The effect of water temperature on the swimming speed of Nile tilapia (*Oreochromis niloticus*) using computer vision technique

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Abstract

Computer vision technique using a Tracker program was applied to determine how water temperature affected swimming speed of Nile Tilapia. The experiment was divided into 3 treatments: treatment 1 with low water temperature (20.43±1.12 °C), treatment 2 with normal water temperature (26.72±0.67 °C), and treatment 3 with high water temperature (34.73±1.88 °C). There were three replicates of each treatment. Average weight and total length of the experimental fish were 115.30±13.50 g. and 18.30±8.97 cm. Each treatment examined swimming performance of the fish according to feeding time. This was divided into 5 stages as; 15 min before feeding (S1), from start of feeding-15 min (S2), 16-30 min (S3), 31-45 min (S4), and 46-60 min (S5) after feeding. Swimming speed performance and sprint swimming speed were examined in all stages but average sprint swimming speed was tested only in stage 2. Results showed that S2 and S3 of treatment 2 had the highest average swimming speeds at 4.95±2.38 cm/s and 0.87±0.09 cm/s, respectively which were significantly different ($P<0.05$) from results in treatments 1 and 3. Average sprint swimming speed (S2) was only observed in treatment 2 at 7.00±4.00 cm/s. Findings indicated that the computer vision technique using a Tracker program was useful for studying fish behaviors.

Keywords: computer vision, swimming speed, temperature, tilapia

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Introduction

Nile Tilapia (*Oreochromis niloticus*) originated in Africa and are considered the world's most important economic fish. Their global production is estimated to be more than 5 million tons and the Global Aquaculture Advocate expects output to increase by 1.5-2.5 million tons over the next 7 years. The Nile Tilapia farming industry plays an important role in both the economic and social lives of people around the world (Paiboon, Saenphet, & Saenphet, 2018).

The problem of rising global temperatures over the past few years has received a lot of attention and also impacted the production of tilapia. Nile Tilapia are native to tropical and subtropical climates and can tolerate water temperatures up to 42 °C (Chervinski, 1982; Philippart, & Ruwet, 1982). However, optimum water temperature for tilapia culture is 26-32 °C (Azaza, Dhraïef, & Kraïem, 2008). Water temperatures above 32 °C affect stress levels and impact eating and growth performance of tilapia (Pandit, & Nakamura, 2010). Stress and acute stress may also result in severe physical and behavioral changes in fish. Stress is usually examined using the blood sampling method (Barton, & Iwama, 1991; Maxime, Nonnotte, Peyraud, Williot, & Truchot, 1995; Vijayan, Pereira, Grauf, & Iwama, 1997; Mancera et al., 2008). But collecting blood samples also causes stress and creates rapid stress hormones during sampling. As a result of these problems, fish stress assessment techniques without touching or catching have been developed and applied. Computer vision technique is now used by many researchers to observe the behavior of aquatic animals (Suzuki, Takagi, & Hiraishi, 2003; Kane, Salierno, Gipson, Molteno, & Hunter, 2004; Pratt, Smokorowski, & Muirhead, 2005; Xu, Miao, Liu, & Cui, 2005; Xu, Liu, Cui, & Miao, 2006; Mueller, Brown, Hop, & Moulton, 2006; Miller, & Gerlai, 2007; Stien, Brafland, Austevollb, Oppedala, & Kristiansen, 2007; Waggett, & Buskey, 2007; Grubich, Rice, & Westneat, 2008; Liua et al., 2014; Müllera, Nunesa, Silveiraa, Lorob, & Rosemberga, 2017). Results can be used as indicators of fish stress levels resulting from changing environments such as ammonia and dissolved oxygen. Most studies require programming and image processing techniques to improve images before analysis. This requires a skilled person and is time consuming.

Tracker (v.5.0.6) is an open source program developed by scientists for video analysis, in particular physics (Douglas, 2019). It offers the combination of using a computer to store data and a modeling program that has the function of marking the position of the object in each frame of the video, derived from recording with a video camera. It can analyze the movement of the object by allowing the program to display results such as position, speed, acceleration and time. Therefore, the objective of this research was to

study the effect of water temperature on swimming speed of Nile Tilapia during the periods before and after feeding using computer vision combined with the Tracker program. Results will be useful as a warning system of water quality alternations from changes in fish behavior. This technology may also be applied in toxicology, animal welfare and precision aquaculture.

Methodology

Fish sample preparation

Thirty (average 115.30±13.50 g in weight and 18.30±8.97 cm in length) fish from the Kamphaeng Saen Fisheries Research Station, Faculty of Fisheries, Kasetsart University, Kamphaeng Saen Campus, Kamphaeng Saen District, Nakhon Pathom Province, were transported to the Department of Aquaculture laboratory, Faculty of Fisheries, Kasetsart University Bang Khen Campus. Before the experiment, the fish were raised in 3 (1,000 L) tanks, 10 fish per tank for a period of 14 days to allow them to become familiar with laboratory conditions. Three air stones and two filters were provided in each tank and 20% by volume of water was exchanged every day to control water quality in the appropriate range as dissolved oxygen (DO), water temperature, pH, total ammonia-nitrogen (TAN), and nitrite-nitrogen (NO_2-N) at levels> 3 mg/L, 25-32 ° C, 7.5-8.5, <1 mg/L, <0.1 mg/L, respectively (Begum, Mondal, Ferdous, Zafar, & Ali, 2014). The fish were fed with floating pellets with at least 35% protein twice a day at 8:00 am and 5:00 pm. The method of feeding involved placing all the feed in the feeding ring to train the fish to become accustomed to swimming to the feed. After the acclimatization period, nine fish were randomly selected and placed in nine experiment glass tanks, size 50 cm × width 30 cm × height 40 cm, filled with 300 mL of water. One air stone was added to each tank for air supply. Sediment suction was performed and 50% of water volume was exchanged every day after completing testing at 05.00 PM. All fish were rested for two weeks prior to commencing the experiments.

Water quality analysis

In each experiment tank, DO and water temperature were measured using a YSI DO meter (YSI 550A), pH was monitored using an Ecosense pH 100A, turbidity was measured using a Turbidimeter (Eutech Instruments TN-100) daily at 07.00 AM. and 05.00 PM before feeding. The TAN and NO_2-N levels were examined in the laboratory according to APHA, AWWA, & WEF (1995). Methods, 3 times on day 1, 3 and 5.

Feeding and fish movement recording

The fish were fed by hand 2 times a day, in the morning at 8:00 AM and in the evening at 5:00 PM, double the rate of Jongjaraunsuk (2017). This allowed the fish to eat sufficiently. All the feed was placed in the feeding ring which was

convenient for storing, and the remaining feed was collected after 1 hr. Fish movement was recorded 15 min before feeding and then every 15 min until 60 min after feeding at the side of the glass tank following the method of Martins, Conceição, & Schrama (2011). The camera used was a CCTV Kenpro Model KP-TVI8004HI. Image size was 1024 × 764 pixels and 24 images per second were recorded with a computer (Dell Inspiron 5520 Intel® Core ™ i7-3632QM 2.20 GHz, 8GB Memory, 1TB HDD) for data analysis as shown in (Figure 1).

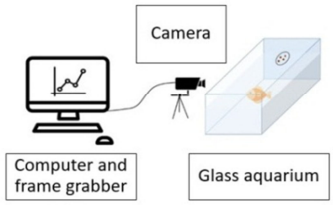

Figure 1 Schematic diagram of the experimental system.

Tracker program application

The procedure began with selecting the video for analysis and setting the frame rate to match the recording speed. Then, fish length was compared with a known length of the glass tank. The coordinate axes were set at the center of the video together with the object chosen to capture movement speed (the eyes of the fish) (Figure 2). The program analyzed the speed by measuring the distance that the object moved in each image (red line) against the time spent in each image (Figure 3).

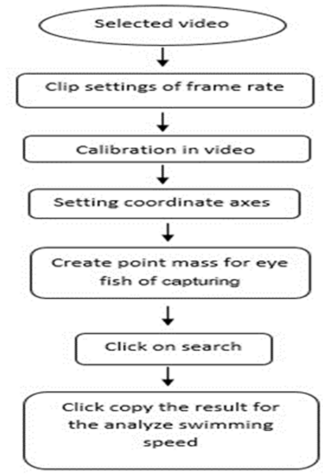

Figure 2 Flow chart showing basic steps in the behavior of swimming speed using the Tracker program.

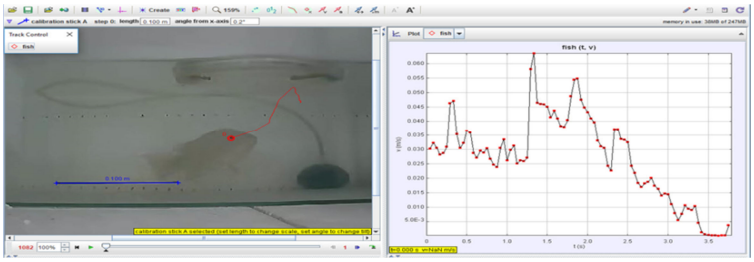

Figure 3 Estimation of the sprint swimming speed to the first pellet.

Experimental design

A completely randomized design (CRD) was performed. The experiment was divided into 3 treatments with three replicates as follows.

Treatment 1, water temperature 20.43± 1.12 °C (in the range 17-24 °C)

Treatment 2, water temperature 26.72± 0.67 °C (in the range 25-32 °C)

Treatment 3, water temperature 34.73± 1.88 °C (in the range 33-40 °C)

The duration of the experiment was 5 days.

Water temperature in treatment 1 was controlled using a cooler (Hailea model HS-28A). Water temperature in treatment 2 was set at room temperature and water temperature in treatment 3 was controlled using a heater (FISH GUARD model SH002 300W). Average swimming speed (cm/s) of each treatment was recorded using the Tracker program which analyzed the average swimming speed behavior from 180 video images every 3 min (modified from Xu, Liu, Cui, & Miao, 2006). Data were calculated by Equation 1.

$$V = S / t \quad (1)$$

where V is the speed of the fish (cm/s), S is the displacement or the distance between first image capture (image 1) and the last point (cm) (image 180), and t is the period from the beginning of the capture until the end of the recording at image 180 (s).

Swimming speed was divided into 5 stages as follows:

Stage 1 (S1) was 15 min before feeding.

Stage 2 (S2) was from the beginning of feeding to 15 min. During this period movement behavior toward the first pelleted feed was studied as Equation 2.

$$V_{\text{Average sprint swimming}} = (v_1+v_2+v_3+\bullet\bullet\bullet+v_n)/N \quad (2)$$

where N is the number of images used for analysis, Vn is calculated from Equation 1, where V is the speed of the fish moving toward the first pelleted feed (cm/s), S is the displacement or the distance between positions that start to capture (start feeding) to the last point (where fish reach the first feed) (m), and t is the period from the beginning of feeding until the time when the fish enters the first feed (s).

Stage 3 (S3) was 16-30 min after feeding.

Stage 4 (S4) was 31-45 min after feeding.

Stage 5 (S5) was the period of 46-60 min after feeding.

Statistical data analysis

Water quality data were analyzed for variance difference using one-way analysis of variance (one-way ANOVA) and compared with mean differences of each experiment by Duncan's multiple range test at $P<0.05$. Average swimming speeds of each stage were analyzed using

multivariate analysis of variance (MANOVA) and mean differences of each experiment were compared using Duncan's multiple range test at $P<0.05$. Sprint swimming speeds were analyzed using one-way ANOVA. Differences between means were also compared using Duncan's multiple range test at $P<0.05$. All analyses were performed using a statistical analysis program.

Results and discussion

Water quality results in (Table 1) showed that DO was significantly different ($P<0.05$). The highest value was recorded in low water temperature (T1) at 7.47±0.35 mg/L, followed by normal temperature (T2) at 6.17±0.23 mg/L and high temperature (T3) at 4.67±0.36 mg/L, respectively. This occurred because DO dissolved better when the water temperature decreased (Jeremias, 2010). Highest pH was found in the low temperature condition at 8.21±0.06, and significantly different ($P<0.05$) compared to normal and high conditions at 7.91±0.18 and 7.70±0.06, respectively. This can be explained because at low temperatures the fish have lower metabolic rates than at normal and high temperatures, resulting in less carbon dioxide which, when combined with water, results in carbonic acid and decreases the pH (Kathleen, 2001). Turbidity at the normal temperature condition was 1.97±1.01 NTU. This was statistically higher ($P<0.05$) than the low and high temperature conditions at an average of 0.86±0.45 and 0.80±0.55 NTU, respectively. At low and high temperature conditions, the fish did not respond to the food provided. This differed from the normal temperature condition in which the fish were fed and waste excretion caused increasing turbidity. This also affected the TAN and NO_2-N levels at normal temperature condition compared to the other two conditions but with no significant difference ($P>0.05$). However, water quality did not exceed the standard value range suitable for tilapia culture and did not affect tilapia behavior (Begum, Mondal, Ferdous, Zafar, & Ali, 2014).

Table 1 Mean concentration (±SD) of water temperature, dissolved oxygen (DO), turbidity, pH, total ammonia-nitrogen (TAN), and nitrite-nitrogen (NO_2-N) in laboratory conditions.

treatments (T)	parameter					
	water temperature (°C)	DO (mg/L)	turbidity (NTU)	pH	TAN (mg/L)	NO_2-N (mg/L)
T1	20.43±1.12^{c}	7.47±0.35^{a}	0.86±0.45^{b}	8.21±0.06^{a}	0.18±0.13^{a}	0.07±0.02^{a}
T2	26.72±0.67^{b}	6.17±0.23^{b}	1.97±1.01^{a}	7.91±0.18^{b}	0.30±0.25^{a}	0.15±0.12^{a}
T3	34.73±1.88^{a}	4.67±0.36^{c}	0.80±0.55^{b}	7.70±0.06^{b}	0.14±0.03^{a}	0.23±0.04^{a}

$^{a, b, c}$ For given water quality, means denoted by different letters in columns are significantly different.

Effect of water temperature on swimming speed during the period before and after feeding

For experiment 1 at low temperature condition in all 5 stages, average swimming speeds were as follows: S1 0.002±0.003 cm/s, S2 0.002±0.003 cm/s, S3 0.006±0.01 cm/s, S4 0.005±0.008 cm/s, and S5 0.002±0.004 cm/s. The red color lines show the direction of fish movement. These show that tilapia swim at slow speeds in the corners of the experiment tanks, as shown in (Figure 4).

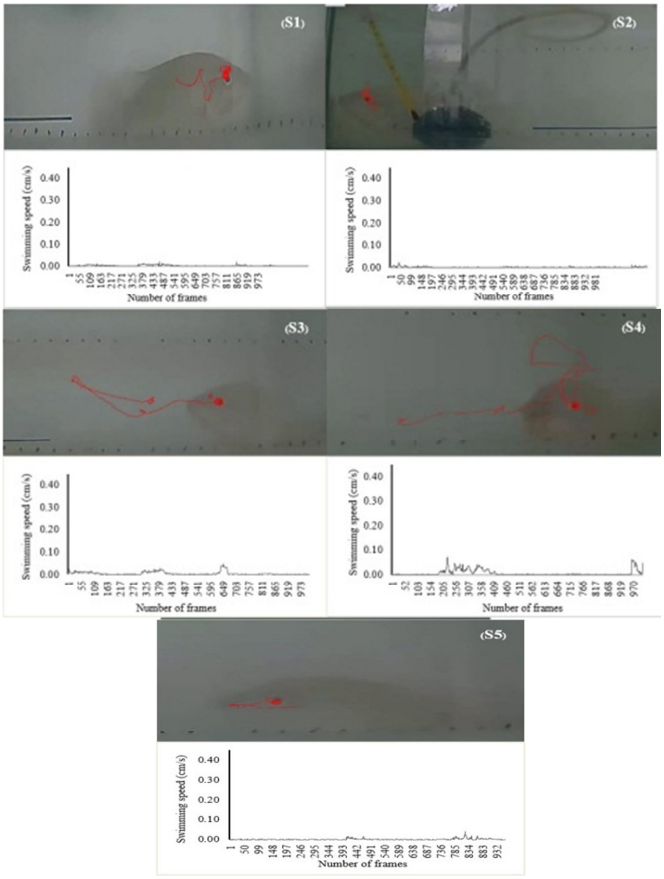

Figure 4 Swimming speeds of Nile Tilapia in low water temperature condition (T1) at S1, S2, S3, S4 and S5.

Average swimming speeds at normal temperature condition in all 5 stages were as follows: S1 0.006±0.014 cm/s, S2 0.016±0.031 cm/s, S3 0.011±0.016 cm/s, S4 0.003±0.005 cm/s, and S5 0.003±0.006 cm/s. During S1, the fish swam around the tank as noticed from the red line but at very low speeds. When the fish were fed (S2), they swam faster and moved around the feed. The fastest swimming speed was recorded when fish swam to the first pellet feed (sprint

swimming), then swimming speed decreased as the fish consumed the feed. When entering S3, the fish still ate the feed for a period of not more than 15 min. When entering S4 and S5, the fish moved less or not at all and swam in the corners of the experiment tanks, as shown in (Figure 5).

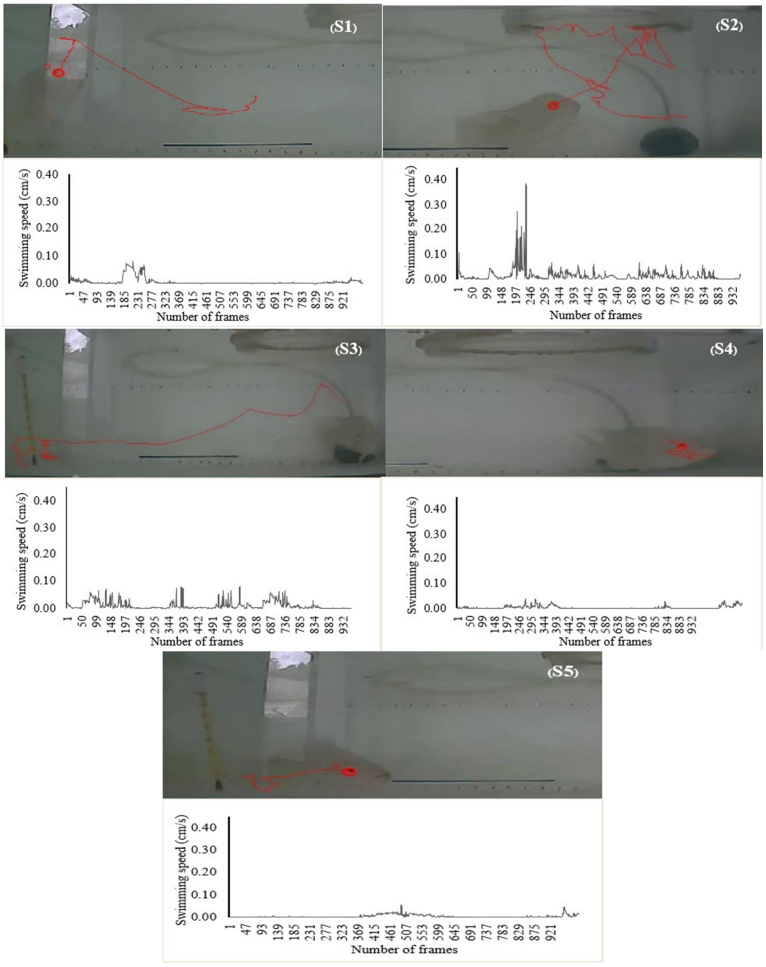

Figure 5 Swimming speeds of Nile Tilapia in normal water temperature condition (T2) at S1, S2, S3, S4 and S5.

For the experiment at high temperature (T3) in all 5 stages, average swimming speeds were as follows: S1 0.007±0.010cm/s, S2 0.006±0.010 cm/s, S3 0.003±0.005 cm/s, S4 0.003±0.006 cm/s, and S5 0.003±0.003 cm/s. These show that fish swam at slow speeds and were always in the corners of the experiment tanks as noticed from the red line and shown in (Figure 6).

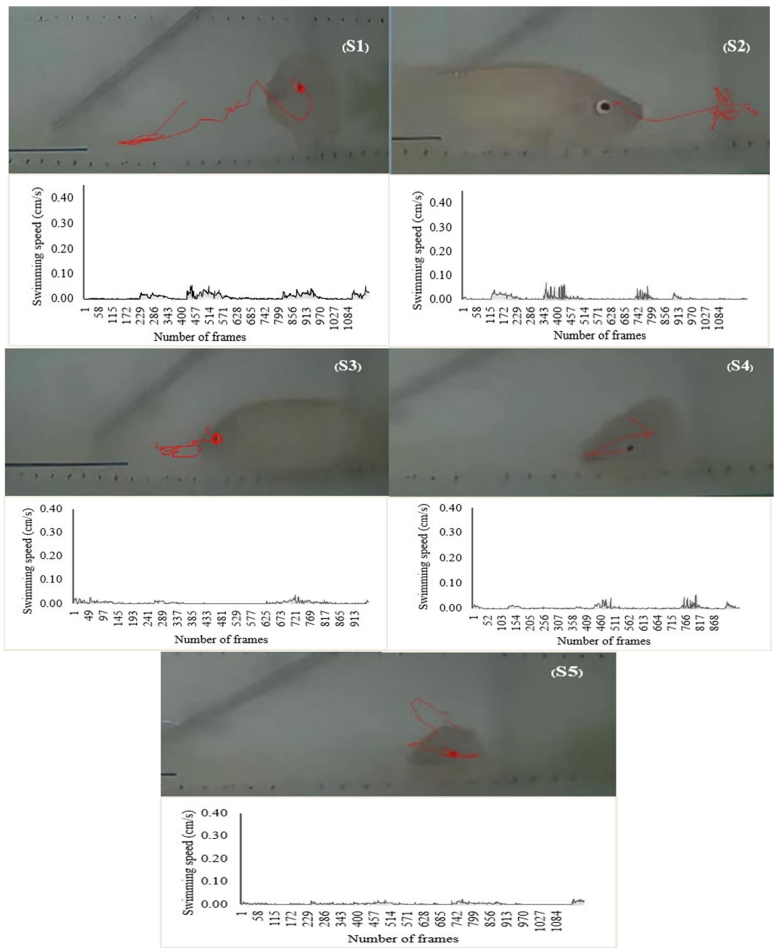

Figure 6 Swimming speeds of Nile Tilapia in high water temperature condition (T3) at S1, S2, S3, S4 and S5.

Analysis results by MANOVA showed that average swimming speeds of low temperature (T1), normal temperature (T2) and high temperature (T3) during the pre-feeding stage (S1) were 0.16±0.09, 0.19±0.11, and 0.21±0.19 cm/s, respectively. During the feeding stage (S2), average speeds of swimming before feeding were 0.26±0.07, 4.95±2.38, and 0.21±0.21 cm/s, respectively. During the period of S4, average swimming speeds were 0.25±0.27 cm/s, 0.24±0.05 cm/s, and 0.26±0.05 cm/s, respectively, while during the 5th stage (S5) average swimming speeds were 0.07± 0.05 cm/s, 0.09±0.05 cm/s, and 0.16±0.08 cm/s, respectively as shown in (Figure 7). Results indicated that average swimming speeds before feeding (S1) in all 3 treatments were not statistically different (P>0.05) when starting to feed until 15 min after feeding (S2). In treatment 2 (T2) at

normal temperature condition, fish showed increased average swimming speed. The speed was significantly different ($P<0.05$) from the low (T1) and high temperature experiments (T3). At normal temperature, fish responded to the feed, resulting in average sprint swimming speed at 7.00±4.00 cm/s which was significantly different ($P<0.05$) from the other conditions, as shown in (Table 2). This was consistent with previous studies (Pitcher, 1993; Felicity, Jobling, & Kadri, 2012), which stated that when fish are hungry, neuropeptide Y (NPY) actively stimulates feed intake. This is produced from the pituitary gland and works with agouti-related protein (AgRP) and ghrelin. NPY is found in the central nervous system and in the pituitary gland and nerves. NPY receptors are found in the brain and the outer organs such as the eyes, stomach and intestines. NPY has a stimulating effect on fish food-seeking behavior, and fish swim at a faster rate to the feed (sprint swimming). The low temperature experiment caused the fish not to respond to the feed and sprint swimming was equal to 0 cm/s or no movement. This result concurred with other papers (Mazeaud, Mazeaud, & Donaldson, 1977; Barton, 2002; Szekeres et al., 2014; Ahmed, Solomon, Alhaji, & Dan kishiya, 2015; Paiboon, Saenphet, & Saenphet, 2018). These papers studied the chemical and physical responses of fish in cold water and reported that the fish produced more cholesterol when water temperature dropped from 25 °C to 21 °C, causing the fish not to respond to the feed. When the temperature falls below 20 °C, the pituitary and adrenal glands produce more lactate and cortisol hormones in the blood. These two hormones are secreted when fish are under stress, causing slow swimming behavior compared to normal temperature. For swimming speed at high temperature, experiment (T3), the fish had no response to the food given and average sprint swimming speed was also 0 cm/s. Elliott (1972); Murugaian, Ramamurthy, & Karmegam (2008); Pandit, & Nakamura (2010) studied the effect of high temperature on feeding of tilapia and reported that the fish responded to food slowly or stopped eating if the water temperature rose above 32 °C. This is because at higher temperature, the fish become stressed and resulted in stomach dysfunction. When the temperature is too high, the enzymes deteriorate and the fish do not have appetite. During the period of 16-30 min after feeding (S3) in the experiment at normal temperature (T2), the fish began to reduce swimming speed. It may be because when the fish become saturated, the pituitary gland will release Cholecystokinin (CCK), Cocaine and Amphetamine regulated transcript (CART), which will inhibit NPY, resulting in stopping fish intake and decreasing swimming speed. However, average swimming speed in this stage was still

greater than the other two conditions and showed a significant difference (P<0.05). At 31-45 min after feeding (S4), average speed of swimming behavior decreased and was similar to swimming speed before feeding in all 3 treatments. Average swimming speed also tended to decrease at 46-60 min after feeding (S5). Statistical analysis showed that average swimming speeds of S1, S3 and S5 were not significantly different (P>0.05).

Different letters (a, b, c) in each swimming stage indicate a significant difference among treatments (T1, T2, T3) (P<0.05).

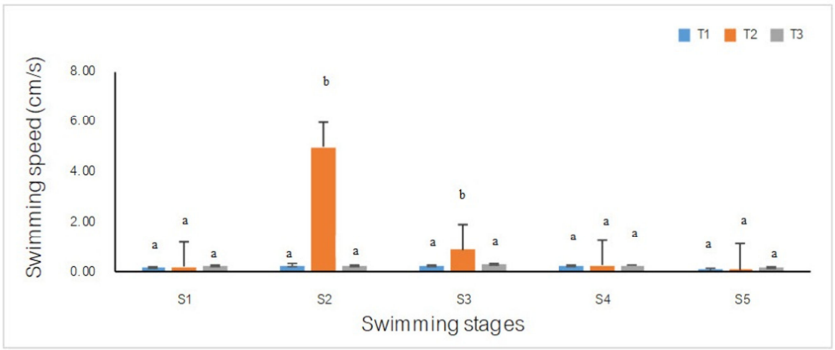

Figure 7 Mean swimming speed of each stages in 3 temperature ranges.

Table 2 Mean (±SD) of sprint swimming speed in 3 temperature ranges.

treatment (T)	T1	T2	T3	*P*-value
average sprint swimming speed (cm/s)	-*	7.00±4.00	-*	<0.05

P-values reported are significantly different.

* non responsive

Conclusions

The Tracker computer technique can be applied to study the behavior of the fish. Here, temperature levels were used as an environmental factor to stimulate the fish eating behavior. At normal temperature, the fish responded to hunger, with average swimming speed in S2 increased to 4.95±2.38 cm/s and sprint swimming speed at 7.00±4.00 cm/s. When they began to saturate, average swimming speed decreased to 0.87±0.09 cm/s and finally, there was no movement. Moreover, this technique can be applied for behavioral study of other aquatic animals. To interpret the behavior more accurately, future studies should be conducted using chemical methods coupled with behavioral studies using this technique.

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