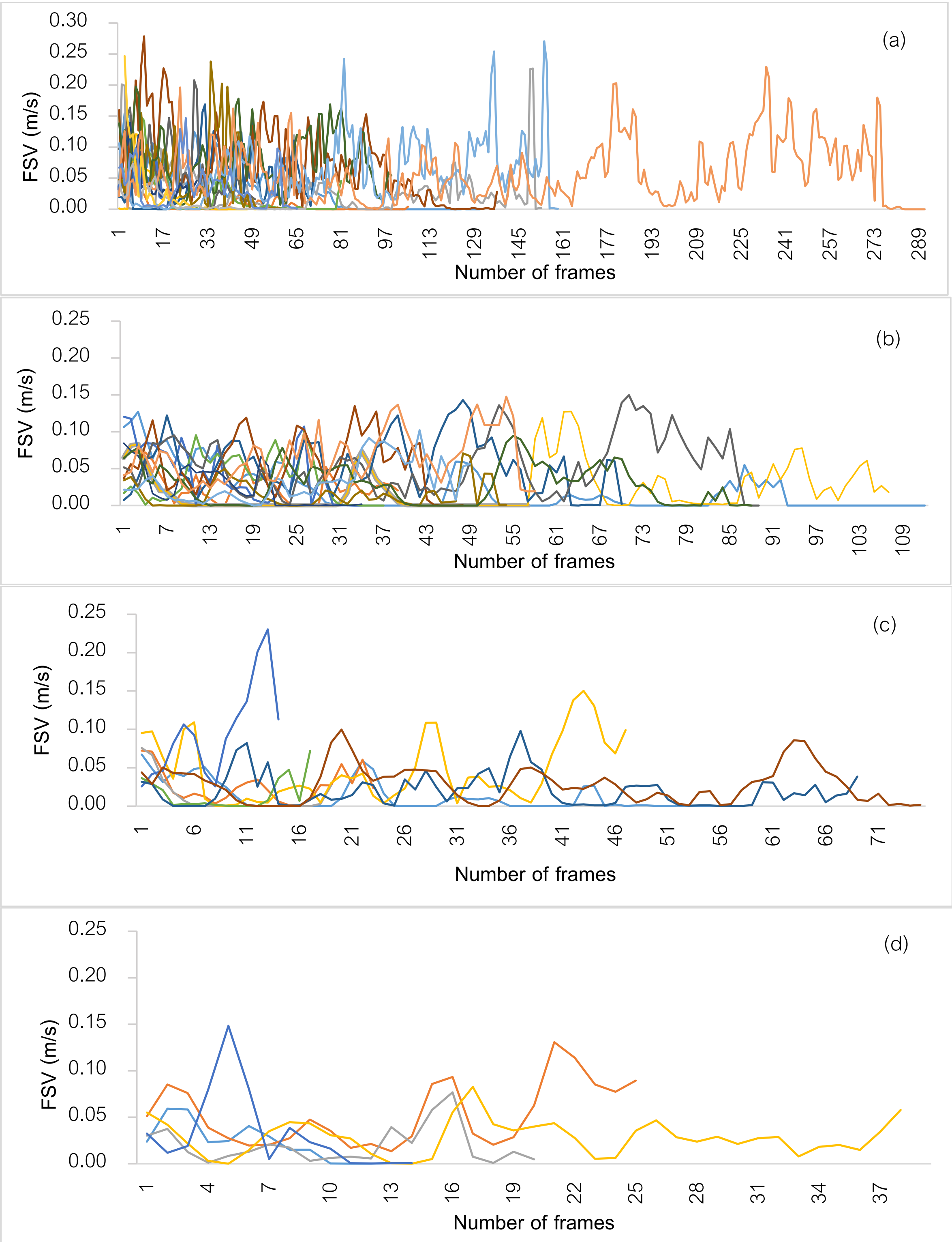

Figure 4 Example for one replication; alteration of FSV under different FPS. All V-sets in every treatment cover at least 11 frames from (a)-(d); 1 FPS with 17 V-sets, 3 FPS with 14 V-sets, 5 FPS with 8 V-sets and 10 FPS with 5 V-sets.

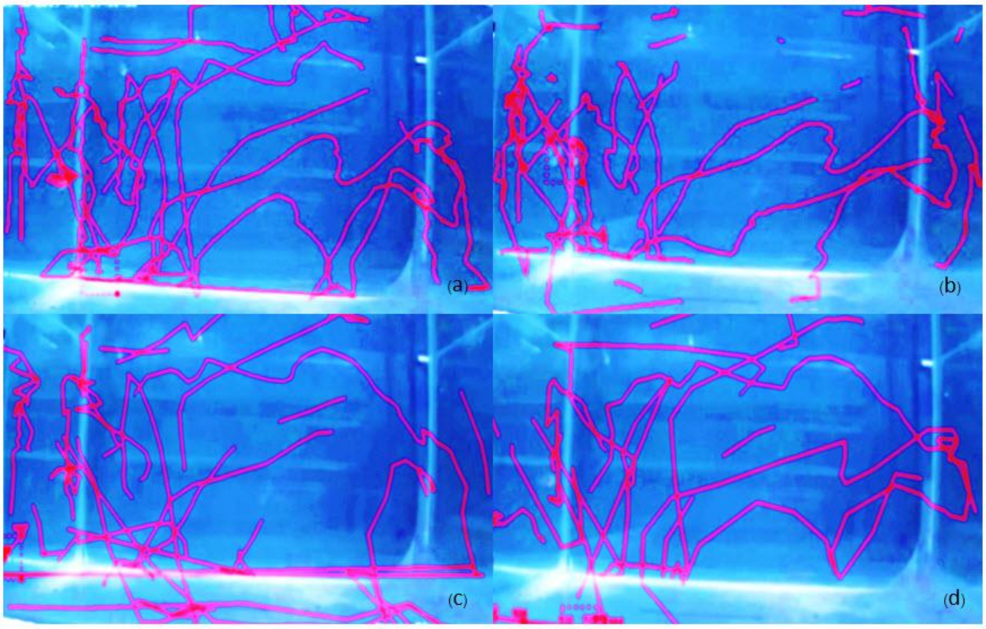

Figure 5 Tracking movement with 1FPS (a), 3 FPS (b), 5 FPS (c) and 10 FPS (d).

2. Effect of different formalin concentrations on FSV using the computer vision technique

Results showed that within a 60-minute period (using 3 FPS), average FSV in the three trials was statistically different ($P < 0.05$). In the first treatment (no formalin applied), the fish had FSV of 0.038 ± 0.005 m/s, while average FSV of treatment 2 (300 mg/L of formalin) was 0.029 ± 0.025 m/s and not significantly different from treatments 1 and 2. Average FSV of treatment 3 was 0.016 ± 0.016 m/s. This was the lowest velocity and significantly different compared to treatment 1 as shown in *Figure 6*.

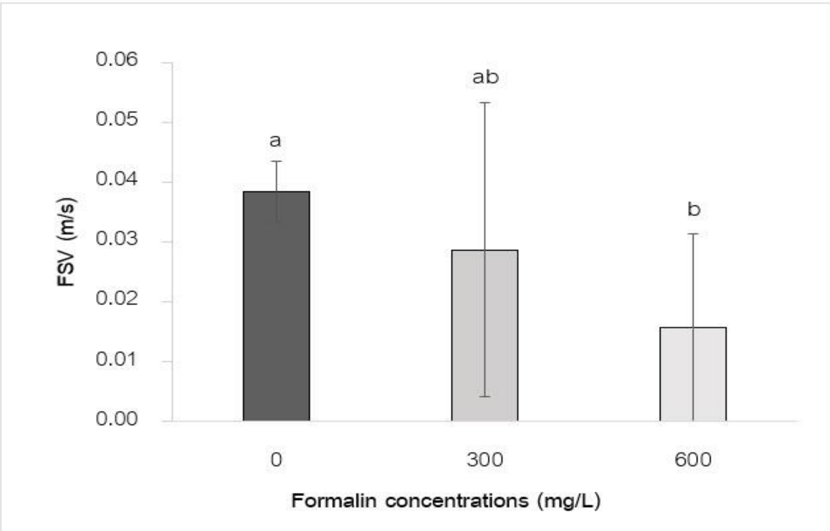

Figure 6 FSV under different formalin concentrations.

The average FSV values were divided into 12 periods of 5-minute intervals. Results showed that average FSV of treatment 1 was relatively constant at 0.038±0.005 m/s over the period of 60 minutes. This was the highest velocity and significantly different ($P < 0.05$) compared to treatments 2 and 3 after time period 6 (30 minutes). In the second treatment, the fish had the highest average FSV, which was statistically different ($P < 0.05$) from the other treatments during the first time period (5 minutes) of the experiment. After that, during time periods 5-12, there was a statistically different reduction in velocity ($P < 0.05$) compared to the first treatment. Average FSV values were between 0.028 and 0.097 m/s in time periods 1 to 4 and then decreased in the range of 0.007 to 0.030 m/s in time periods 5 to 12. Treatment 3 showed average FSV similar to the first and second treatments in the first five time periods but FSV then decreased statistically differently ($P < 0.05$) from the first and second trials. Average FSV was 0.023 to 0.041 m/s during time periods 1 to 5, while during time periods 6 to 12 the fish had average FSV in the range of 0.000 to 0.013 m/s as shown in *Table 2* and *Figure 7*.

Table 2 FSV in five minute time intervals under different formalin concentrations.

Total	Time	T1 (0 mg/L)	T2 (300 mg/L)	T3 (600 mg/L)	P-Value
	0-60	0.038 ± 0.005^{a}	0.029 ± 0.025^{ab}	0.019 ± 0.015^{b}	<0.05
Period	**Time**	**T1 (0 mg/L)**	**T2 (300 mg/L)**	**T3 (600 mg/L)**	**P-Value**
1	0-5	0.035 ± 0.004^{b}	0.097 ± 0.014^{a}	0.029 ± 0.004^{b}	<0.05
2	5-10	0.044 ± 0.005^{ab}	0.052 ± 0.006^{a}	0.041 ± 0.005^{b}	<0.05
3	10-15	0.032 ± 0.003^{a}	0.031 ± 0.011^{a}	0.023 ± 0.004^{a}	>0.05
4	15-20	0.036 ± 0.004^{a}	0.028 ± 0.009^{a}	0.033 ± 0.005^{a}	>0.05
5	20-25	0.032 ± 0.007^{a}	0.030 ± 0.007^{b}	0.038 ± 0.005^{a}	<0.05
6	25-30	0.033 ± 0.003^{a}	0.025 ± 0.001^{b}	0.004 ± 0.003^{c}	<0.05
7	30-35	0.048 ± 0.004^{a}	0.025 ± 0.003^{b}	0.013 ± 0.005^{c}	<0.05
8	35-40	0.039 ± 0.004^{a}	0.013 ± 0.003^{b}	0.002 ± 0.001^{c}	<0.05
9	40-45	0.042 ± 0.004^{a}	0.017 ± 0.007^{b}	0.003 ± 0.006^{c}	<0.05
10	45-50	0.042 ± 0.004^{a}	0.007 ± 0.005^{b}	0.002 ± 0.010^{c}	<0.05
11	50-55	0.038 ± 0.004^{a}	0.016 ± 0.006^{b}	0.000 ± 0.000^{c}	<0.05
12	55-60	0.040 ± 0.004^{a}	0.011 ± 0.002^{b}	0.000 ± 0.000^{c}	<0.05

**Note :* Mean±standard deviation with different superscript letters in the same row were significantly different ($P < 0.05$).

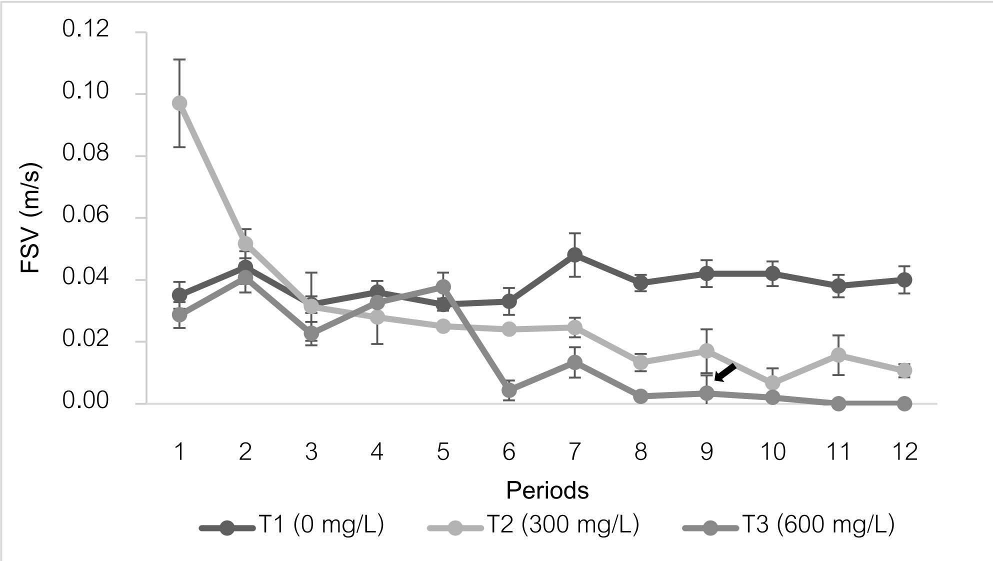

Figure 7 FSV in the 12 time periods of five minutes each under different formalin concentrations. The black arrow indicates mortality starting to occur.

Discussion

Results showed that for 1 FPS, FSV did not differ between treatments and obtained the best V-set and frame/V-set values. However, this took the longest time to process (83.33 minutes) and was therefore unsuitable for studying the FSV of tilapia. This result differed from Xu *et al*. (2006) who used 11 images per 10 seconds (about 1 FPS). They developed a program using Matlab and complex image processing techniques to assess fish stress by the change of area in each image caused by fish movement. This technique also took a long time to process and was unable to study fish swimming direction since the images were analyzed only in the frame area. Results of this study showed that using 3 FPS for processing was more suitable for studying tilapia swimming velocity behavior than 5 or 10 FPS because 3 FPS had V-set and number of frames/V-set that were not statistically different ($P > 0.05$) from 1 FPS. This resulted in more information on the direction of motion than using 5 or 10 FPS. Moreover, the process of studying FSV for a period of 60 minutes, using 3 FPS can be completed 3 times faster than using 1 FPS.

Formalin at both 300 mg/L and 600 mg/L concentrations affected the FSV of tilapia. For treatment 2 (300 mg/L of formalin), average FSV was highest during 0-5 minutes (period 1) as the fish received formalin at a slightly higher concentration than the lethal concentration (LC_{50-24h}) of 191.34 mg/L of juvenile tilapia (Tancredo *et al*., 2019). The fish adapted to the alarm reaction stage. During 5-25 minutes (periods 2-5), the fish were in the stage of resistance, followed by the stage of exhaustion during 25-60 minutes (periods 6-12). Treatment 3 (600 mg/L of formalin) was highly in excess of the LC_{50}. The fish were unable to adapt to the alarm reaction stage and immediately entered the stage of resistance and the stage of exhaustion during periods 6-8.

Death started to occur during period 9. These results were similar to many previous studies. When fish are exposed to high chemical concentration that exceeds the LC_{50} value, they will swim fast during the first 1-2 h. The operculum will be fast-moving, and fish anxiety will increase; they will swim close to the aeration system as a result of the secretion of catecholamine and corticosteroid hormones to adapt to an equilibrium stage (Tancredo *et al.*, 2019). At more than 4 h exposure, an increase in mucus production is observed, promoting a change in water color, reduction of operculum beating, and slow swimming until the beginning of the first deaths (Mazeaud *et al.*, 1977; Kakuta *et al.*, 1991; Barton, 2002; Santos *et al.*, 2012; Tancredo *et al.*, 2019). However, these circumstances also depend on the chemical concentration of the water and the health of the fish (Sharp *et al*., 2004).

Results showed that using computer vision techniques to study swimming velocity indicated changes in tilapia stress levels and this new technique was far superior to traditional methods (blood sampling) or observation of fish behavior through established points (ventilatory frequency; VF). The VF was applied since there is a positive correlation with the amount of cortisol produced (Barreto & Volpato, 2004). However, the VF method cannot determine the level of stress. Computer vision techniques using the Tracker program can quickly assess the stress levels of aquatic animals using low investment and reduced labor. The Tracker technique can be used as a real-time monitoring system of stress caused by environmental disorders.

Conclusion

Computer vision techniques using the Tracker program can apply to study tilapia swimming velocity and may apply for studying the stress level of the fish.

Acknowledgements

The authors are grateful to the Department of Aquaculture, Faculty of Fisheries Kasetsart University for supporting laboratory operation. We also wish to thank Miss. Rawiwan Panlima for her assistance in data collection.

References

Azaza, M.S., DhraÏef, M.N., & KraÏem, M. (2008). Effect of water temperature on growth and sex ratio of juvenile Nile tilapia *Oreochromis niloticus* (Linnaeus) reared in geothermal waters in southern Tunisia. *Journal of Thermal Biology, 33*, 98-105.

Baganz, D., Staaks, G., & Steinberg, C. (1998). Impact of the cyanobacteria toxin, microcystin-LR on behaviour of zebrafish, *Danio rerio*. *Water Research, 32,* 948–952.

Barreto, R.E., & Volpato, G.L. (2004). Caution for using ventilation frequency as an indicator of stress in fish. *Behavioural Processes* ,*66*, 43-51.

Barton, B.A., & Iwama, G.K. (1991). Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annual Review of Fish Diseases*, *10*, 3-26.

Barton, B.A. (2002). Stress in Fishes: A Diversity of Responses with Particular Reference to Changes in Circulating Corticosteroids. *Integrative & Comparative Biology, 42*, 517-525.

Buchmann, K., & Kristensson, R.T. (2003). Efficacy of sodium percarbonate and formaldehyde bath treatments against *Gyrodactylus derjavini* infestations of rainbow trout. *North American Journal of Aquaculture*, *65*, 25-27.

Douglas, B. (2019). *Open source physics*. Retrieved October 6, 2019, from https://physlets.org/tracker.

Food and agriculture organization. (2016). *The State of World Fisheries and Aquaculture*. Rome.

Global-Aquaculture. (2016). *Global Fish Production Data and Analysis*. Retrieved May 9, 2019, from http://refhub.elsevier.com/s0044-8486(19) 30693-3/rf0125.

Handy, R.D., Sims, D.W., Giles, A., Campbell, H.A., & Musonda, M.M. (1999). Metabolic trade-off between locomotion and detoxification for maintenance of blood chemistry and growth parameters by rainbow trout (*Oncorhynchus mykiss*) during chronic dietary exposure to copper. *Aquatic Toxicology, 47*, 23-41.

Israeli-Weinstein, D., & Kimmel, E. (1998). Behavioral response of carp (*Cyprinus carpio*) to ammonia stress. *Aquaculture, 165,* 81–93.

Kakuta, I., Namba, K., Uematsu, K., & Murachi, S. (1991). Physiological response of the fish, *Cyprinus carpio*, to formalin exposure – I. Effects of formalin on urine flow, heart rate, respiration. *Comparative Biochemistry and Physiology, 100*, 405-444.

Kolding, J., Haug, L., & Stefansson, S. (2008). Effect of ambient oxygen on growth and reproduction in Nile tilapia (*Oreochromis niloticus*). *Canadian Journal of Fisheries and Aquatic Sciences, 65*, 1413-1424.

Kristiansen, T.S., Ferno, A., Holm, J.C., Privitera, L., Bakke, S., & Fosseidengen, J.E. (2004). Swimming behaviour as an indicator of low growth rate and impaired welfare in Atlantic halibut (*Hippoglossus hippoglossus* L.) reared at three stocking densities. *Aquaculture, 230*, 137–151.

Maxime, V., Nonnotte, G., Peyraud, C., Williot, P., & Truchot, J.P. (1995). Circulatory and respiratory effects of hypoxic stress in the Siberian sturgeon. *Respiration Physiology, 100*, 203-212.

Mazeaud, M.M., Mazeaud, F., & Donaldson, E.M. (1977). Primary and secondary effects of stress in fish. *Transaction of the American Fisheries Society*, *106*, 201-212.

McFarlane, W.J., Cubitt, K.F., Williams, H., Rowsell, D., Moccia, R., Gosine, R., & McKinley, R.S. (2004). Can feeding status and stress level be assessed by analyzing patterns of muscle activity in free swimming rainbow trout (*Oncorhynchus mykiss* Walbaum)?. *Aquaculture, 239*, 467–484.

Nogita, S., Baba, K., Yahagi, H., Watanabe, S., & Mori, S. (1988). Acute toxicant warning system based on a fish movement analysis by use of AI concept. In Artificial Intelligence for Industrial Applications. In *Proceedings of the International Workshop*. (pp. 273–276).

Picón-Camacho, S.M., Marcos-Lopez, M., Bron, J.E., & Shinn, A.P. (2012). An assessment of the use of drug and nondrug interventions in the treatment of *Ichthyophthirius multifiliis* Fouquet, 1876, a protozoan parasite of freshwater fish, *Parasitology*, *139*(2),149–190.

Sabra, F.S., & El-Sayed, E.D.M. (2015). Pesticides toxicity in fish with particular reference to insecticides. *Asian Journal of Agriculture and Food Science*, *3*(1), 40-60.

Santos, R.F.B., Dias, H.M., & Fujimoto, R.Y. (2012). Acute toxicity and histopathology in ornamental fish amazon bluespotted corydora (*Corydoras melanistius*) exposed to formalin, *Brazilian Academy of Sciences, 84*, 1001-1007.

Shankar, K.M., Kiran, B.R., & Venkateshwarlu, M. (2013). A review on toxicity of pesticides in fish. *International Journal of Open Scientific Research*, *1*(1), 15-36.

Sharp, N.J., Diggles, B.K., Poortenaar, C.W., & Willis, T.J. (2004). Efficacy of Aqui-S, formalin and praziquantel against the monogeneans, *Benedenia seriolae* and *Zeuxapta seriolae*, infecting yellowtail kingfish *Seriola lalandi* in New Zealand, *Aquaculture, 236*, 67-83.

Shezifi, Y., Kimmel, E., & Diamant, A. (1997). The electrophysiological response of fish to hypoxia. *Aquacultural Engineering*, *16*, 253–259.

Tancredo, K.R., Ferrarezi, J.V., Marchiori, N.D.C., & Martins, M.L. (2019). Ecotoxicological assays to determine the median lethal concentration (LC_{50}) of formalin for fish. *Aquaculture international, 27*, 685-694.

Tran-Duy, A., Van Dam, A.A., & Schrama, J.W. (2012). Feed intake, growth and metabolism of Nile tilapia (*Oreochromis niloticus*) in relation to dissolved oxygen concentration. *Aquaculture Research, 43*, 730-744.

Vijayan, M.M., Pereira, C., Grauf, E.G., & Iwama, G.K. (1997). Metabolic responses associated with confinement stress in tilapia: the role of cortisol. *Comparative Biochemistry and Physiology, 116*, 89-95.

Wang, M., & Lu, M. (2016). Tilapia polyculture: a global review. *Aquaculture Research, 47*(8), 2363–2374.

XU, J., Liu, Y., Cui, C., & Miao, X. (2006). Behavioral responses of tilapia (*Oreochromis niloticus)* to acute fluctuation in dissolved oxygen levels as monitored by computer vision. *Aquacultural Engineering*, *35*, 207-217.