Thesis for the Degree of Master of Fisheries Science

Visual Sensitivity and Behavioral Analysis of Nile Tilapia *Oreochromis niloticus* to LED

by

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LED 광에 대한 틸라피아의 시각민감도 및 행동 분석

Advisor: Prof. Hyeon-Ok Shin

by

Esther Kagoya

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Table of Contents

List of Figures

List of Tables

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Abstract

Oreochromis niloticus has been found sensitive to environmental light and water temperature, which directly affected its growth and productivity. In order to analyze the effect of light and temperature on *Oreochromis niloticus*, the effect of light intensity, light sensitivity and light colour on feeding, behavior and stress have been studied. In this study, spectral sensitivity using electroretinogram was investigated. Furthermore, the effect of temperature and light colour on behavior of *Oreochromis niloticus* using CCTV monitoring system and light emitting diodes as the light source was also assessed. It was found that, *Oreochromis niloticus* was more sensitive to green-yellow light with spectral sensitivity peak of 574 nm wavelengths. It was also found that *Oreochromis niloticus* was more active under red light compared to green. However, red light has been found to make growth of fish inhibiting. It was also found that there was no significant difference in fish activity under water temperature from 23 to 35 ^{o}C. The highest activity was observed at 37 ^{o}C however, fish stopped feeding at this temperature. Therefore, study suggested that suitable environmental condition to cultivate *Oreochromis niloticus* would be under the green LEDs with water temperature ranging from 23 to 30^{o}C.

Key words: visual sensitivity, light emitting diodes (LED), temperature, behavior and electroretinogram (ERG)

Introduction

Aquatic organisms' growth, culture and survival are influenced by a number of factors such as temperature and light including wave length, intensity and photoperiod which are ranked as key and deeply influence the physiology of fish (Lorenzen, 2000).

In nature, wavelength of light penetrating water varies greatly i.e. light attenuates with depth and its spectral composition changes differentially underwater. While the long end of the visible spectrum penetrates relatively shallow waters, the short end becomes predominant in deeper. Spectral composition is a main characteristic of light hence most species of fish have well-developed color sight, and are said to be very sensitive to colored light i.e. oceanic and deep-water fishes tend to be sensitive to blue light, where as fresh water and shallow marine fishes tend to be sensitive to green light (Bowmaker, 2008). Influence of light on aquatic organisms has been expressed in various ways, such as successful larval rearing depends on environment and all three characteristics of artificial environment (wavelength, Intensity and photoperiod). Furthermore, survival and growth rate of most fishes are higher with blue and green light (Ruchin et al., 2002; Volpato and Barreto, 2001; Castro et al., 2009). Therefore, fish vision and spectrum perception are strongly adapted to each species natural habitat and living ethology), therefore, these influence cultured fish growth performance, behavior and physiological status (Volpato and Barreto, 2001; Delabbio, 2015). Fish behavior varies depending on species in response to temperature and light (Villamizara et al., 2011). However, light of high intensity can be stressful or even lethal to fish.

Incandescent and fluorescent lighting systems are traditional artificial sources for environmental lighting in fisheries, however, these have been found inefficient and not cost effective. Therefore, these have been replaced by light emitting diodes (LEDs) in aquaculture and fisheries due to their narrow bandwidth outputs and their ability to permit intensity and spectrum manipulation to simulate the environmental conditions that match the target species' sensitivities. LEDs are new lighting technology that can be manufactured to output specific wavelengths (Migaud et al., 2007). In addition, LEDs have lower power requirements, lower electrical running costs, and a longer life span than the standard metal halide bulbs (Migaud et al., 2007). These have been found effective in fisheries i.e. green and blue LEDs which are both short wavelengths increased antioxidant materials against oxidative stress in the yellowtail clown fish, *Amphiprion clarkia* whereas red LED affects physiological function and induces oxidative stress in yellowtail clownfish (Shin et al., 2011). Furthermore, blue LED has been shown to play a role in reducing stress in the *Oreochromis niloticus* (Volpato and Barreto, 2001).

Oreochromis niloticus is an African freshwater cichlid and one of the world's most important food fishes. It's the predominant cultured species worldwide (FAO, 2014) due to its tolerance to a wide range of environmental conditions. Popma and Masser (1999) monitored tolerance of tilapias to high salinity, high water temperature, low dissolved oxygen, high ammonia concentrations and diseases. It's omnivore that feeds on both plankton and aquatic plants typically during daytime hours, similar to other fishes, it exhibits the behavioral response to light as a main factor contributing to its feeding activity. In addition, *Oreochromis niloticus* generally feeds and reproduces in shallow waters, as harmful gases such as carbon dioxide, hydrogen sulfide and ammonia plus temperature fluctuations found in deep waters create problems for its physiology. The species is thermopile and therefore, can only survive if temperatures are not lower than fatal.

Oreochromis niloticus has expressed different visual pigments through ontogeny (Sabbah et al., 2012). Effects of Wavelength, light intensity and photoperiod have been monitored in this species in relation to light intensity and aggressive interactions (El-Sayed and Kawanna, 2004, Elsbaay, 2013). It has also been found that red light stimulates its feeding motivation, blue light prevent stress, minimize mortality and increase weight in Oreochromis niloticus.(Gilson et al., 2013)

Many studies have evaluated temperature and light in relation to health, stress, feeding, reproduction, aggressive interactions, growth and visual sensitivity in fish (Beitinger, 1990; Schreck, 1990; Plumb, 1994; Stoskopf, 1994). However, a few studies have focused on visual sensitivity using electroretinogram with LEDs as light source. Furthermore, effect of temperature and light spectrum on behavior of *Oreochromis niloticus* has not yet been studied. Therefore, in this study visual sensitivity of *Oreochromis niloticus* using electroretinogram (ERG) was investigated with LED as light source. Also behavior of

Oreochromis niloticus under varying temperature and wavelength was analyzed. It was hypothesized that *Oreochromis niloticus* is sensitive to light of wavelengths ranging from 400 to 660 nm (Lisney et al., 2010) and that its behavior is influenced by light spectrum and temperature.

Materials and Methods

2.1 Experiment on Visual sensitivity

In this experiment, visual sensitivity of *Oreochromis niloticus* to LEDs as a light source was assessed using ERG. Ten adult fish from aquaculture farm were used in the study. Fish were stabilized in an experimental tank, and temperature was gradually increased by one degree until 21^{o}C under the natural photoperiod for 7 days of acclimatization prior to the experiment. Fish were fed once a day on commercial feed (protein 52 %, ash < 15 %, fat 10 %, calcium 1.5%, fiber < 3% and phosphorus < 2.5%).

2.1.1 Light source

Before the experiment, 12 LED packages (L5 × W5 × H1.5 mm) were prepared and mounted on the epoxy board (L80 × W80 × H1.5 mm) (Figure 2). Luminous intensity, spectral power curves, irradiance and peak wavelengths of each LED were measured using Stella net spectrometer at different current levels (Figure 1). LED wavelengths used in the experiment ranged from 405 to 660 nm (Table1).

Table 1. Specification of LEDs for lighting system

No.	Wavelength (nm)	Model No.	Voltage (V)	Current (mA)	Light Intensity (W/m^2)
1	405	LVH1056	3.3	60	1.41
2	432	LRU1056	3.0	60	1.99
3	465	LBH1056	3.3	60	0.97
4	505	LCH1056	3.2	60	0.24
5	520	LGH1056	3.2	60	0.40
6	540	LRU1056	2.8	60	0.264
7	542	LRU1056	2.8	60	0.304
8	574	LYG1056	2.0	60	0.025
9	591	LYH1056	2.0	60	0.074
10	610	LAU1056	2.0	60	0.203
11	640	LRH1056	2.1	60	0.203
12	660	LRU1056	2.0	60	0.794

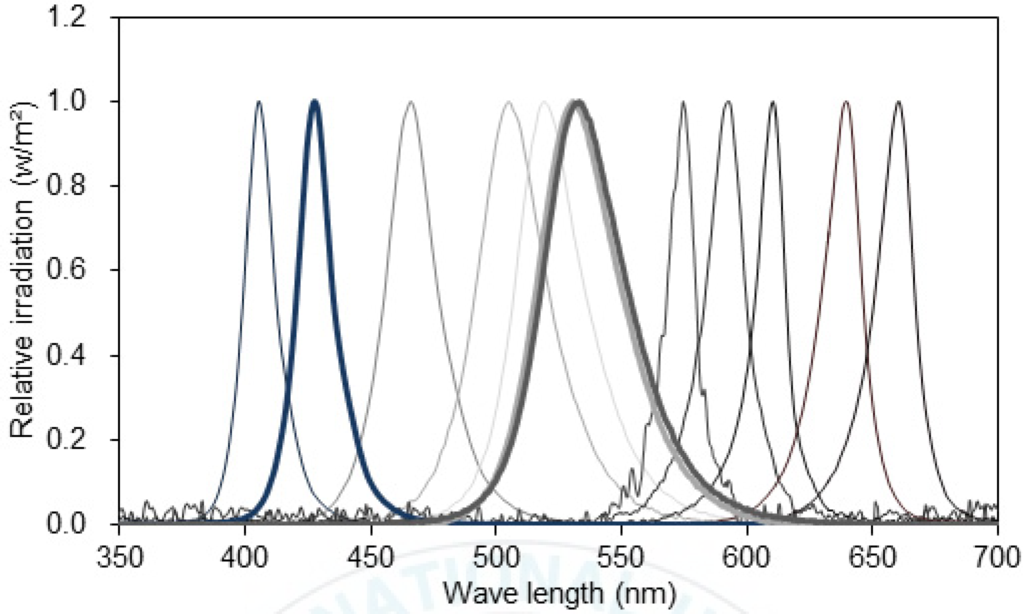

Figure 1. Measured relative spectral sensitivity curves of LEDs used in the experiment.

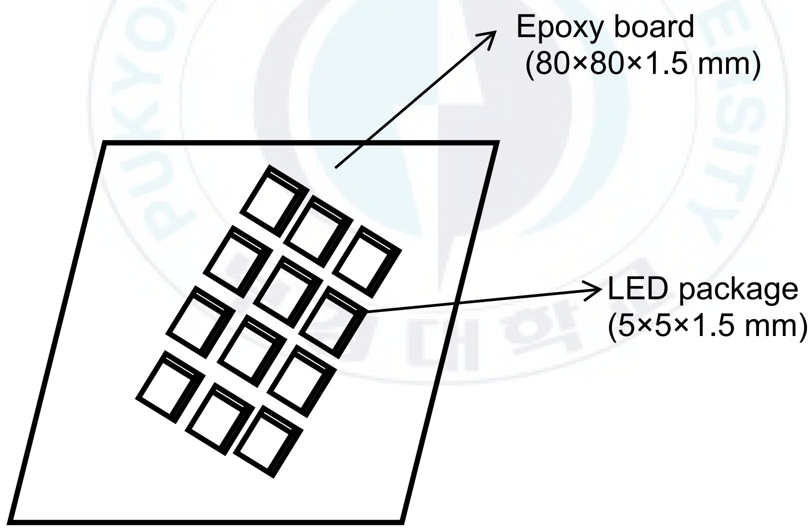

Figure 2. Arrangement of LED packages attached on an epoxy board.

2.1.2 Light intensity

During ERG experiment, electric current was adjusted using a resistor and light intensity of the stimulus was varied in 8 discrete steps (Table 2) by a programmable logic controller (PLC) automatically for each LED package (Figure 3), LED packages were selected manually.

Table 2. Discrete steps used to vary light intensity

Number	R1	R2	R3	R4	R5	R6	R7	R8
Resistance (kΩ)	59.2	50.0	44.0	33.0	25.0	16.6	8.2	3.3

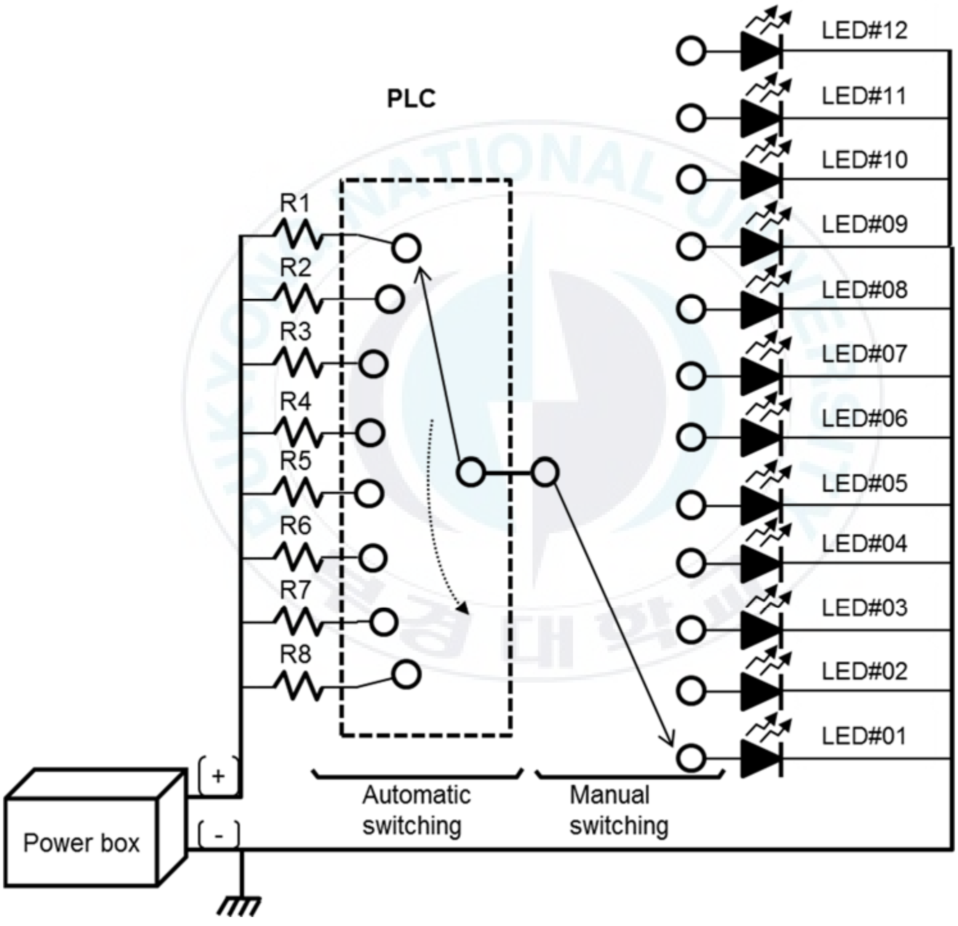

Figure 3. Circuit diagram of a prototype automatic light controller.

2.1.3 ERG recording

Before recording, five fish were measured weight and total length. The average of weight and total length was 14.5 ± 6.0 g and 87 ± 13 mm, respectively. Fish was dark-adapted for 2 hours in a bucket containing 2.5 liters of fresh water, and anesthetized by an intramuscular injection of gallamine triethiodide (mg/kg, Ringer's solution). The optimum amount and concentration (0.0375mg/ml for 10g fish) of the gallamine triethiodide in Ringer's solution was determined to suit individual fish. Anesthetized fish was placed in a faraday cage. It was shielded with opaque clothes, and fish was allowed to dark-adapt for 30 minutes more.

During ERG recordings, the body of the fish remained out of the water and the fish was artificially ventilated by pumping aerated fresh water of 20℃ temperature over the gills to keep it alive. It was done manually by placing a small inlet tube in the fish mouth through which aerated freshwater was flowing through to fish gills and recycled continuously.

ERGs were recorded from the faraday cage by using two silver-wire electrodes. Recording electrode was bent to avoid damage and placed on the cornea so that it rested on the retina. The reference electrode was a silver wire (bent to hold its position) placed on the cranium of the fish. Electrodes were positioned using a micromanipulator. Electrical signals were amplified with a differential amplifier (P400, Physiolab, Korea) and amplified signals transmitted simultaneously to a data logger (PA20, R10N, Japan) and a laboratory computer. LEDs were used as light source each with different peak emissions ranging from 405 to 660 nm (Figure 3 and Table 2).

The attenuated light was positioned such that light was projected onto the entire pupil of the experimental fish. The stimulus light system was placed inside the faraday cage and dim red light was used to provide ambient illumination while setting up electrodes on the body of dark-adapted fish.

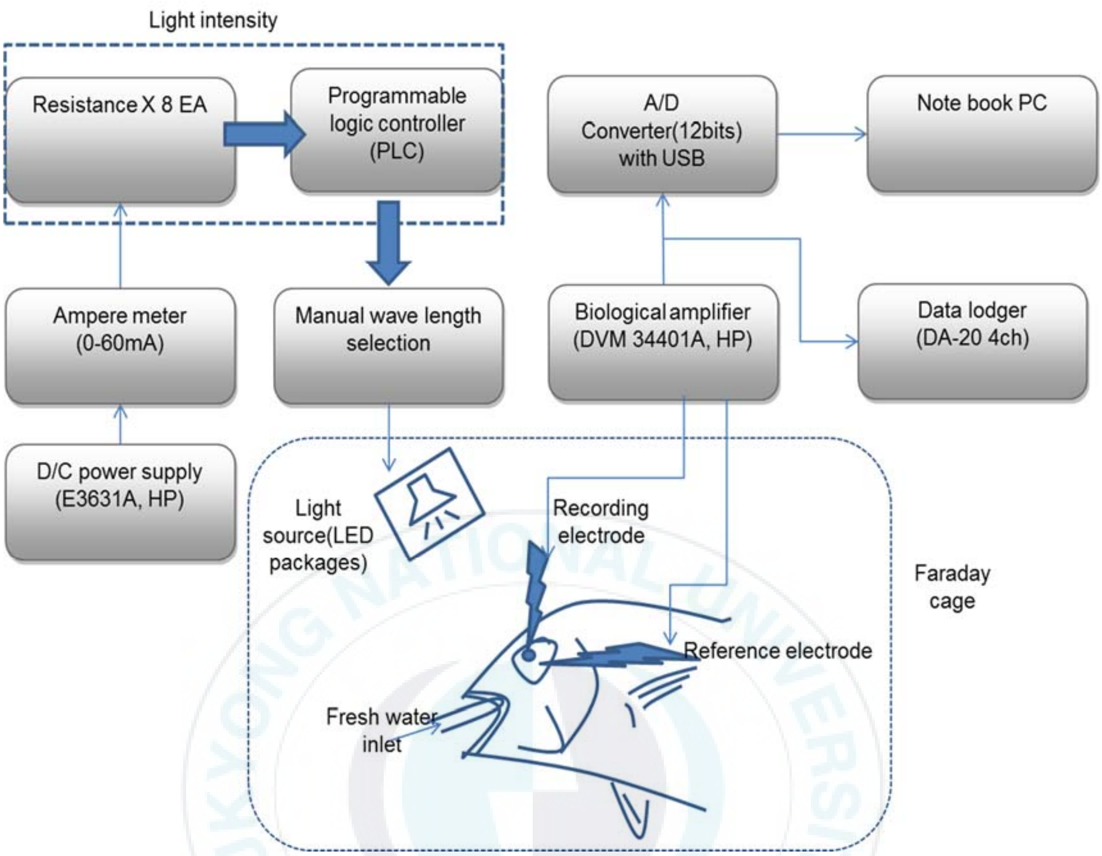

Figure 4. Schematic representation of equipment setup for ERG recording.

2.1.4 Visual spectral sensitivity

In each recording session, ERGs were recorded in response to narrow-band stimuli of increasing intensity (each flash for 500 m/s). To maintain the dark-adapted state, at least 20 seconds were used to separate the stimulus presentations. Twelve sessions were conducted one for each wavelength (Figure 3 and Table 2). Equal-intensity light stimulus was recorded between each session for correcting the change of ERG voltage with time. To determine retinal sensitivity, b-wave amplitude was measured. Responses versus light intensity curves were constructed for the different wavelengths, and these curves were then used to interpolate the threshold of the incident light intensity required to generate criterion responses in *Oreochromis niloticus* (n =5). The criterion responses were determined for each fish by selecting values above the baseline recording of responses; it was done because of the variation in ERG voltage among individuals, and the narrow intensity ranges of stimuli among some LEDs for a detectable ERG voltage. Relative spectral sensitivity was calculated using the inverse ratio of the threshold light intensity. Spectral sensitivity peak wavelength was estimated from ERG data by fitting Stavenga et al. (2010) template to averaged relative sensitivity data.

$$S_{\alpha} = A \exp\left[-a_0 x^2 (1 + a x)\right] \qquad (1)$$

With $x = \log_{10} (\lambda/\lambda_{max})$; λ is the wavelength and λ_{max} the peak wavelength.

2.2 Experiment on behavior

Five fish (18.6 ± 10.3 g in weight, 9.9 ± 2.0 cm in total length) were used in analyzing behavior under varying temperature and wavelength using CCTV camera and LEDs as light source.

Acclimatization was carried out for 2 days at 21^{o}C and 22^{o}C under natural photoperiod prior to start of the experiment in a recirculation aquaculture system (RAS) tank. First, fish behavior was recorded under natural photoperiod (white light) for duration of 3 hours (13:00, 14:00 and 15:00p.m.) daily for 17 days whereas temperature was being increased by 1^{o} C. In the second experiment, fish behavior was recorded under different light colors (white: 13:00p.m., red: 14:00p.m, and green: 15:00p.m.) for a duration of one hour each for 7 days. Experiment carried out in white colour was used as control. Temperature was also being increased by 1^{o}C per day from 28^{0} to 34^{0} C because *Oreochromis niloticus* prefers temperatures between 31 to 36^{0}C (Nach and Novotny, 1995). Fish were fed once a day on commercial feed throughout the experiment.

The monitoring system (CCTV Camera) composed of four infrared cameras (CNB-D2000, CNB technology INC, Korea) and was linked to a real time digital video recorder (N-0441L, NADATEL CO. LTD., Korea). CCTV camera was used to record the image, movement of fish and time for one hour (40 minutes on-light stimuli and 20 minutes off-light stimuli) on a four channel real time image recorder after which videos were retrieved for analysis using PVStudio 3D software (version 2.3, DAIKIN COMTEC., Japan). Fish position was determined manually by a mouse click between the fish eyes. Data was exported to excel and x-y coordinates were recorded. Fifteen-minute sample data recorded within the first 30 minutes of the experiment for every one hour were used to analyze behavior obtained by measuring the distance accumulated for each wave length. Cartesian coordinate for two points was used to estimate moving distance during 15 minutes as below;

$$\mathrm{d} = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2} \tag{2}$$

Moving distance was used to obtain speed of fish which was used to estimate swimming activity of fish as below;

$$\text{Swimming activity (TL/s)} = \frac{\text{Fish speed (cm/s)}}{\text{Fish total length (cm)}} \tag{3}$$

2.3 Statistical analysis

The parameter values for electroretinogram analysis were determined by method of least-squares in Microsoft excel. Students T-tests and Post-Hoc were performed when ANOVA tests were found significant ($p<0.05$) to analyze behavior data using. All statistical analyses were performed using statistics software (SPSS Ver. 16 for Windows, IBM, USA)

Results

3.1 Visual sensitivity of *Oreochromis niloticus*

3.1.1 Light emitting diode (LED) output

As illustrated in Figure 5, peak wavelength of LED was directly proportional to electric current. Peak wavelength increased with the increase in electric current from 10 to 60 mA for the different LED packages. Maximum peak wavelength was observed when the electric current was at the standard LED current (60 mA).

Relationship between LED light intensity and electric current was computed using linear regression. In the result, the LED light intensity was directly proportional to electric current (Figure 6 and 7). In addition, the short wavelengths (405 ~ 465 nm) showed high illumination, whereas the long wavelengths (505 ~ 640 nm) showed low illumination with minimum in observed at 574 and 591 nm.

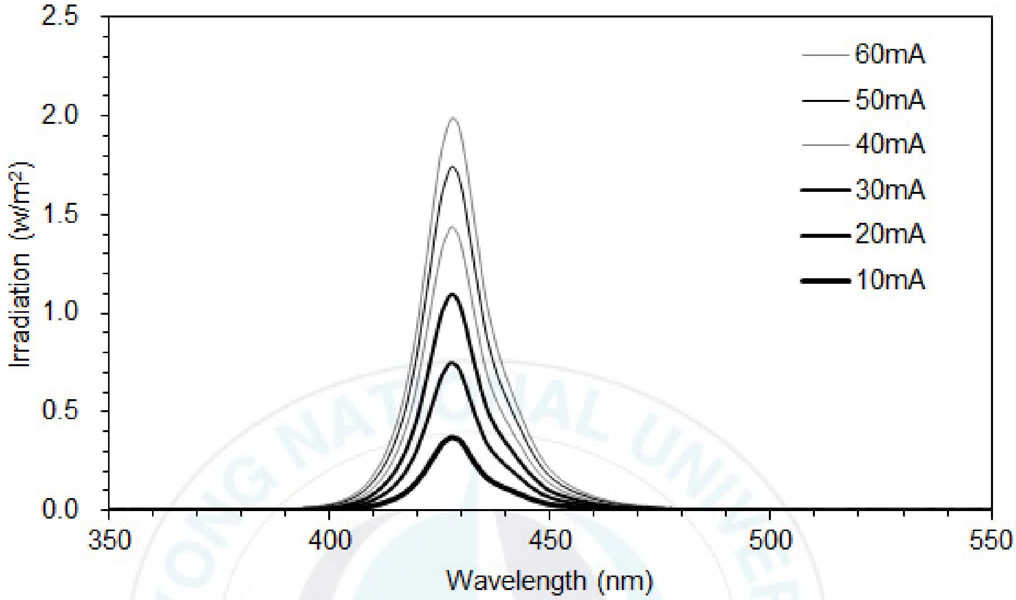

Figure 5. Measured relative spectral power curves from 428 nm LED package.

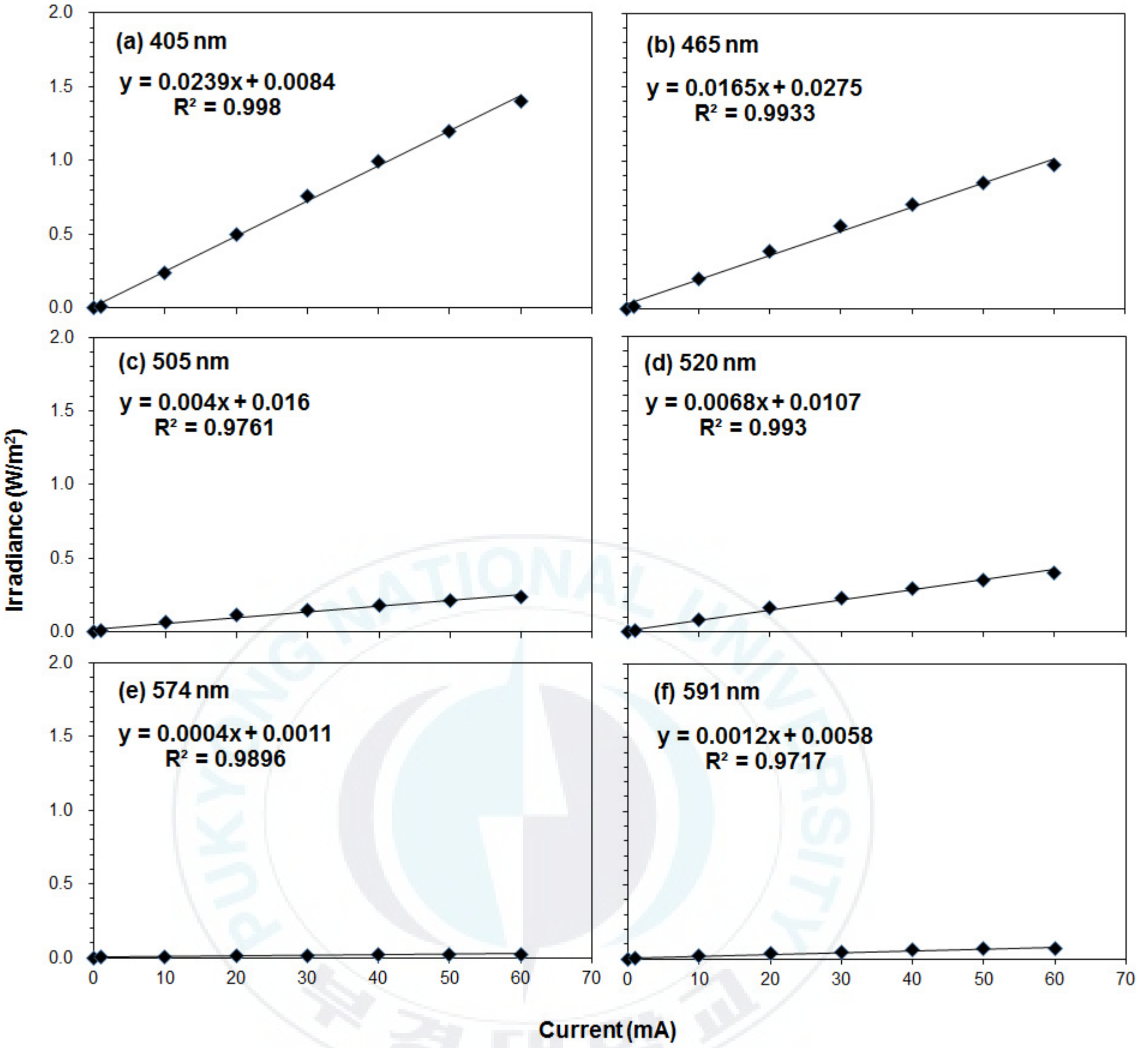

Figure 6. Relationship between LED light intensity and electric current used in the experiment for wavelengths ranging 405 ~ 591 nm.

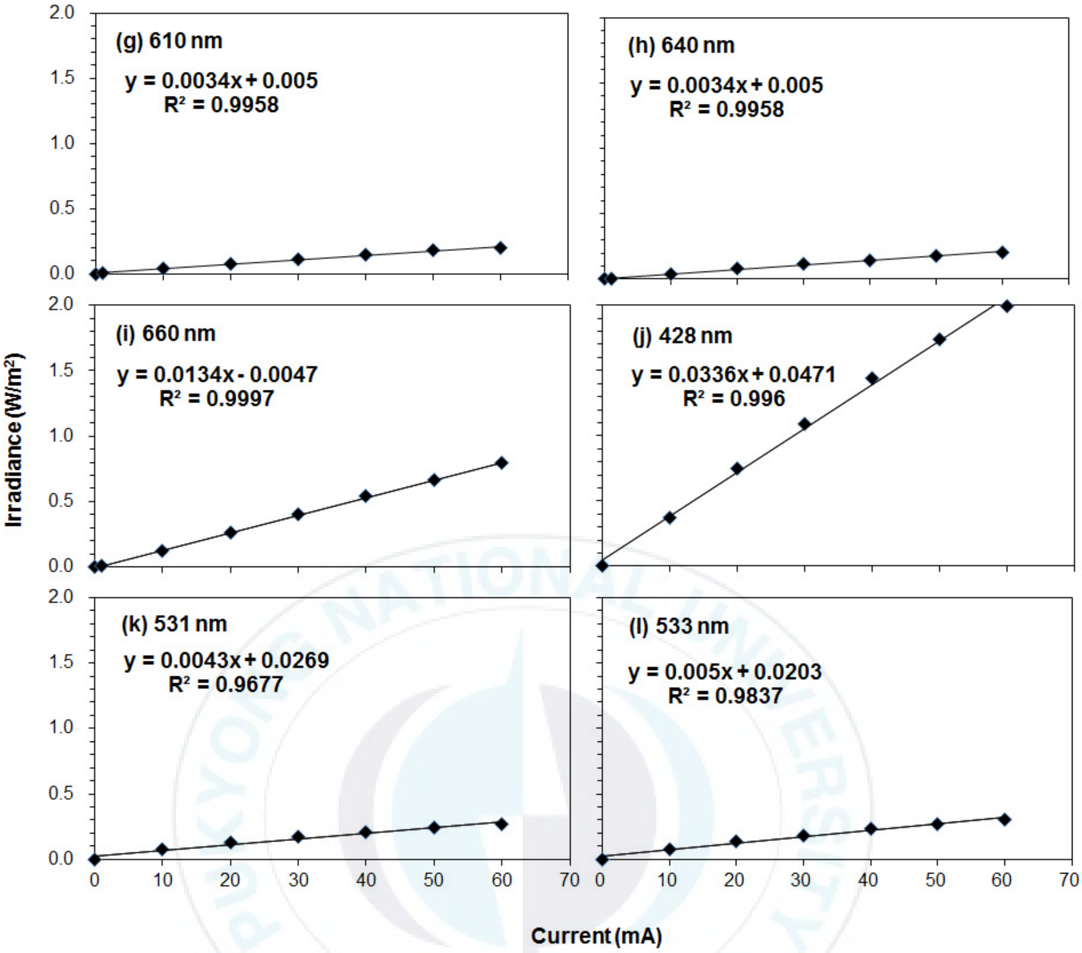

Figure 7. Relationship between LED light intensity (irradiance) and the electric current used in the experiment for wavelengths ranging 591 ~ 660 nm.

3.1.2 ERG signal

In the study, 3 of the 4 typical bands (a-wave, b-wave and d-wave) of the ERG wave in dark-adapted *Oreochromis niloticus* were observed. It was shown that ERG started from a-wave and followed by b-wave and lastly d-wave (Figure 8). However the dominant wave bands obtained from the experiment were a-wave and b-wave which were used to measure to measure amplitude response of fish to light of different wavelength as shown in Figure 8.

ERG signals were detected from all LED packages at different light intensities. Wavelength 505 nm showed higher amplitude, whereas 405 nm showed the least (Table 3).

As seen in Figure 9, response amplitude of *Oreochromis niloticus* increased with the increase of light intensity. It also indicated that response amplitude depended on the wavelength in use. The responded amplitude of *Oreochromis niloticus* was high at 505 nm and low at 405 nm for fish (n=5; W=26.0 g; TL=11.0 cm).

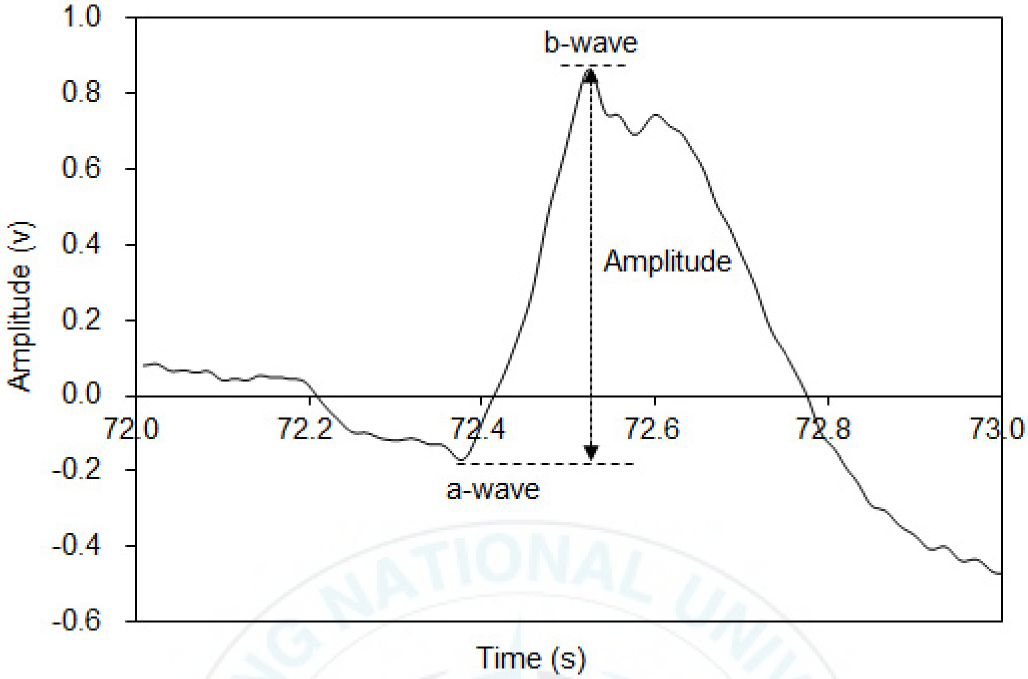

Figure 8. Sample of measured ERG waveform in dark-adapted *Oreochromis niloticus*.

Table 3. Relationship between LED intensity radiated to *Oreochromis niloticus* and measured ERG response amplitude

Wavelength	Light intensity (log quanta/cm^2/s)		ERG amplitude (v)	
	Min	Max	Min	Max
405	-1.47	-2.01	0.07	0.73
428	-1.31	-1.09	0.164	1.216
465	-1.55	-1.35	0.35	1.94
505	-1.79	-1.70	0.60	2.16
520	-1.96	-1.81	0.33	1.39
531	-1.57	-1.51	0.27	1.73
533	-1.69	-1.60	0.27	1.73
574	-2.95	-2.86	0.38	0.67
591	-2.23	-2.15	0.42	0.82
610	-2.29	-2.13	0.49	1.11
640	-2.29	-2.14	0.49	0.69
660	-	-2.33	0.74	1.10

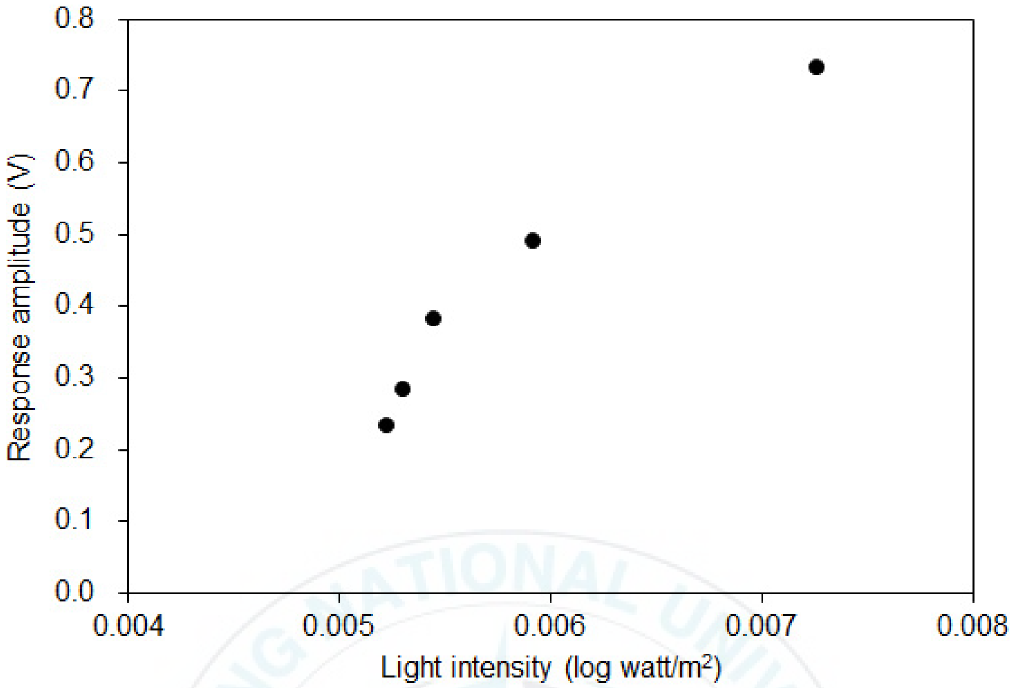

Figure 9. Relationship between response amplitude and log intensity of dark-adapted *Oreochromis niloticus* (n=5; W=26.0 g; TL=11.0 cm) at 625 nm of wavelength.

3.1.3. Visual threshold

Visual threshold refers to the minimum light intensity that can be detected by the dark-adapted eye. Visual threshold of *Oreochromis niloticus* was inversely proportional to the LED wavelength. Visual threshold increased with decrease in wavelengths. The visual threshold of the test fish was high in the short wavelengths and low in the long wavelengths. *Oreochromis niloticus* responded to high light intensity under the short wavelength, whereas its response to light intensity under the long wavelength was low with minimum visual threshold at 574 nm. In contrast, *Oreochromis niloticus* showed high visual threshold at 660 nm wavelength for all the fish used in the experiment. In Figure 10, *Oreochromis niloticus* responded to light intensity of -2.0 W/M^2 at 405 nm wavelength, and light intensity was increased to -1.5 W/M^2 at 432 nm. However, light intensity was slightly decreased to -1.8 W/M^2 at 465 nm, -2.0 W/M^2 at 505nm and -2.2 W/M^2 at 520 nm. There was further decrease in light intensity to -2.3 W/M^2 where it was constant for both 540 and 542 nm and finally dropped to -3.0 W/M^2 at 520 nm. It was followed by an increase in visual threshold to -1.31 W/M^2 at 591 nm, and then increased to -1.67 nm at 610 nm. There was almost similar increase in light intensity to -1.69 W/M^2 and finally toped at 0 W/M^2 at 660 nm wavelength.

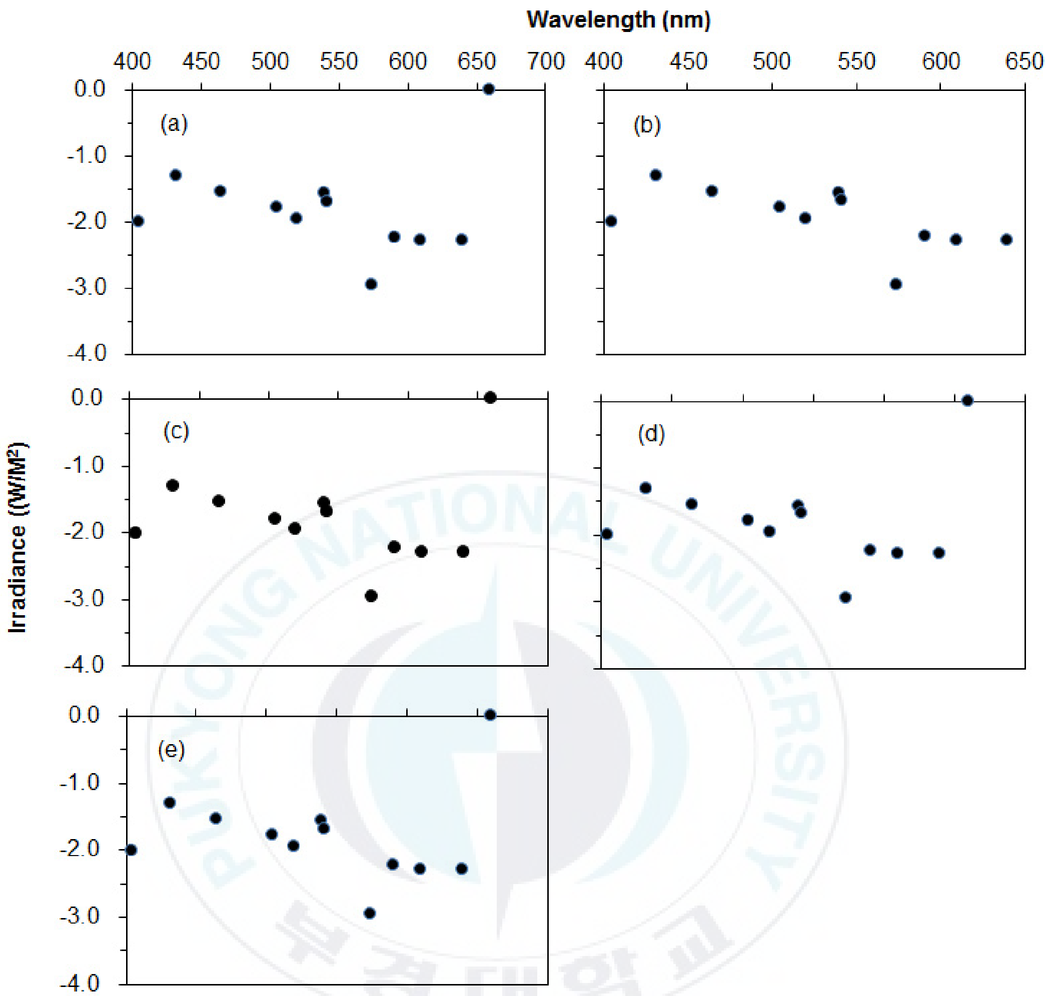

Figure 10.Visual threshold of *Oreochromis niloticus* from LED wavelength of weight and total length (a) 9.0 g and 8.0 cm, (b) 15.2 g and 9.2 cm, (c) 11.4 g and 8.0 cm, (d) 11.1 g and 7.5 cm and (e) 26.0 g and 11.0 cm.

3.1.4. Spectral sensitivity of *Oreochromis niloticus*

Spectral sensitivity is the relative efficiency of detection of light or other signal, as a function of the frequency or wavelength of the signal. In Figure 11, it was shown the average sensitivity of *Oreochromis niloticus* (n=5) to LEDs of wavelength ranging from 405 to 660 nm. It showed maximum average sensitivity of 1.0 quanta/cm²/s at 574nm whereas 660 nm wavelength showed minimum sensitivity 0f -0.27 quanta/cm²/s. Although fish was more sensitive at 574 nm and less sensitive at 660nm wavelength, fish tended to be more sensitive to the long wavelength than the short wavelength.

Nonlinear regression analysis was used to estimate parameters of Stavenga et al. (2010) and estimates were; A= 0.998, a_0 =9.22e^3, and a_1 =6.534 at maximum wavelength (574 nm). Using estimated parameters, final was expressed as shown in equation (4).

$$S_{\alpha}= 0.998 \exp [-9.22e^3 x^2 (1+6.534x)] \tag{4}$$

By fitting Stavenga et al. (2010), template to average sensitivity data obtained by electroretinogram, peak spectral sensitivity of *Oreochromis niloticus* was estimated at 574 nm whereas the minimum was at 432 nm. *Oreochromis niloticus* showed visual capability to wavelengths between 400 ~ 640 nm with increased sensitivity to long wavelengths and least sensitive to short wavelengths, however fish was not sensitive at 660 nm wavelength (Figure 12.)

There was a good fit between averaged spectral sensitivity data and the model (Pearson correlation, R^2=0.81). In addition, there was a shift in the curve to the long wavelength of the light spectrum with the sensitivity curve ranging from 480 to 640 nm wavelengths. However, the sensitivity curve extended to the short wavelengths and increased as 405 nm as shown in Figure 12.

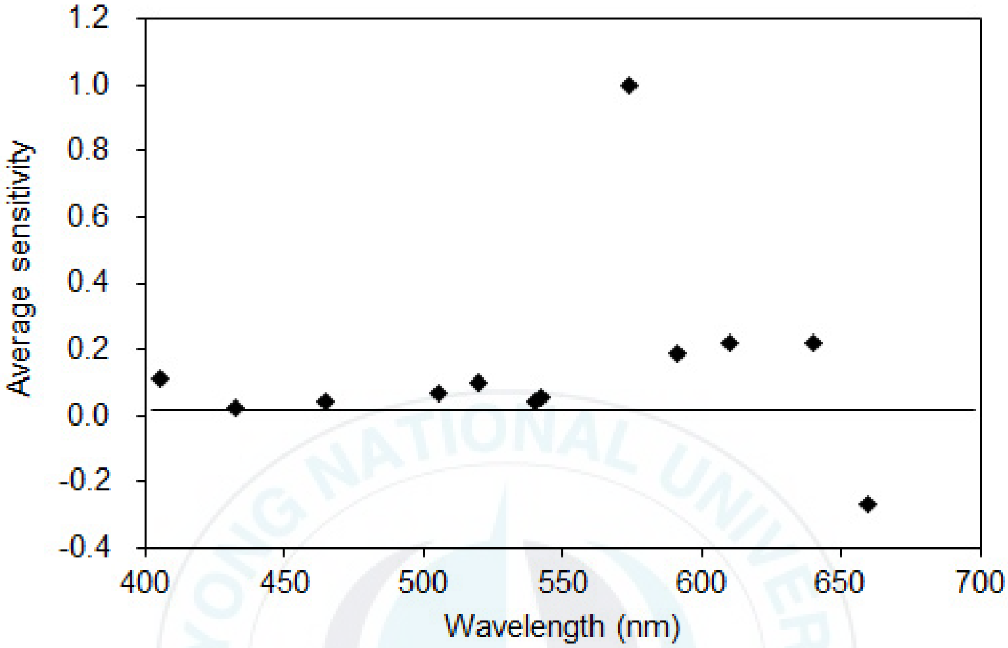

Figure 11. Averaged spectral sensitivity of *Oreochromis niloticus* (n=5).

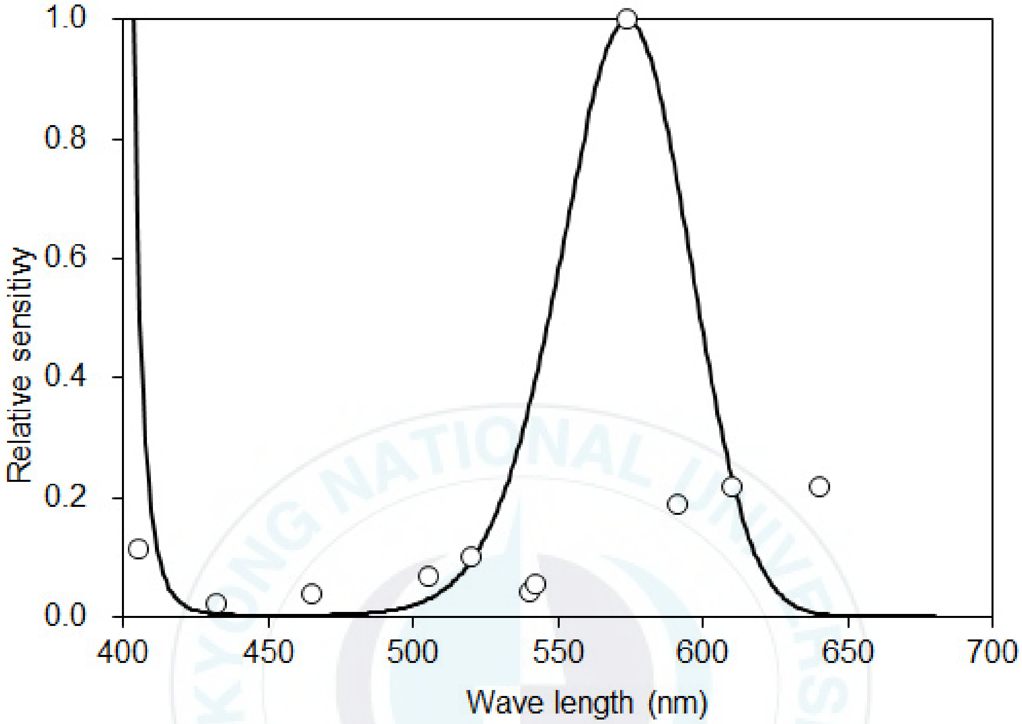

Figure 12. Relative spectral sensitivity of *Oreochromis niloticus,* White circles indicate measured sensitivity based on threshold light intensity. Solid line indicates curve fitted to the template of Stavenga et al. (2010).

3.2 Behavior of *Oreochromis niloticus*

At the beginning of the experiment, movement distance was 32 cm at water temperature of 23 °C under the natural photoperiod. Then, it slightly decreased until it reached 24 cm at 25 °C temperatures. Movement distance of the fish was highest at 26 °C temperatures; it was followed by a decrease at 27°C. After a moderate fall, there was a slight increase again. After being stable between 28 ~ 30 °C for three days, movement distance dropped until it reached its lowest at 33 °C before increasing again (Figure 12). However, there was no significant relationship between movement distance and water temperatures (??)

Similar pattern was observed for swimming speed of *Oreochromis niloticus* as an indicator of fish activity with movement distance (Figure 13). Fish was more active at water temperature between 23 and 30 °C and 37 °C whereas fish was less active at water temperature between 31 and 34 °C. However, there was no significant relationship between fish swimming speed and different water temperatures, even though fish stopped feeding at 37 °C (Pearson correlation, R^2=-0.07).

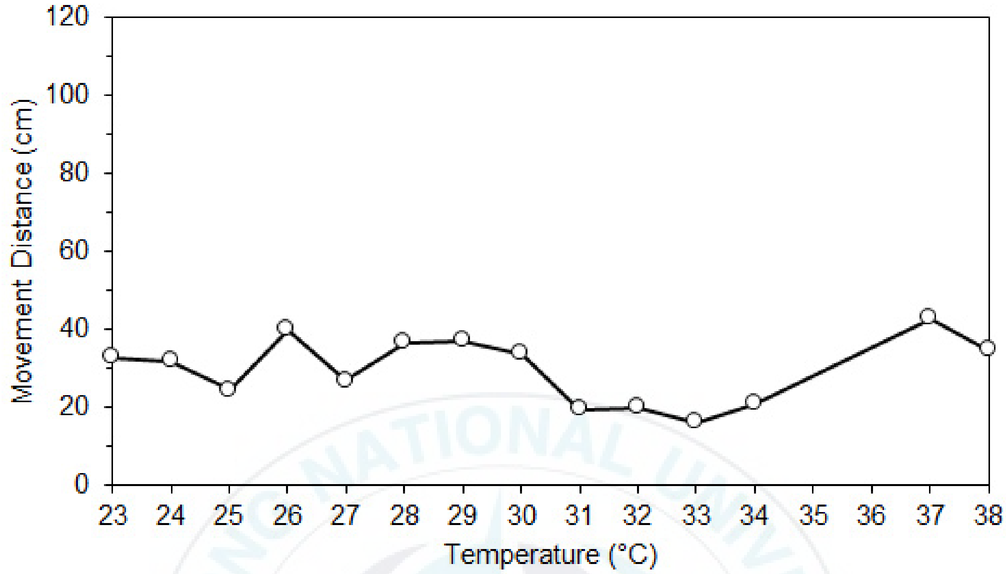

Figure 13. Movement distance of *Oreochromis niloticus* with varying temperature under the natural photoperiod.

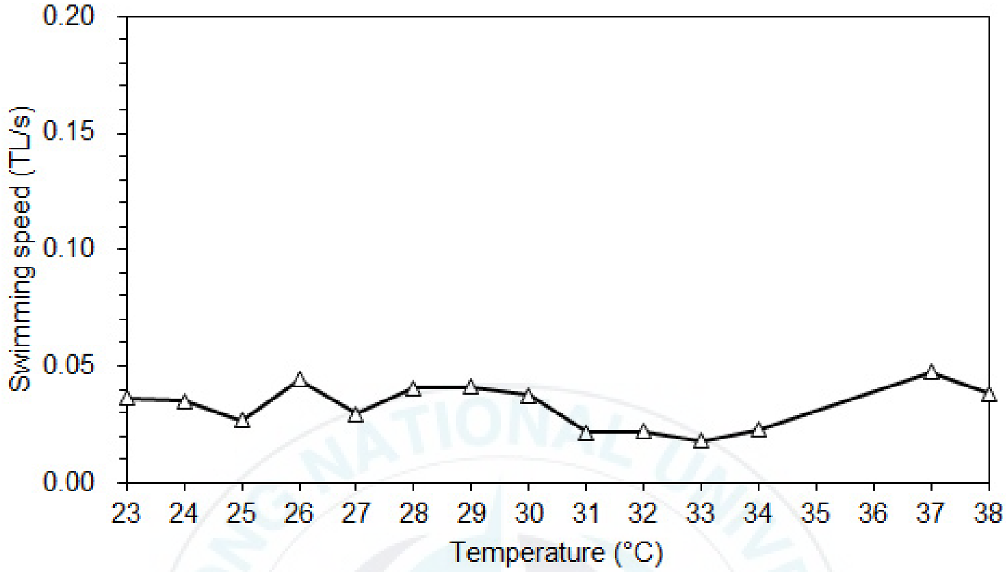

Figure 14. Swimming speed of *Oreochromis niloticus* with varying temperature under the natural photoperiod.

To determine effect of changing temperature and wavelengths on behavior of *Oreochromis niloticus,* movement distance and swimming speed were used to measure fish activity under varying temperature and wavelengths (white, green and red). The results indicated that movement distance of *Oreochromis niloticus* was influenced by different wavelengths (One-way ANOVA, $F = 53.26$, $p<0.001$). Fish activity was high in red color than green (Post-Hoc Turkey, M=61.5, SD=6.0, $p<0.001$, Table 4). In general, there was significant difference between fish activity under different light colors.

Oreochromis niloticus showed higher activity in red, followed by green and lastly white (Figure 14). For swimming speed, *Oreochromis niloticus* followed the same pattern as movement distance (Figure 15).

In the study, we examined whether there was a significant difference in fish activity between wavelengths (control, red and green) in relation to movement distance. The test revealed a statistically high significant difference in activity of fish among wavelengths. The fish activity was the highest in red colour followed by green and lastly white colour as control group. Also using groups, red and control and green and control groups had the highest statistical difference with mean difference of 61.52 ± 6.05 cm and 39.79 ± 6.05 cm whereas control and green had the least static difference with mean difference of 21.73 ±6.05 cm.

Table 4. Difference in *Oreochromis niloticus* reaction (movement distance) to wavelengths using Post-Hoc Turkey's test

Subject	Wavelength	Mean Difference	Std. Error	Sig.
Control	Red	-61.517*	6.048	.000
	Green	-21.730*	6.048	.006
Red	Control	61.517*	6.048	.000
	Green	39.787*	6.048	.000
Green	Control	21.730*	6.048	.006
	Red	-39.787*	6.048	.000

*. The mean difference is significant at the 0.05 level.

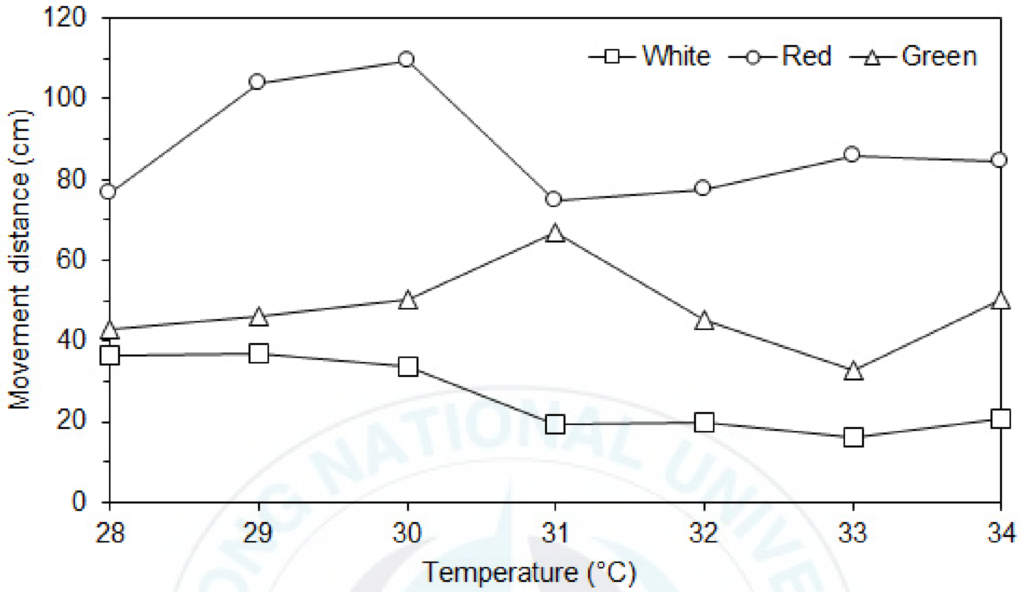

Figure 15. Effect of temperature and wave length on moved distance of *Oreochromis niloticus.*

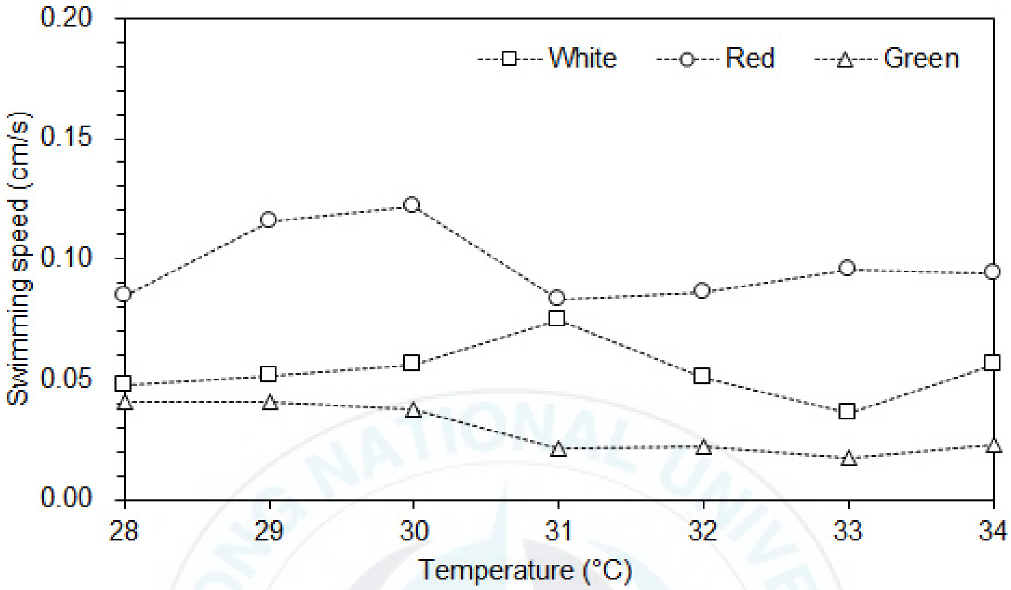

Figure 16. Effect of wavelength and temperature on *Oreochromis niloticus* activity in relation to swimming speed.

Discussion and Conclusion

The eyes are major light receptor organs for fish. Light in the environment stimulates photoreceptors in the retina of the fish's eye, which changes chemical signals into electrical ones that are interpreted by the fish's brain. The pineal gland is also an ultrasensitive light sensor closely connected to the brain. The light stimuli receive cues biochemical, physiological and behavioral activities of the fish on a daily and seasonal basis.

Animal behavior allows organisms to adjust to external and internal stimuli in order to the best meet the challenges to survive in a changing environment. It is a selective response that is constantly adapting through direct interaction with physical, chemical, social and physiological aspects of the environment (Shrivastava et al., 2011). Some specific behaviors were well reported as indicators of stress and pain in fish, like feeding, agonistic interactions, swimming activity or biting and rubbing the site of stimulation (Beitinger, 1990; Schreck, 1990; Plumb, 1994; Stoskopf, 1994). Holland et al. (1990) reported that activities of fishes was divided into relatively few distinct behavior patterns, which were set into motion by various environmental stimuli and were oriented by gradients of light, currents and temperature.

4.1. Visual sensitivity in *Oreochromis niloticus*

The spectrum and intensity of environmental light place constraints on the visual system (Sabbah et al., 2012), and influence the success of visual tasks such as foraging (Gilson et al., 2013) and mate choice (Bowmaker, 2008).

The ERG has been used to assess spectral sensitivity by determining the electric potential of the retina. In light adapted retinas, ERG waveforms include a-wave generated by the photoreceptor hyperpolarization upon initiation of a light stimulus, and a b-wave, originating from Müller cell and bipolar cell depolarization.

It was hypothesized that *Oreochromis niloticus* was sensitive to the wavelength between 400 to 650 nm wavelengths, and that it was more sensitive to long wavelengths.

Results of this study indicated that visual capability of *Oreochromis niloticus* range from 405 ~ 640 nm with sensitivity peak of 574 nm. It was within the green-yellow colour of the light spectrum and in the long wavelength. *Oreochromis niloticus* had four sensitivity peaks in the range 380 ~ 420, 440 ~ 480, 500 ~ 600 and 600 ~ 680 nm (Lisney et al., 2010) and long wavelength spectral range over which the sensitivity in adults was reduced in the range of 523 ~ 560 nm and 560 ~ 625 nm (Sabbah et al., 2012). In addition, species spectral sensitivity peak shifted to the long-wave lengths with 465 nm and 505 nm wavelengths having the highest amplitude. It is because *Oreochromis niloticus* is fresh water species that's to say turbid fresh water absorbs chiefly blue rays and transmits red rays. Depending on the properties of suspended particles, fresh waters have greenish, yellowish or even brownish colors (Nickyurchenk, 2013). Also, using cardiac conditioning experiments *Oreochromis niloticus* showed sensitivity to 865 nm NIR (Matsumoto et al., 2005). Furthermore, Bowmaker (2008) found out that fresh water fish tend to be sensitive to green light and maximum wavelength of majority of cells being 573 nm which is approximately in accord with results of this study. Therefore, sensitivity peak of 574 nm in this study is in the range 500 to 600 nm and 560 to 625 nm wavelengths Lisney et al. (2010) and Sabbah et al. (2012) respectively.

This study showed that there was a good fit between averaged spectral sensitivity data and the model; however, some data did not fit the model well and it could be because *Oreochromis niloticus* has more than one sensitivity peak. Other data were supposed to fit to different sensitivity curves with different peak sensitivities as shown by the extension of the sensitivity curve towards short wavelengths at 405 nm (Figure 12). Linsey et al.(2010) measured four sensitivity peaks in the range 380 ~ 420, 440 ~ 480, 500 ~ 600 and 600 ~ 680 nm, and Sabbah et al. (2012) found that long wavelength spectral range over which the sensitivity in adults was reduced is 523 ~ 560 nm and 560 ~ 625 nm.

It can also be attributed to the inconsistence in distance between light source and fish eye made during the experiment and differences in eye size and electrode position of each test fish, which influence b-wave amplitude that is used to measure response amplitude in electroretinogram analysis. Therefore, as vision of fish shifted from cons to rods during dark adaption, fish became relatively more sensitive to long wavelengths and rods dominated electroretinogram signals with b-wave.

4.2. Activity of *Oreochromis niloticus* under varying wavelength and temperature

Temperature exerts a major influence on the biological activity and growth of aquatic organisms. The higher the water temperature, the greater the biological activity in fish, insects, zooplankton, phytoplankton and other aquatic animals. As temperatures get too far above or below the preferred range, it has a great possibility to increase the mortality of aquatic species.

Oreochromis niloticus is one of the tropical species that prefers to live in shallow water. In this study, it was observed that temperature was inversely proportional to fish activity**.** *Oreochromis niloticus* activity decreased with the increase in temperature, although slightly high activity was observed from 23 ~ 30^{o}C and less activity above 30^{o}C water temperature. It was also observed that *Oreochromis niloticus* stopped feeding at 37^{o}C. However, there was no significant difference in fish activity at different water temperature. This could be because *Oreochromis niloticus* preferred wide range of temperature. Similar results were obtained by Popma and Masser (1999) who found out that tilapia were more tolerant than other cultured freshwater fish to high salinity, high temperature, low dissolved oxygen, high ammonia concentrations and diseases. This could also be attributed to fish adaption to low temperatures. The test fish used in this study was obtained in the middle of winter. It means that fish had already adjusted to cold conditions that resulted into cold tolerance of fish to temperature lower than 23 ^{0}C which was the minimum water temperature used in the experiment. Acclimatization to lower temperatures before cold stress can improve the cold tolerance ability of *Oreochromis niloticus.* Fish reared under mid-summer conditions dies between 13.6 ºC and 8.6 ºC while those reared under autumn conditions dies between 11.7 ºC and 7.5 ºC (Charo-Karosa et al., 2005). Furthermore, Cnaani et al. (2000) found out that tolerance of the fish to lethal temperatures is 31^{0}C depending upon environmental effects, history of the fish and genetic effects. In addition, similar to the research of Nach and Novotny (1995), lower and upper lethal temperatures for *Oreochromis niloticus* are 11 ~ 12°C and 42 °C, respectively, while the preferred temperature ranges from 31 to 36 °C. However, water temperature used in this study ranged from 23 to 38 °C hence no significant difference in fish activity.

Spectral composition is a main characteristic of light. Most species of fish have well-developed color sight, and are therefore, very sensitive to colored light. From electroretinogram experiment, results were shown that *Oreochromis niloticus* was more sensitive to 474 nm wavelengths. However, it did not reveal how fish reacted and what color fish preferred. Therefore, behavior analysis using natural period, red and green color was further carried out to understand fish visual system due to its sensitivity to the long wavelengths.

Behavior study revealed that *Oreochromis niloticus* was more active under red light followed by green and less active under white. Similar to Volpato et al. (2001), red light motivates feeding in *Oreochromis niloticus*. The fish showed sensitivity to the long wavelengths that was to say spectral sensitivity studies revealed that *Oreochromis niloticus* was sensitive to 865 nm NIR (Matsumoto et al., 2009). However, red light has been found not to increase fish growth (Volpato et al., 2001). It affected fish physiology and increase stress (Shin et al., 2012) and the reverse was true for green LEDs. Shin et al. (2012) reported that green and blue light of the LEDs, both of which were the short wavelengths, increased antioxidant materials against oxidative stress in the yellowtail clown fish, *Amphiprion clarkii.* In addition, Bowmaker (2008) reported that fresh water and shallow marine fishes tend to be sensitive to long wavelengths with maximum of 573 nm light (green yellow spectrum). From the study, swimming activity of *Oreochromis niloticus* under green was higher than in control group (the natural photoperiod). Hence, *Oreochromis niloticus* was stable under green light compared to red. However, according to Levine and Macnichol (1982), if the visual environments of fish were blue, green or near infrared, the visual system of *Oreochromis niloticus* is better under green light compared to red and white. Green has also been shown to improve fish welfare in other species, such as *Sardinops caerulea* (Loukashkin et al., 1959) and *Brycon cephalus* (Volpato et al., 2001).

Therefore, the best environmental condition to cultivate *Oreochromis niloticus* would be under the green LEDs with water temperature ranging from 23 to 30^{0}C in order to prevent stress and to increase productivity and economic gains with minimal effects on the environment.

In order to obtain perfect fit of sensitivity data with the model, constant distance between fish eye and light source should be observed. For behavior studies, minimum experimental temperature should range between 11 to 15 ^{0}C depending on the season and study area in order to better understand the effect of temperature and wavelengths on fish behavior.

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