Behavioural Response Detection in Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1758) With Different Formalin Concentrations Using Tracker Software-Based Computer Vision Techniques

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Abstract

Changes in fish behaviour caused by stress are difficult to measure. In this study, tracker software-based computer vision techniques were applied, with formalin used as a stressor. At different formalin concentrations, stress responses of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), were examined for fish swimming velocity (FSV) and behaviour. Seven treatments included 1 (control) without formalin, with treatments 2–7 consisting of 100, 200, 300, 400, 500 and 600 mg.L^{-1} formalin concentration, respectively. Three (25 × 51 × 31 cm, width × length × height) glass tanks were 80 % filled with water for each trial. Each tank contained three fish with weights of 0.5–1.0 g, and the FSV of each fish was recorded for 120 min after exposure to formalin. Average FSV statistically differed ($P < 0.05$) at different formalin concentrations. Treatment 1 (control) gave the highest FSV at 0.038 ± 0.005 m.S^{-1} followed by treatments 2 (100 mg.L^{-1}) and 3 (200 mg.L^{-1}) at 0.020 ± 0.013 and 0.018 ± 0.020 m.S^{-1}, respectively. Treatments 4 (300 mg.L^{-1}), 5 (400 mg.L^{-1}), 6 (500 mg.L^{-1}) and 7 (600 mg.L^{-1}) recorded 0.007 ± 0.010, 0.006 ± 0.090, 0.004 ± 0.008 and 0.003 ± 0.007 m.S^{-1}, respectively. Differences in FSV at each concentration interval were applied to indicate the behavioural expression of fish response to stress in phase III (tertiary responses). Results indicated that computer vision techniques were suitable for studying Nile tilapia behaviour, with possible applications in other aquatic animals. Highlights of this technique included continuous real-time results to monitor fish stress using a non-invasive method.

Keywords: tilapia, swimming velocity, swimming behaviour, computer vision

Introduction

Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), is the second most commercially farmed fish in the world after carp (Li et al., 2023) in more than a hundred nations, with production increasing from 2.6 million tons in 2010 to 4.5 million tons in 2018 (FAO, 2022). During intensive aquatic farming, the commercial pelleted diet not consumed by fish is converted into ammonia, nitrate, phosphate, carbon dioxide and organic solids in suspension (FigueirÓ et al., 2018). Accumulation of these remaining nutrients causes changes in pond water quality, affecting plankton growth and promoting algae bloom (Molisani et al., 2015). Regular water quality monitoring is essential and necessary to reduce disease and fish mortality from water quality problems (Li et al., 2016).

Multi-parameter water quality monitoring for terrestrial and aquatic animals is now commonly used to study the diverse effects of behavioural expressions (Zala and Penn, 2004; Huntingford et al., 2012). A standard method for water quality monitoring is by assessing fish behaviour. In aquatic animals, fish behaviour refers to an external reaction to environmental changes that induce factors leading to behavioural disorders (McFarlane et al., 2004; Kristiansen et al., 2004). Fish swimming behaviour is more sensitive to toxic stress than lethality or growth (Little and Finger, 1990). Therefore, continuous monitoring of swimming velocity as behavioural responses can be used to assess fish stress due to disease and water quality parameters. If changes in these

parameters are detected early, the aquaculturist can take immediate mitigation measures to reduce production losses (Xu et al., 2006).

Previously, traditional fish stress measurement methods relied on an experienced technician's blood sampling. However, environmental changes can rapidly increase hormonal stress parameters. Thus, copious research has concentrated on developing other forms of behavioural monitoring, such as observation or manual methods (Yu et al., 2015) using fluorescent markers to locate the fish and analyse their behaviour (Marti-Puig et al., 2018) or using video-based recognition and manual analysis to track markers in fish (Delcourt et al., 2013). These methods are invasive, leading to increased stress behaviour; they require manual operation, are time-consuming and laborious, and results are susceptible to subjective factors. Moreover, the results are inaccurate and behavioural trajectories cannot be plotted (Xu et al., 2020).

Recently, computer technology is utilised to detect stress in fish by continuous in situ non-invasive and non-contact measurement methods (Shezifi et al., 1997; Israeli-Weinstein and Kimmel, 1998; Xu et al., 2006; Xu et al., 2020). Computer vision techniques allow qualification of aquatic animal stress levels using automated, non-invasive, and cost-effective methods to assess chemical toxicity on behavioural changes (swimming) (Baganz et al., 1998; Handy et al., 1999; Papadakis et al., 2012; Marti-Puig et al., 2018; Jang et al., 2022).

When using a video-based mode to analyse fish behaviour, the motion of each fish is first captured, and then, a quantitative analysis of the video images is performed to determine the motion trajectory. Conventional methods typically obtain fish trajectory information by manually annotating each image frame, but this has low efficiency and accuracy. The development of fish tracking based on computer vision technology has provided new effective ways to conduct studies in this research area (Blaser and Gerlai, 2006; Fontaine et al., 2008; Qian et al., 2016; Zhiping and Cheng, 2017).

However, most computer vision techniques to study fish behaviour are complicated, which hampers wide-scale applications. The present study applied a computer vision technique using the open-source video analysis tracker software designed by Douglas Brown (Claessens, 2017). A tracker is used for analysis and video modelling tools (Wee and Leong, 2015). Utilising this software makes it easier for students to investigate the centre of mass and changes in position, speed and acceleration with time while also visualising the concept of motion in real-time (Hockicko, 2011). Behavioural changes in tilapia can be studied by measuring swimming velocity using different levels of formalin concentration as the stimulants. Formalin, an aqueous solution of formaldehyde stabilised with methanol, is commonly used as a disinfectant (Leal et al., 2018) for disease management in many fish species, replacing malachite green (Buchmann and Kristensson, 2003). Formalin is easily metabolised by aquatic organisms and has low bioaccumulation potential (Picón-Camacho et al., 2012) but can cause high toxicity to fish when used in excess (Tancredo et al., 2019).

Materials and Methods

Ethical approval

This study was approved by the Ethics Committee of Kasetsart University, Bangkok, Thailand (Approval no. ACKU 62-FIS-006).

Fish sample and study site

The experiment was conducted in the Aquacultural Engineering Laboratory at Kasetsart University, Bangkok. Nile tilapia mono-sex of 0.5–1.0 g fingerlings were obtained from the Kamphaeng Saen Fisheries Research Station, Faculty of Fisheries, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom. A flow-through system was used consisting of two rectangular 1,000 L tanks, each equipped with three air stones to maintain dissolved oxygen (DO), water temperature, pH, total ammonia-nitrogen (TAN) and nitrite-nitrogen (NO_2-N) at >3 mg.L^{-1} (Kolding et al., 2008; Tran-Duy et. al, 2012), 25–32 °C (Azaza et al., 2008), 7.5–8.5 (Lawson, 1995; El-Sherif and El-Feky, 2009), <1 mg.L^{-1} (Hargreaves and Tucker, 2004) and <1 mg.L^{-1} (Lawson, 1995), respectively.

The DO and water temperature were monitored daily using a dissolved oxygen meter (Pro 20i, YSI, USA). The level of pH was also recorded daily using a pH meter (pH100A, YSI, USA). TAN and NO_2-N were monitored every three days in the laboratory following the guidelines of the American Public Health Association (APHA, 2005). Daily feed containing at least 35 % protein was given by hand until satiation at 0800 and 1700 h. After the acclimatisation period of one week, three fish were randomly selected and placed in each experimental glass aquarium 25 × 51 × 31 cm (width × length × height), filled to 80 % water level and equipped with one air stone. Sediment suction was performed, and 40 % of the water volume in each tank was exchanged daily after feeding at 1700 h. The fish were rested for 3 days before commencing the experiments.

Experimental design

Experiments were conducted following a completely randomised design (CRD) for seven treatments with three replicates as follows; no formalin (control) and 100, 200, 300, 400, 500 and 600 mg.L^{-1} of 37 % formaldehyde (Sigma-Aldrich ®).

Image analysis apparatus

A closed-circuit television camera (CCTV; KP-

TVI8004HI, Kenpro, Thailand) with an image size of 1,024 × 764 pixels was used to record 24 images per second with a digital video recorder (DVR; 4CH. HDCVI DAHUA#XVR4104HS-I, Advice IT Infinite Public Co., Ltd., Thailand). An Acer Aspire E15 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) was used for data analysis, as shown in Figure 1.

Video recording

The fish movement was recorded in each experiment from when the formalin solution was added for 120 min. The video clips were saved from the DVR to the personal laptop. Fish behavioural monitoring began by selecting the video for analysis (Fig. 2a). Then, using a calibration stick (Fig. 2b), the FSV values were compared with the known length of the video screen. A 10-cm line was drawn on the front wall of the glass aquarium (Fig. 2c), with the coordinate axes set at the centre of the video screen to create a point mass, with the object chosen to capture the movement speed of the fish's body. The search button was pressed to capture the velocity of the fish (Fig. 2d). The program analysed the velocity of the fish by measuring the distance the object moved in each frame.

Fig. 1. System overview of posture tracking for fish behavioural monitoring. Closed-circuit television images of fish from the glass aquarium were transmitted to the digital video recorder. Image analysis tracker software analysed fish movement and displayed the result via a user interface in the program. Note: A 10-cm straight line was a marker to capture the fish swimming velocity.

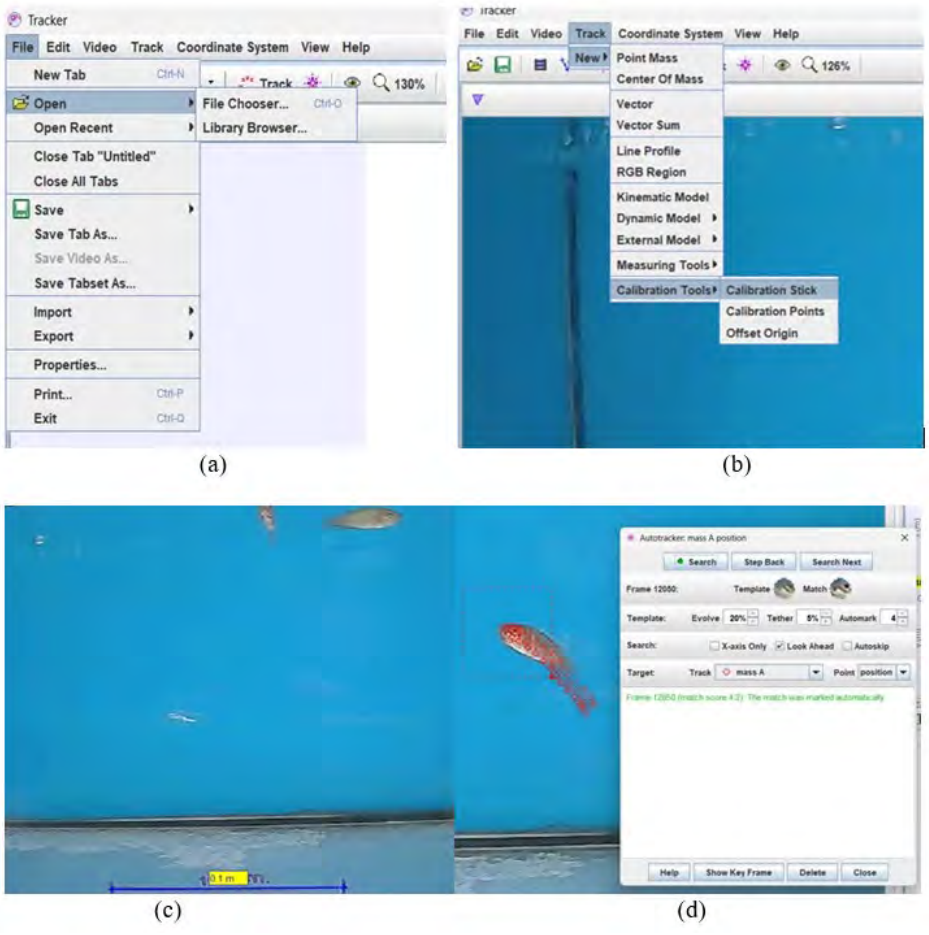

Fig. 2. The process of using the tracker program for the detection of behavioural response in Nile tilapia, *Oreochromis niloticus* (a) select the video for analysis, (b) use a calibration stick, (c) draw a line and set the distance (in this study 0.10 m) and (d) press the search button to capture the velocity of the fish.

Behavioural response

The theory of fish stress identifies three response phases: primary, secondary and tertiary (Barton, 2002). The primary and secondary phases are internal responses, when fish encounter external stimuli in the form of physical stressors such as handling, capture, confinement, transport, chemical stressors such as contaminants, pollutant exposure, acidification and perceived stressors such as stimuli evoking the response presence of a predator. Primary and secondary phase responses occur in vivo.

In the primary phase, fish increase in corticosteroid and catecholamine hormone alterations in neurotransmitter activity (Donaldson, 1981; Wendelaar Bonga, 1997; Mommsen et al., 1999). The secondary phase involves metabolic changes such as increased glucose or lactate and decrease tissue glycogen (Pickering, 1981; Iwama et al., 1998; Mommsen et al., 1999). Both these response phases are not observable through external fish behavioural manifestations (Barton, 2002). In the tertiary phase, the response is expressed by whole-animal performance characteristics such as growth, swimming capacity, disease resistance or modified behavioural patterns (Wedemeyer and McLeay, 1981; Wedemeyer et al., 1990). The tertiary phase is divided into three stages of behavioural expression according to the general adaptation syndrome as stage 1: alarm reaction, stage 2: resistance, and stage 3: exhaustion. The alarm reaction occurs when the fish becomes aware of any external abnormalities; this stage is usually the longest. The stage of resistance is when the fish tries to adapt and resist external anomalies, and the stage of exhaustion occurs when the fish is no longer able to adapt and resist external abnormalities (Selye, 1950).

Data analysis

Data were analysed for average fish swimming velocity (FSV) as follows:

FSV ($m.s^{-1}$) from the animation image using tracker software was calculated by Eq. (1):

$$FSV_{average} = V_1 + V_2 + V_3 + \ldots + V_n / N \quad (1)$$

where N is the number of images used for analysis.

$V_{1\text{-}n}$ was calculated from Eq. (2):

$$V = S/T \quad (2)$$

where V is the velocity of the fish ($m.S^{-1}$), S is the displacement or distance between the fish image capture and the last point (meters), and T is the period from the beginning of the capture until the end of the recording in seconds.

Each V must have a minimum of 10 displacements (10 sec) according to Xu et al. (2006), as shown in Eq. (3):

$$\begin{aligned} V_1 &= S_{f1} + S_{f2} + S_{f3} + \ldots + S_{fn} \quad (3)\\ V_2 &= S_{f1} + S_{f2} + S_{f3} + \ldots + S_{fn}\\ V_3 &= S_{f1} + S_{f2} + S_{f3} + \ldots + S_{fn}\\ &\ldots\\ &\ldots\\ V_n & \end{aligned}$$

The tracker program was used to determine the average FSV of the Nile tilapia, at three frames per second (3 FPS), according to Jongjaraunsuk and Taparhudee (2021). Each experiment was recorded continuously for 120 min, and the track was then divided into 24 intervals of 5 min each. Average FSV values (from 120 min) were analysed for variance difference using one-way analysis of variance (one-way ANOVA), and mean differences of each experiment were compared by Duncan's multiple range test at $P < 0.05$. The time interval calculated FSV results to determine the likelihood of general adaptation syndrome level, performed by comparative analysis at all time intervals of each trial. All analyses were performed using IBM SPSS Statistics version 26.

Results

Average fish swimming velocity (FSV) over 120 min

Results showed that FSV in treatment 1 (control) was stable at 0.032 to 0.048 $m.S^{-1}$. In treatments 2–7 (100–600 $mg.L^{-1}$ formalin), FSV decreased with increasing recording time and formalin concentration, as shown in Figure 3.

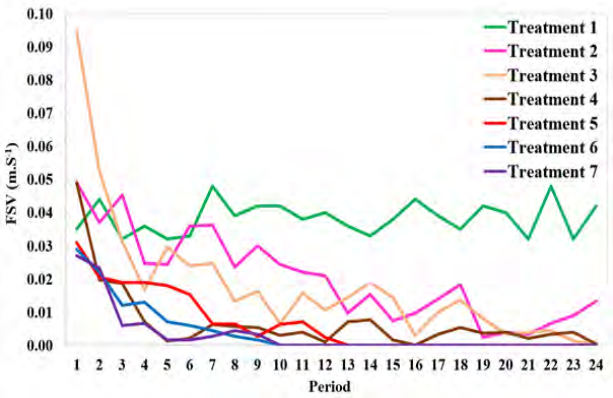

Fig. 3. Average Nile tilapia, *Oreochromis niloticus*, swimming velocity at each treatment with formalin at different concentrations. Note: Treatments 1, 2, 3, 4, 5, 6 and 7 comprised 0, 100, 200, 300, 400, 500 and 600 $mg.L^{-1}$ formalin concentration.

Treatment 1 gave the highest average FSV, with statistical differences ($P < 0.05$) from the other treatments. FSV for treatment 1 was 0.038 ± 0.005 $m.S^{-1}$, followed by treatments 2–3 at 0.020 ± 0.013, 0.018 ± 0.020 and treatments 4 to 7 at 0.007 ± 0.010, 0.006 ± 0.09, 0.004 ± 0.008 and 0.003 ± 0.007 $m.S^{-1}$, respectively as shown in Figure 4.

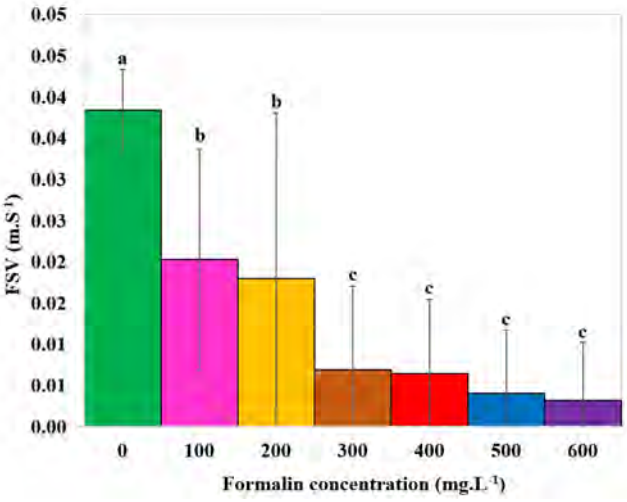

Fig. 4. Average fish speed velocity after Nile tilapia, *Oreochromis niloticus*, were exposed to different formalin concentrations. Each treatment was recorded for 120 min with standard deviation (SD). Note: Different letters on each bar indicate significant differences at $P < 0.05$. The SD value is high because swimming velocity varied greatly due to stress response.

Average FSV at five-minute recording intervals

The mean FSV of period 1 was not statistically different from the mean period 2 ($P > 0.05$) but the mean FSV of period 1 was different from periods 3–24 ($P < 0.05$). The mean FSV of period 2 was statistically different from the mean FSV of periods 8–24 ($P < 0.05$) and the mean FSV of period 3 was statistically different from the mean FSV of periods 19–21 and 23–24 ($P < 0.05$). The mean FSV was divided into four groups: group 1 (periods 1–2), group 2 (periods 2–7), group 3 (periods 3–18 and 22) and group 4 (periods 4–24), as shown in Table 1 and Figure 5.

The average FSV for each 5 min recording interval showed that the first treatment (control) was stable, with a minimum average velocity of 0.032 m.S^{-1} and a maximum of 0.048 m.S^{-1} (Fig. 6a).

For treatment 2 (100 mg.L^{-1} of formalin), in the first 9 periods (0–45 min), fish had an average FSV of 0.034 ± 0.005 m.S^{-1} predicted to be stage 1 of adaptation (alarm). During the 10–18 (>45–90 min) period, the fish had an average FSV of 0.016 ± 0.002 m.S^{-1} predicted to be stage 2 of adaptation (resistance). During the 19–24 (>90–120 min) periods, the fish entered stage 3 of adaptation (exhaustion), with an average FSV of 0.006 ± 0.002 m.S^{-1} (Fig. 6b).

For treatment 3 (200 mg.L^{-1} of formalin), in the first 7 periods (0–35 min), fish had an average FSV of 0.039 ± 0.005 m.S^{-1} predicted to be stage 1 of adaptation (alarm). During the 8–18 (>35–90 min) periods, the fish had an average FSV of 0.012 ± 0.005 m.S^{-1} predicted to be stage 2 of adaptation (resistance). During the 19–24

Table 1. Average fish swimming velocity of 24 periods were classified into 4 subsets using Duncan's new multiple range test.

Period	Subset of alpha = 0.05			
	1	2	3	4
1	0.044	-	-	-
2	0.032	0.032	-	-
3	-	0.027	0.027	-
4	-	0.022	0.022	0.023
5	-	0.021	0.021	0.021
6	-	0.020	0.020	0.020
7	-	0.020	0.020	0.020
8	-	-	0.014	0.014
9	-	-	0.014	0.014
10	-	-	0.014	0.014
11	-	-	0.011	0.011
12	-	-	0.011	0.011
13	-	-	0.011	0.011
14	-	-	0.010	0.010
15	-	-	0.010	0.010
16	-	-	0.008	0.008
17	-	-	0.008	0.008
18	-	-	0.114	0.114
19	-	-	-	0.007
20	-	-	-	0.007
21	-	-	-	0.007
22	-	-	0.086	0.086
23	-	-	-	0.005
24	-	-	-	0.005

Note: Periods 22 = FSV increased towards periods 19–24 in treatment 2 (100 mg.L^{-1}).

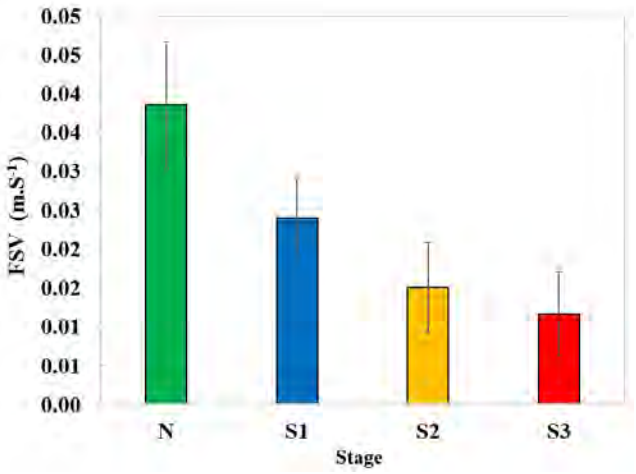

Fig. 5. Fish swimming velocity (FSV) (mean ± SD) of Nile tilapia, *Oreochromis niloticus*, from four subsets of alpha (0.05). Note: N = normal condition as the average FSV from periods 1 and 2. S1 = stage 1; alarm reaction as the average FSV from periods 2, 3, 4, 5, 6, and 7. S2 = stage 2; resistance as the average FSV from periods 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 18, and 22. S3 = stage 3; exhaustion as the average FSV from periods 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, and 24.

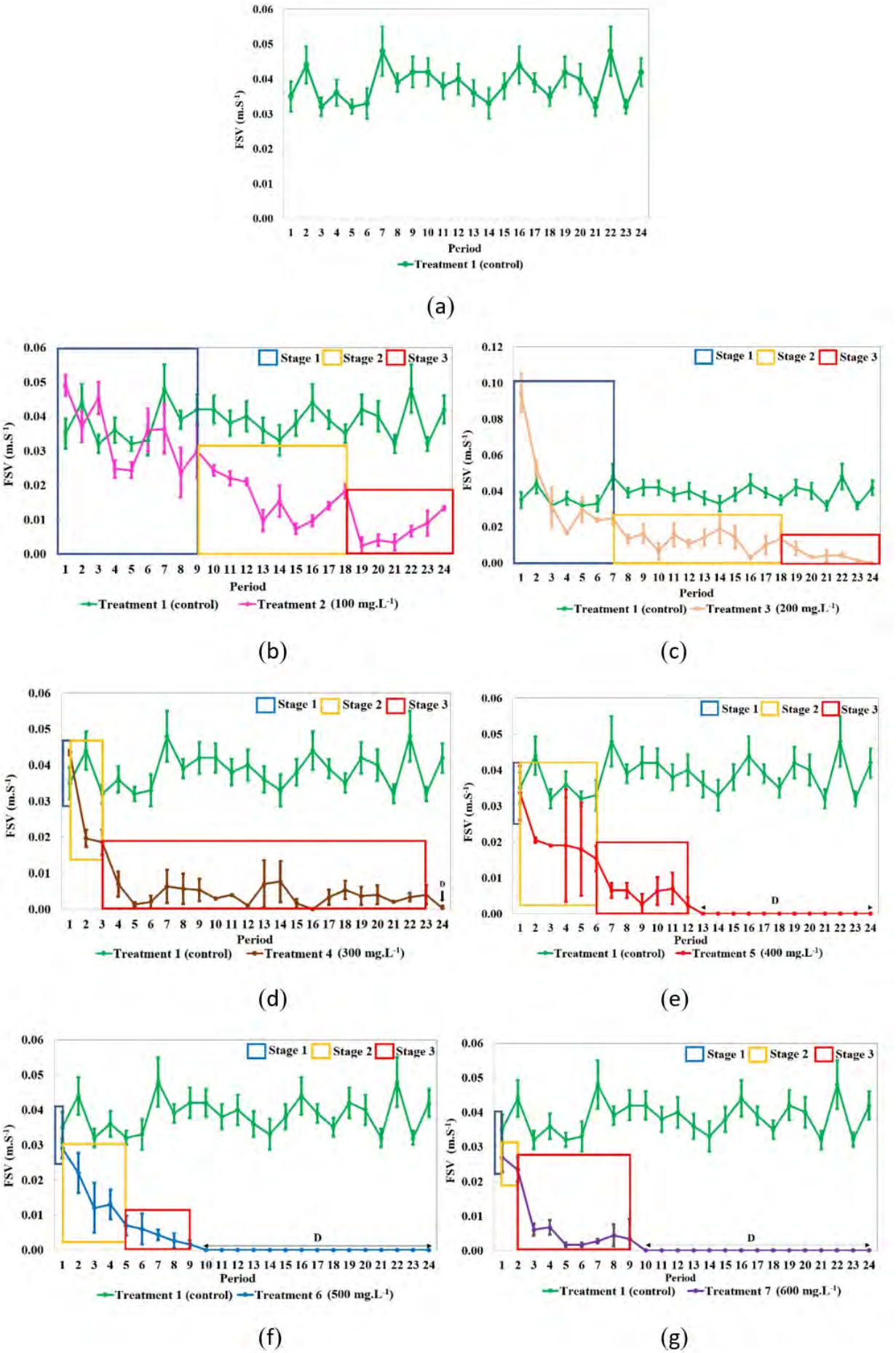

Fig. 6. Average FSV for each 5 min time interval (a) treatment 1 (control without formalin), (b), (c), (d), (e), (f), and (g) treatments 2–7 with formalin concentrations of 100, 200, 300, 400, 500 and 600 mg.L^{-1}, respectively. Note: The black arrow and alphabet "D" represents periods of mortality.

periods (>90–120 min), the fish entered stage 3 of adaptation (exhaustion) with an average FSV of 0.004 ± 0.002 m.S^{-1} (Fig. 6c).

For treatment 4 (300 mg.L^{-1} of formalin), in the first period (0–5 min), fish had an average FSV of 0.049 ± 0.001 m.S^{-1} was predicted to be stage 1 of adaptation (alarm). At the 2 and 3 periods (>5–15 min), the fish had an average FSV of 0.019 ± 0.003 m.S^{-1} predicted to be stage 2 of adaptation (resistance). During periods 4–23 (>15–115 min), the fish entered the stage of adaption (exhaustion) with an average FSV of 0.004 ± 0.002 m.S^{-1}, while death occurred in period 24 (>115–120 min) (Fig. 6d).

For treatment 5 (400 mg.L^{-1} of formalin), in the first period (0–5 min) fish had an average FSV of 0.031 ± 0.008 m.S^{-1}, predicted to be stage 1 of adaptation (alarm). During periods 2–6 (>5–30 min), the fish had an average FSV of 0.018 ± 0.007 m.S^{-1} as stage 2 of adaptation (resistance). At the 7–12 (>30–60 min) periods, the fish entered stage 3 of adaption (exhaustion) with an average FSV of 0.005 ± 0.003 m.S^{-1}. The death occurred from interval 13 (Fig. 6e).

For treatment 6 (500 mg.L^{-1} of formalin), during the first time (0–5 min), the fish entered stage 1 of adaptation (alarm) with an average FSV of 0.029 ± 0.003 m.S^{-1}. During the 2–5 (>5–25 min) periods, the fish had an average FSV of 0.014 ± 0.005 m.S^{-1} as stage 2 of adaptation (resistance). During the 6–9 (>25–45 min) periods, the fish entered stage 3 of adaptation (exhaustion) with an average FSV of 0.004 ± 0.002 m.S^{-1}. The death occurred at interval 10 (Fig. 6f).

For treatment 7 (600 mg.L^{-1} of formalin), during the first period (0–5 min), the fish had an average FSV of 0.027 ± 0.004 m.S^{-1} as the first stage of adaptation (alarm). During the second period (>5–10 min), the fish had an average FSV of 0.023 ± 0.004 m.S^{-1} as the second stage of adaptation (resistance). At the 3–9 (>10–45 min) periods, the fish entered the stage of exhaustion with an average speed of 0.004 ± 0.002 m.S^{-1}, followed by death starting at interval 10 (Fig. 6g).

Discussion

In treatment 1 (control), the fish swam around the tank and constantly engaged in social behaviour towards each other. During the stages of 1–2 adaptation, the fish became anxious. They moved vertically, swimming up and down but still observed common social behaviours. When entering the third stage of adaptation, fish movement slowed down and were located on the bottom and at the corners of the tanks, as shown in Figures 7a–7j.

After exposure to formalin, the average FSV over 120 min showed that all treatments were statistically different ($P < 0.05$) from the control. Treatment 2 is the recommended formalin concentration for the prevention of disease in aquatic animals at 60 min (Tancredo et al., 2019). The short observation period of 120 min may have caused the FSV of treatment 2 to be lower than the control, but no deaths were recorded.

Treatment 3 was close to the lethal concentration ($LC_{50\text{-}24h}$) of 191.34 mg.L^{-1} of juvenile Nile tilapia at 24 h (Tancredo et al., 2019). However, since the study was conducted over only 120 min, no mortality was observed, although the fish moved slightly throughout the experiment. This result was consistent with Santos et al. (2012), who reported that fish exposed to formalin near the lethal concentration level usually presented agitation and erratic swimming with slight movement, mainly on the bottom of the experimental glass tank. Vertical swimming behaviour was also found in early stress-induced stages (Xu et al., 2006).

For treatments 4, 5, 6 and 7, average FSV values were lower and statistically different ($P < 0.05$) from treatments 1, 2 and 3 because the fish were exposed to high concentrations of formalin. Acute toxicity caused agitation and erratic swimming before slow and unpredictable swimming behaviour (Xu et al., 2006; Santos et al., 2012; Jimmy et al., 2014), with mortality in treatments 4, 5, 6 and 7 occurring at 5 min intervals periods 24 (115–120 min), 13 (60–65 min), 10 (45–50 min) and 10 (45–50 min), respectively. The death occurred at a high concentration of formalin because its toxicity caused pathological damage with gill dysfunction, osmoregulatory and respiratory imbalance, disorganisation of liver arrangement and necrosis in the kidney (Santos et al., 2012; Reardon and Harrell, 1990).

When assessing fish swimming direction, treatments 4, 5, 6 and 7 recorded vertical direction and slower movement at the bottom of the aquarium than treatments 1, 2 and 3, especially treatment 7. These results concurred with Calfee et al. (2016), who reported that stress-induced fish tended to move around the edge of the container and presented agitated and vertically violent movements (Xu et al., 2006; Jimmy et al., 2014).

Stress response results showed that fish presented whole-animal performance characteristics and exhibited all three behavioural levels of adaptation. However, when this study's three stages of adaptation compared FSV with the control treatment, it was not possible to determine velocities at the adaptation level. FSV is associated with fish stress and results from many factors, including fish genetics, the duration of stress, fish maturity and even schooling. (Selye, 1950; Wedemeyer et al., 1990).

This study showed that computer vision techniques to monitor swimming velocity were useful as indicators of fish stress response levels. Results may be applied in conjunction with or to replace traditional blood sampling or manual marking stress. Manual methods cause stress in the fish during sampling or marking processes and may result in changes in the

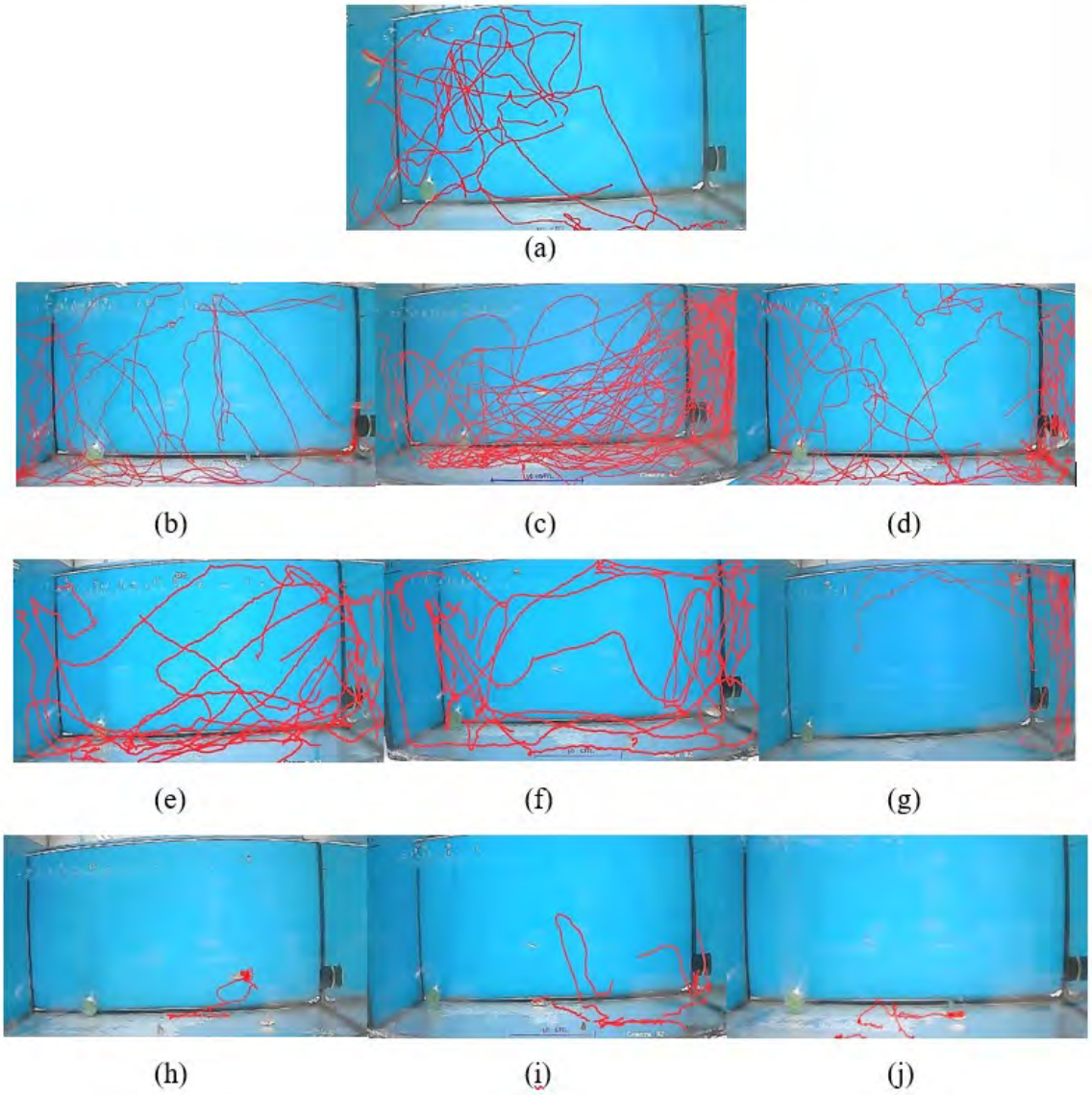

Fig. 7. Swimming behaviour of Nile tilapia, *Oreochromis niloticus*, at different stages of adaptation (a) normal, (b), (c), (d), (e), (f) and (g) stages of 1–2 adaptation and (h), (i) and (j) as stage 3 of exhaustion. Note: Highlights of this technique provided continuous real-time results to monitor fish stress using a non-invasive approach.

parameters to be measured (Sadoul and Geffroy, 2019). Tracker techniques are easy to use. Many researchers have applied the techniques of tracking objects using more than one camera or 3D tracking. But accuracy and ease of use remain limited, especially with more than one object to the tracking (Nimkerdphol and Nakagawa, 2008; Wu, 2010; Mendelson and Techet, 2015; Stewart et al., 2015). Moreover, much of the workflow is still complex and has individual usage. Therefore, it is recommended that researchers use this tracker software because it is convenient and easy to use.

For practical applications in fish farms, further studies are needed to investigate different ages and sizes as fish size and age influence stress-induced behaviour (Koakoski et al., 2012). Furthermore, a study should evaluate images at wide angles to cover a broader scope of the whole culture system. Using unmanned aerial vehicles (UAVs) could complement image acquisition in inspecting, counting, assessing weight or monitoring the movement of aquatic animals (Raoult and Gaston, 2018; Schaub et al., 2018; Rieucau et al., 2017). However, using UAVs in aquaculture systems has not yet been successfully implemented. The object tracking patterns should be changed from the side view to the top view to match the use of UAVs. A previous comparative study showed no difference in FSV when the program implemented tracking objects from the top view (Jumnienboon et al., 2020).

Conclusion

This study examined fish swimming velocity (FSV) and behaviour of 0.5–1.0 g Nile tilapia, *Oreochromis niloticus*, using different formalin concentrations of 0 (control), 100, 200, 300, 400, 500 and 600 mg.L^{-1} in a glass aquarium for 120 min. Tracker software-based computer vision techniques were applied. Results showed that this technique identified continuous

change in the swimming velocity of fish over time. When the concentration of formalin increased, FSV decreased. Differences in FSV at each concentration interval could be applied to indicate the behavioural expression of fish response to stress, particularly in phase III (tertiary responses). Results suggested that tracker software-based computer vision techniques can accurately measure tilapia swimming velocity to determine stress levels. This technology should be used with unmanned aerial vehicles for large operational study areas, using top-view image analysis for practical applications. However, further studies are needed in farms to investigate fish behaviour using fish of different ages and sizes.

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Conflict of interest: The authors declare that they have no conflict of interest.

Author contributions: Wara Taparhudee: Conceptualization, writing and supervision. Roongparit Jongjaraunsuk: Data collection, data analysis, writing original draft, reviewing and editing.

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