INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
20–0048/202x/00–0–000–000
DOI: 10.17957/IJAB/15.1459
http://www.fspublishers.org

Full Length Article

The Behaviour and Retinular Response of Yellowstripe Scad, *Selaroides leptolepis* to the different Colors of Light Emitting Diode

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Received 09 January 2020; Accepted 16 March 2020; Published ________________

Abstract

The objective of this research was to analyse the behavioural and the retinular response of scad through both laboratory observation and a fishing trial as scientific evidence to designate the effective LED light for lift net fisheries. Different colors were used in a laboratory experiment, including green, orange, and white LED. Laboratory experiment were conducted in three illumination levels, low, medium and high intensity. Light intensity strongly affects fish behaviour and activity. Green light improves the vision of fish and the ability to maintain schooling behaviour. Using the same LED colors, the adaptation stage of cone cells increases as the light intensity grows. The exposure of high-intensity LEDs rapidly induces the cone cells into photopic adaptation. The same results are found in both fishing trials and laboratory observations. Regarding the ability to induce good light adaptation and stable swimming patterns, it is argued that green LED lamps are preferable to substitute fluorescent lamps currently used in the lift net fishing. The combination of both green and white LED can be more effective light attractant to Yellowstripe scad for lift net fishing. © 2020 Friends Science Publishers

Keywords: Behavioural response; LED fishing; Retinular response; *Selaroides leptolepis*

Introduction

Light fishing is one of the most effective and advanced fishing methods to catch commercial small pelagic species in both small-scale and large-scale fisheries. In the common fishing practices, fishers use either fixed or mobile fishing gear (Ben-Yami 1976; Wang *et al*. 2010; Yamashita *et al*. 2012; Ortiz *et al*. 2016; Solomon and Ahmed 2016; Nguyen *et al*. 2017; Nguyen and Winger 2019). However, artificial light consumes a significant amount of energy due to the use of numerous high-powered lamps. One of the prominent small-scale light fishing practices in Indonesia is lift net fishing, which uses a fluorescent lamp as the typical light sources. Fish production of lift net fishing in 2017 reached 48% from total production of small-scale light fishing in Indonesia (Ministry of Marine Affairs and Fisheries Republic of Indonesia 2018). The main targets of lift net fishing are anchovy, scad, sardinella and squid, which proportion of scad ranged between 10–45% from the total catch (Guntur and Munataha 2015; Rudin *et al*. 2017). The application of fluorescent lamp in lift net fishing has several problems, including short lifetime, high fuel consumption and low effectiveness to control the fish behaviour during fishing operation. Fishing operation using fluorescent lamps in fixed lift net consumed 5.20 to 7.00 L/night (mean 6.33 ± 0.54 SD) while light emitting diode (LED) lamps consumed 3.30 to 5.30 L/night (mean 4.11 ± 0.61 SD). However, it is argued that differences in fluorescent lamp quantities and wattages significantly affect the fishers' income (Susanto *et al*. 2017a).

Light emitting diode as the latest efficient light source technology, has the potential to be applied as an artificial light source for fishing with light (An *et al*. 2017; Susanto *et al*. 2017b; Nguyen and Winger 2019). This lamp provides the maximum illumination power combined with lower energy consumption, longer lifetime, higher efficiency, better chromatic performance, and lower environmental impact compared to traditional lighting technology (Matsushita *et al*. 2012; Matsushita and Yamashita 2012; Yamashita *et al*. 2012; Breen and Lerner 2013; Hua and Xing 2013; Yeh *et al*. 2014; An *et al*. 2017; Nguyen and Winger 2019). Furthermore, the light distribution of LEDs, colour and intensity considerably affect fish behavioural and retinular response. Therefore, understanding the behaviour of target species in response to LEDs is an important step to develop an efficient LEDs for lift net fisheries.

We illuminate scad (*Selairoides leptolepis*) by three different low powered LED light sources including green, orange and white lamps, as well as dark conditions to investigate the behavioural and retinular response. Furthermore, in order to determine the LEDs performance for lift net fishing, we focused to construct the basic evidence regarding the behavioural and the retinular response of scad through fishing trial. This information is important to develop efficient and effective LEDs for lift net fishing in Indonesia.

Materials and Methods

Fish and tank experiment

For the laboratory experiment, the behaviour monitoring was conducted in a black fiberglass of rectangular prims open tank (150 W x 200 L x 50 H cm) and the water depth was maintained at 30 cm. The tank was placed in the controlled dark room at State College of Fisheries Serang City, to secure no natural light existing during experiment. The tank was divided into six zones and marked at the bottom in 10 cm intervals as the calibration scale (Fig. 1). Being closer to the light source zone 3 and 4 are light zones (bright zones), while zone 1,2,5, and 6 are the dark zones. The LEDs were assembled approximately 20 cm from sea water level at experiment tank. The experimental setup was installed by following the researches of Marchesan *et al.* (2005), Pignatelli *et al.* (2011), Cha *et al.* (2012) and Utne-Palm *et al.* (2018).

The running water systems were installed to ensure the fish remain alive in optimum water quality during observation. Scad (12.45 cm in average total length (TL), N=60) were collected using a guiding barrier trap at Banten Bay and transferred to the laboratory for adaptation and acclimatization period for seven days. The scad were exposed to normal daily light-dark cycle (12 L: 12 D, sun light was used in daytime). The water salinity in all tanks was maintained at 30–33‰, the temperature was at 29–31°C and dissolved oxygen ranged from 5.9–6.1 mg/L. Before and after the experiments, fish were fed two times per day with *Arthemia* spp. Therefore, the behaviour experiment was conducted on static water circulation. All of the experiments were performed during the night to minimize the influence of light from any endogenous circadian effects on the fish behavioural and retinular responses.

Light source and behavioural methods

Lamps were assembled using four dual inline package (DIP) LEDs (Shenzhen Yuliang Optoelectronics Technology Co. Ltd) mounted on the metal housing (11L x 5W x 7H cm), powered by 4 V DC supply. The experiments were conducted in three colors of LEDs *i.e*., green [approximate peak wavelength = 565 nm], orange [approximate peak wavelength = 600 nm], and white [approximate peak wavelength = 450 nm and 545 nm]. Therefore, each of which consists of three illumination levels *i.e*., 20, 35, 50 lux. Light intensities were measured using ILT 5000 research radiometer at 15 cm distance bellowed sea water level of the tank. The intensity of green LEDs at 20, 35 and 50 lux is 24, 54 and 90 $\mu W.cm^{-2}$, respectively. In the same order of illumination level, the intensity for orange LEDs was 20, 21 and 24 $\mu W.cm^{-2}$ while for the white LEDs was 15, 76 and 94 $\mu W. cm^{-2}$.

The order of the experimental procedures is as follows. Firstly, before being tested 30 scad were left at the experimental tank at least three days to acclimate and were subjected to a 12 L:12 D photoperiod (light from 06:00 to 18:00; dark from 18:00 to 06:00). Ambient illumination at 15 cm deep below seawater-level was about 16 $nW.cm^{-2}$ in light conditions. All experimental sessions started at 19:00 and ended at 22:00. Secondly, before the experiments were started and between each experiment, fish were kept in the dark state for 30 min to ensure their retina in a scotopic condition. Subsequently, each lamp was turned on for 30 min to allow fish to respond and adapt to the light. Visual observation and video recording using the infrared camera were conducted during the dark and lighted conditions. Three replications were conducted for each experiment (Marchesan *et al.* 2005). A total of 540 min of video recording was analysed for each color to define fish proportion, scad swimming speed around illumination zone, mean of nearest neighbour distance (MNND), and swimming pattern of the fish schooling.

Retinular response to irradiance changes

We used eighteen fish for the retinal adaptation experiment using the following procedure. Firstly, two fish were taken from the storage tank and placed in a cylinder tank with diameter 50 cm and height 43 cm. After 30 min set in the dark state, each LED lamp was illuminated for approximately 30 min and at the end of each treatment, eye specimens from both fish were collected. Subsequently, each specimen was fixed in Bouin's solution and infiltrated with paraffin. Tissue samples were cut in cross-sections of 4–6 μm thickness and were stained with haematoxylin and eosin for examination under the microscope. This histological process followed the procedures from Arimoto *et al.* (2010) and Jeong *et al.* (2013).

Fishing experiment

The result of laboratory experiment was applied to define the suitable LEDs color for the fishing trial which was conducted using a fixed lift net in Banten Bay during peak fishing season on August 2018. The fishing trial was conducted at 10 m water depth as common fishing ground in Banten Bay. According to the laboratory experiment, scad are especially responsive to white and green LEDs.

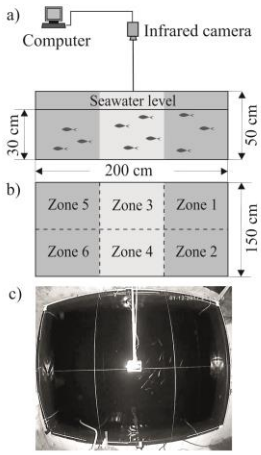

Fig. 1: The open tank experiment for the behavioural response. The side view of experimental tank set up (**a**), top view (**b**), and infrared camera view (**c**)

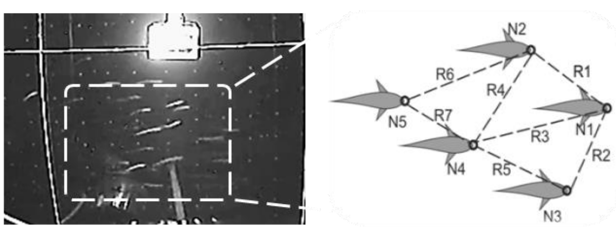

Fig. 2: Illustration of NND method from the image recording. N1 to N5 represent the number of fish. R1 to R7 represent the planar distances between each fish (head) and its closest neighbour

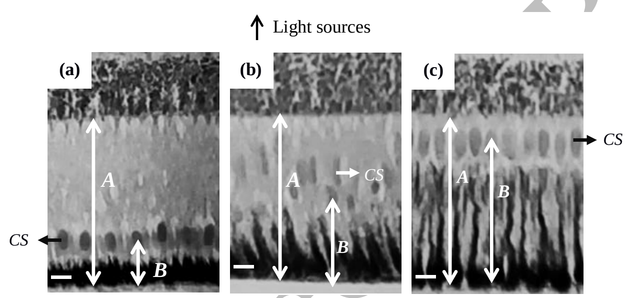

Fig. 3: Photomicrographs show various states of retinal light adaptation of *Selaroides* spp. *A* thickness from the limiting membrane to the surface of the retina and *B* thickness of cone cell migration. **a**. Dark adapted, **b**. transitional stage, and **c**. light adapted. *CS*: *cone cell. Scale bar* = 10 μm

Therefore, both lamps were compared to a fluorescent lamp, the light source used in the existing lift net fishing. We conducted the fishing experiment using 50 W of a fluorescent lamp (typical lamp for lift net fishing) and 1.4 W of LEDs due to the similar light output in the seawater column (12-15 μW.cm^{-2}; measured using ILT 5000 at 1 m below sea water). Ten fish were collected from each lamp making it 30 fish in total. Subsequently, all fish's eyes went through the histological procedures, followed by the examination of retinular adaptation under the microscope.

Data analysis

In order to determine the degree of fish preference on each light stimulus, the proportion of fish at each zone was analysed by counting the number of gathered fish in each zone per minute observation (Kim and Mandrak 2017). Social behaviour was determined by the mean nearest neighbour distance (MNND), which is the average of the planar distances between each fish (head) and its closest neighbour (Parrish *et al*. 2002). It was used to define the effect of different colors and intensity of schooling characteristics of fish (Torisawa *et al*. 2007; Jolles *et al*. 2017). The MNND was analysed using images that converted from the movie at the beginning (< 10 min), intermediate (11–20 min), and the end of observation (21–30 min). The distance was analysed with Kinovea 0.8.15 at the center head of fish (Fig. 2).

The degree of retinal light adaptation was represented in the adaptation ratio (%) (Arimoto *et al*. 2010), which was calculated by $(B/A) \times 100$ (%), where *A* (μm) represents the distance between the limiting membrane and the surface of the retina, and *B* (μm) represents the migration of cone cells when it was stimulated by light. *A* and *B* were measured using photomicrographs (Fig. 3). Swimming patterns at both LEDs colors and irradiance levels were analysed by using video tracking and trajectory software (Kinovea 0.8.15). Videotapes were preliminary observed at 4X speed, to obtain the first qualitative swimming pattern and the characteristic of each stage. In all experiments at every repetition, three dominant swimming patterns from each LED's stimulus were chosen at the beginning, intermediate and the end of the observation period. Parameters used to analyse the swimming speed of each pattern in total length per second (TL/s) are (1) time-lapse in each pattern; (2) number of frames; (3) distance of each pattern. One-way ANOVA was applied to analyse the effect of different light stimuli (as explanatory variable) to proportion, MNND, and swimming speed during observation (as response variable). Post-hoc comparisons, wherever significance was found, were conducted using Tukey HSD test with the significance level was set at $P < 0.05$.

Results

Fish preference to light stimuli

The scad responded to the different light colors. At all illumination levels of each color, fish showed high aggregation levels to the light zone. It was indicated by higher fish proportions in zone 3 and 4 than dark zone as presented in Fig. 4. However, there was not a significant difference in fish proportions at the dark condition for all zones (zone 1 to 6). The fish proportion at the dark condition between the range 14 and 19%. The proportion tended to increase related to the aggregating of light intensity, especially at the light zone. At the low intensity,

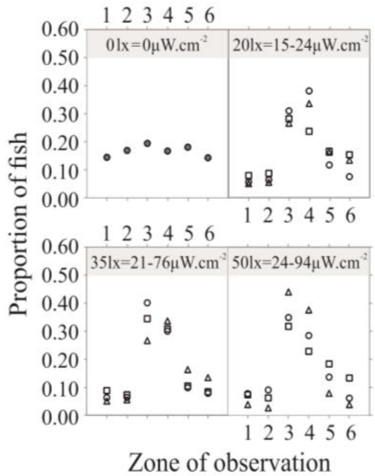

Fig. 4: The proportion of fish related to the color and light intensity. The proportion of scad observed during replicated color treatments (green-circle; orange-square; white-triangle;) and dark condition (filled circle)

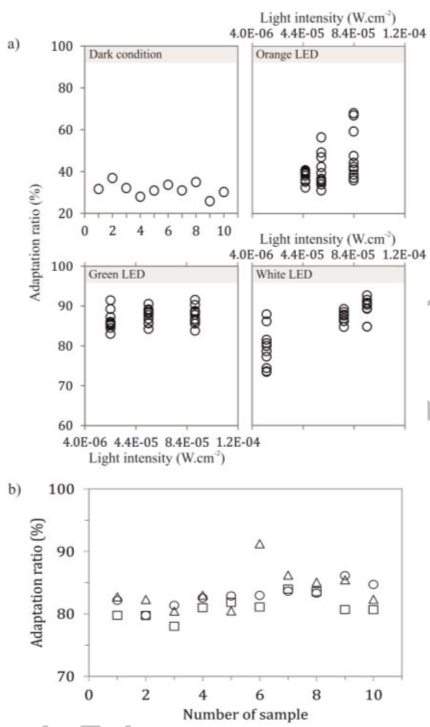

Fig. 5: The light adaptation of scad retina cells to different color and light intensity in laboratory experiment (**a**) and the light adaptation of the retina cells collected from the fishing experiment (**b**). The cone index of fluorescent-circle, white LED-square, and green LED-triangle

the proportion of fish at zone 3 and zone 4 was ranged between 22–32%. However, the proportion was increased to 34–44% at high intensity level. There was also evidence to suggest that light intensity (in the range 15–94 μW.cm^{-2}) influences the behaviour of the fish by modifying swimming aggregations and preference in all LEDs colors.

Relationship between the retinular response and irradiance change

The light adaptation of scad was influenced by LEDs color and intensity. The degrees of adaptation of scad at scotopic adaptation are in the range between 26 and 34%. Adaptation ratio increased with increasing light intensity at each LEDs color (Fig. 5a). The green LEDs generated the highest adaptation ratio with a slight increase, range 83% (low intensity) to 93% (high intensity). However, the degree of light adaptation with orange and white LEDs produced various tendencies. The adaptation ratio of orange LEDs at low intensities is 32% and increase to 67% for the high light level. Furthermore, white LEDs generated higher adaptation ratio than orange LEDs with the ratio between 73% (low intensity) to 92% (high intensity).

Swimming behaviour

The swimming behaviour of scad was strongly affected by light intensities at each LED color. There was a significant decrease of MNND in all treatment with increasing intensity. The farthest individual distance generated with orange LED was 14.8 cm, while the closest distance was found at a white LED of 8.2 cm. In all experiments, the decline of MNND has a relationship with increasing swimming speed. Scad has the fastest swimming speed at green LED approximately 3.0 TL.s^{-1}, while the lowest speed was initiated at low intensity of white LED approximately 1.4 TL.s^{-1} (Fig. 6).

The different LED treatments also influenced the pattern of swimming behaviour. In all dark conditions, fish swam randomly around the experiment tank. There are inconsistent and irregular swimming patterns of scad during the dark observation period. The light exposure caused changes in swimming behaviour. In low intensity, the different color of LEDs did not affect the behavioural patterns. Moreover, the increasing light intensity induces the transformed swimming pattern in all colors. The fish swam randomly and inconsistently at a high intensity of the white LED. However, the scad showed a consistent response to orange and green LED with different radii in swimming patterns. Its radius with green LED is closer to the main light zone than orange LEDs, as presented in Fig. 7. Moreover, there were constant and stable swimming patterns related to the time elapsed at the green LEDs, approximately after 20 min of observation. The specifics of fish swimming behaviour are presented in Table 1.

Fishing trials

There are 1,082 scads were caught during fishing experiment. The light adaptation of the retina collected during the fishing trial is shown in Fig. 5b. The cone index was found to be between the range of 78 and 91%. The green LED generated a higher mean adaptation ratio (84%) than white LED (81%) and a fluorescent lamp (83%).

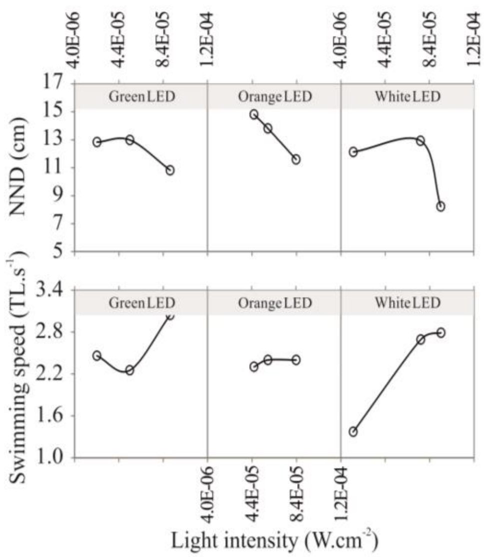

Fig. 6: The swimming behaviour of scad to different LED colors and intensities. The mean nearest neighbor distances (a) and swimming speed (b)

However, there were no significant differences in cone index from the sea experiment ($P > 0.05$).

Discussion

The highest number of fish proportion in the bright observation zone indicated the scad as a phototaxis fish that attracted by light. The proportion was superior to the bright zones. However, these zones have a smaller area than shadow zones. There were significant differences in the fish proportion between colors and intensities, whereas the proportion in the bright zone at each color gradually increased with rising intensity. The brightness level influences the level of fish activity. Thus, it would have been relevant to increase the fish proportion in all bright zones (Marchesan *et al.* 2005; Utne-Palm *et al.* 2018). This condition is an adaptation response to maintaining the formed characteristic of scad schooling behaviour, related to the exposure of light intensity in their environment (Woodhead 1966; Martin and Perez 2006).

There were significant differences in light adaptation ratio between dark and light state ($P < 0.05$). The degree of light adaptation ratio has a positive association with an increase of light intensity (Susanto *et al.* 2017c). With all colors used in the experiment, the adaptation stage of cone cells increases with expanding intensity. The exposure of high-intensity LEDs induces the cone cells into photopic adaptation rapidly. Thus, the light adaptation ratio was increased (Tamura and Niwa 1967; Nakano *et al.* 2006; Migaud *et al.* 2007). The green LEDs generated the highest adaptation ratio of scad in all intensity levels. Thus, it would have been relevant to conclude that the maximum sensitivity of the Carangidae fish family, including scad, has peak sensitivity between 494–500 nm (Munz and McFarland 1973).

Light intensity has prevailing influence on the visual ability of fish. However, scad's ability to use vision to maintain the schooling characteristic during light level increases necessitates phototaxis. Moreover, there were significant differences in swimming behaviour, including swimming patterns and MNND in the different light conditions, whereas the fish activity increased with rising intensity. The MNND has a negative relationship with light intensity, whereas the MNND decreased with increasing intensity levels. However, the swimming speeds of scad showed different tendencies with MNND during expanding light levels. The fish swam faster at the high intensity at all LED colors. The high intensity induced fish easier to maintain the direction and orientation of their schooling, due to an increase in their swimming speed at all treatments (Miyazaki *et al.* 2000). Similar tendencies were found at the swimming speed of Atlantic salmon *Salmo salar*. Its speed was increased from 0.2 BL/s to 0.5 BL/s related to the increase of the light level at sea cage observation (Hansen *et al.* 2017).

The swimming patterns of scad in the green and orange LEDs have similar tendencies. However, the swimming radius at the green LED was closer to the center of the light zone than an orange LED. It was related to the visual adaptation level and the spectral sensitivity of scad. The scad have more reactive to the green light because it is suitable with peak sensitivity level. The fish have a proper response to green light due to an increase in visual ability and significant influence on fish capability to maintain their schooling characteristics. Exposure of green light in different light intensities was induced the stable and consistence swimming pattern. In one example of schooling during increased light intensity, increasing visual ability influenced each individual, enabling them to maintain their distance and formation relative to the rest of the school during swimming (Glass *et al.* 1986; McMahon and Holanov 1995; Miyazaki and Nakamura 1990).

Even though the orange light has longer wavelength than green light, it has lower of photon energy. It causes the scads to have less reactive and induce wider swimming radius than green light. However, the swimming pattern of scad in orange light was relatively stable and consistence during observation. In other example, orange light has similar influenced with green light to the schooling characteristic of *Mugil cephalus*, *Sparus auratus*, and *Lithognathus mormyrus* (Marchesan *et al.* 2005).

The light adaptation of scad from the fishing experiment has similar tendencies with laboratory observations. The green LED generated a higher degree of adaptation than the white LED and fluorescent lamp at the same intensity. The information on the retinular response and adaptation to light source was utilized in studying the relationship between a light fishing procedure, light color, and light intensity to develop an efficient use of the LED

Table 1: Fish behaviour related to the time elapsed observation

Time elapsed (minute)	Fish Behavioural Response
0 - 5	There was no schooling and swimming behaviour pattern at the beginning treatment. Fish swam in all directions due to orientation and adaptation period related to the light color and intensity at the experimental tank.
6 - 10	The fish started to school with several swimming patterns. However, there were an unstable direction and swimming speed.
11 - 20	There was a stable and consistent swimming pattern. The swimming speed was increased related to time-lapse.
21 - 30	The swimming patterns were stable and consistent with steady swimming speed. The radius of swimming was stable and relatively closest to the center of the light zone.

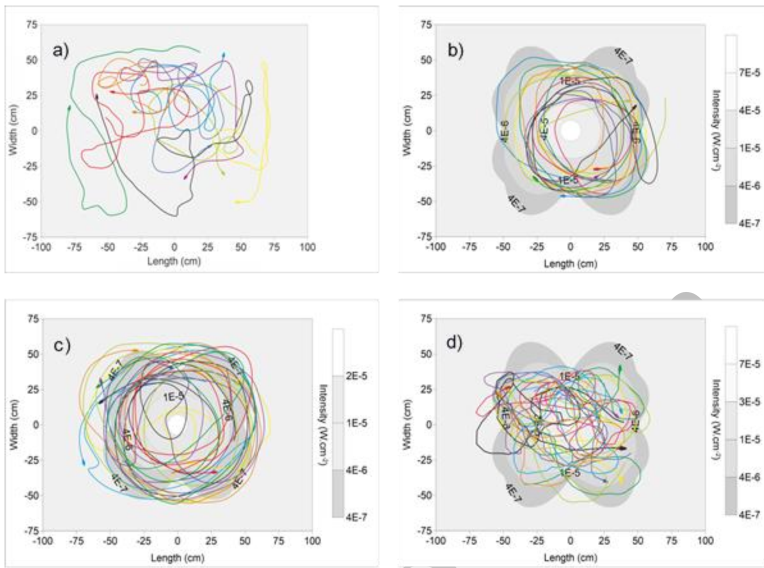

Fig. 7: The individual swimming pattern of scad in different color and LEDs intensity. The swimming pattern in dark condition (**a**), green LED (**b**), orange LED (**c**) white LED (**d**). Different fish are color-coded

fishing lamp (Jeong *et al.* 2013). In this research, we compare the characteristics of scad swimming behaviour, and light adaptation between experimental tanks and fishing trials to determine the suitable low powered LED color as a light source when fishing. From these results, the green LED, which induces good light adaptation and stable swimming patterns, is suitable enough to substitute fluorescent lamps currently used in lift net fishing.

The combination in both green and white LED can be a more effective light attractant to scad fishing at lift net fisheries. In lift net fisheries, white LED is useful when gathering scads and other target species at the initial fishing operation. However, the swimming pattern of fish school at white LED was random and unstable, due to the light source having to change with green LED when focusing the fish at a catchable area. Green light would be more suitable to keep fish close to the light source. Moreover, this light not induces stress behaviour for long exposure time (Shin *et al.* 2013; Stien *et al.* 2014; Sierra-Flores *et al.* 2015). The application of green light in focusing of fish can reduce uncaught fish during hauling process and improve the fishing efficiency and effectiveness in lift net fishing.

The LED innovation as artificial light has several advantages. The LED provides maximum illumination power, combined with minimum energy consumption, long lifespan, high efficiency, better chromatic performance, and reduced environmental impact compared to traditional lighting technology (Matsushita *et al.* 2012; Matsushita and Yamashita 2012; Yamashita *et al.* 2012; Breen and Lerner 2013; Hua and Xing 2013; Yeh *et al.* 2014; Nguyen and Tran 2015; An *et al.* 2017). However, further fishing trials are recommended to validate the effectiveness of both white and green LED as a light stimulus for gathering and focusing scad at lift net fisheries.

Conclusion

Present investigation showed that the white LED is a suitable enough to attract fish to catchable area while the green LED is an effective color for focusing and control behaviour of scad in catchable area. We conclude that combination of white and green LED can be a more effective light attractant to scad fishing at lift net fisheries.

Acknowledgments

This research is supported by the Ministry of Research, Technology and Higher Education, The Republic of Indonesia through graduate team research grant number 1549/IT3.11/PN/2018.

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