Different wavelengths of light influence daily swimming activity in silver catfish (*Rhamdia quelen*)

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Abstract

The aim of this study was to investigate the daily rhythms of swimming activity in *Rhamdia quelen* maintained under different wavelengths of light. Thirty fish were equally divided into ten 100 liters tanks. The water temperature was maintained at 25±0.2ºC. In each tank, an infrared photocell was installed. The tanks were equipped with Red, Green and Blue (RGB) LED strips placed at the top. Fishes were exposed to different 7 day-periods of 12/12 light/dark hours. In each period, a different light color was used: white (150 lux), red (12 lux), green (180 lux), blue (50 lux), and again white (150 lux). The application of cosinor-based techniques for the analysis of time series showed a daily rhythm of swimming activity in all tanks, in all experimental conditions. Acrophase was diurnal during the first white light schedule, on the second day of red light it was observed at the beginning of the light phase. During the green and blue schedules, acrophase was observed during the dark phase of the experimental photoperiod. In the second white schedule, it shifted in the middle of the light phase. Robustness was variable among the different lighting schedules and days of monitoring. The application of two-way of repeated measure analysis of variance showed a statistical effect of experimental lighting and day of monitoring on all rhythmic parameters. In conclusion, *Rhamdia quelen* lives in the deep regions of rivers, this could be the reason because the green lighting creates a reasonable environment that does not disturb the biological clock driving the swimming activity in this fish species. This information could be useful for application in aquaculture to improve fish welfare, reduce costs, and increase productivity.

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Introduction

In the aquatic environment, light has been demonstrated to be an important physical factor influencing fish throughout the various stage of life. The absorbance properties of the water column influence light characteristics making the underwater photo-environment complex.[1] In nature, each 24-hour daily cycle consists of a single light period followed by a single dark period, both of which change in duration throughout the year. As one period lengthens, the other shortens. During twilight, the quality of light changes in the amount of light, the spectral composition of light, and the source of light. Changes in the light spectrum (red *vs.* far-red) during these transitions from one period to another may also allow organisms to better discriminate day and night lengths.[2–3] The visible light that causes photoperiodic responses is within a spectrum that goes from violet to far red. Some wavelengths of radiant energy reaching an organism are preferentially absorbed by the receptor molecules over others.[2] As Light-Emitting Diodes (LEDs) can provide specific wavelengths, studies conducted on the lighting effects using LEDs instead of natural light or halide lights have highlighted their possible use as effective light sources in fish farming.[1,4]

In all living organisms, the circadian timing system synchronizes physiology and behaviour to the varying demands of activity and rest. In mammals, the circadian master clock is located in the Suprachiasmatic Nuclei (SCN) of the hypothalamus, and it receives light information from the retina through a dedicated pathway.[1] Although the retina-SCN-pineal axis has been identified in fishes, its function is not well understood.[6–7] The SCN has been anatomically identified in zebrafishes.[8] The presence of a pineal gland equipped with light-sensitive receptors has been suggested in fishes. This implies that the pineal gland of fish has the function of photoreceptor regulating melatonin secretion in a circadian manner.[8,9] Studies conducted in pinealectomized catfishes reported a loss of daily rhythmicity of fish behaviour. In addition to a pineal clock, a homolog of the suprachiasmatic nucleus found ventrally in the hypothalamus may play a circadian role in fishes.[10,11] It has been supposed that in teleosts the pineal gland produces melatonin rhythmically driven by an endogenous molecular lock that employs an adjustment system consisting of photoreceptors. Watanabe *et al.*[8] by the investigation of *per*2 expression supposed the existence of a circadian rhythm pacemaker in the SCN of teleost whose function is somewhat widespread. The two principal environmental factors that synchronize the biological clock are daily light-dark cycles and daily thermocycles.[12] Light is characterized by a complex set of characteristics including spectrum (quality), intensity (quantity), and photoperiod (periodicity).[13]

In fishes, most physiological and behavioral processes exhibit daily rhythm.[10,11] Pronounced activity rhythm has been observed in synchrony with the environmental cycles of their habitat. Also, when fiddler crabs have been housed in tanks equipped with a actigraph recording system in laboratory conditions, locomotor activity continues for several days with elements of tidal, daily, and semilunar rhythmicity.[5] In most fish species housed in constant conditions, rhythmicity dampened out quickly in others the rhythm persisted at least for a few cycles.[5]

Across all continents, with the growing demand for food, and the decline of natural fish stock, the production of captive fish has been increasing. Among the continents in which fish production has moved towards industrialization, South America reached a high production and exportation. In South America, aquaculture has an important impact on the local economy with a large international presence. In this area, various aquatic organisms are produced, including marine fish, continental fish, shrimp, shellfish, and frogs. In particular, in Argentina, the native catfish *Rhamdia quelen* has been introduced in aquaculture in the last few years. It lives in the deep regions of rivers and can reach up to 3 kg and a length of 50 cm. This fish is omnivorous and adaptable to a large temperature range. These characteristics together with rapid growth, resistance to stress, and feasible reproduction in captivity make this species a good candidate for aquaculture production.[14,15]

To reduce production costs and improve fish growth and survival in aquaculture, environmental factors are commonly manipulated.[13] LEDs are used because they have longer life spans and lower power requirements than standard metal halide bulbs, reducing electrical running costs.[16]

The aim of this study was to improve the basic knowledge about the effects of different wavelengths of light on the swimming activity of fish kept in captivity through the monitoring of *Rhamdia quelen* subjected to 12:12 light/dark cycles using different light colours. Finding the wavelength that better favours the behaviour that fish have in nature can deepen the knowledge of the fish's biological rhythm useful in aquaculture to improve fish welfare, reduce costs, and increase productivity.

Materials and Methods

This study was carried out in the Chronobiología laboratory of the Facultad de Ciencias Veterinarias dependent on the Universidad Nacional del Litoral (Argentina), with the approval of Comité Asesor de Ética y Seguridad de la Facultad de Ciencias Veterinarias (455/19). Thirty female *Rhamdia quelen*, with a body weight of about 200-250 g, provided by a fish farm "Pez Campero" located in the city of Paraná, Entre Ríos (Argentina), were enrolled.

They were equally divided into ten cylindrical 100 L tanks with fresh water, equipped with an individual filter, an oxygen diffuser and three PVC tubes (32 mm in diameter, 200 mm wide) arranged to form a triangular shelter. The experiment was performed in an isolated room without windows, to avoid natural lighting, under strictly controlled environmental conditions. Before starting the experiment, a 30-day period of acclimation was performed, to avoid changes in the behaviour and physiology due to the state of fear induced by isolation.[17] The water temperature was controlled via an electronic thermostat; it remained stable at 25ºC with oscillations of ±0.2ºC between day and night.

Food (1% of fish body weight) was provided once a day, at irregular intervals, to avoid any periodic routine acting as a synchronizer. The tanks were cleaned every 3 or 4 days, siphoning off the waste and remaining granules, and 20% of the water volume was replaced every two weeks. Opaque plastic seals were used to ensure isolation conditions and prevent the pieces from seeing each other. In each tank, an infrared photocell (Omron, E3S–AD62, Hong Kong) was installed 10 cm from the bottom. When a fish swam into the area and passed through the light beam, a signal was generated which was recorded on the computer as a motion event. The computer was programmed to log accumulated motion events in 10-minute periods.

To determine the daily activity rhythms of *Rhamdia quelen*, the fishes were exposed to five different periods of 7 days each with a photoperiod of 12/12 Light/Dark (L/D). In each period, a different light colour was used: cold white, red, green, blue, and again cold white. For this purpose, the tanks were equipped with Red, Green and Blue (RGB) LED strips placed at the top, at 120 cm from the floor, providing an average light intensity at the water surface level of 150 lux using the white light, 12 lux using the red light, 180 lux using the green light, and 50 lux using the blue light, and the photoperiod program was controlled automatically using a programmable digital clock (Datamicro, Orbis, Beniparrell, Spain). A lux meter with specific calibration for the light emission spectrum of the LEDs with silicon sensor (TM-201L LUX/FC LED Light, Tenmars, Taipei, Taiwan), was used for the recording of light intensity. The lux meter was calibrated to standard incandescent lamp 2856K and corrected LED day white-light spectrum, and have an accuracy of ±3% for white light emitted by LEDs and an accuracy of ±8% for other visible light source. Data recording was performed starting from the first day of each period, without the application of days of acclimatization when the colour of light was changed.

Statistical analysis

The chronobiology software El Temps (v.1, 179 Prof. Díez-Noguera, University of Barcelona, Spain) was used to analyse the data collected to obtain the actograms and the average daily waves.

A trigonometric statistical model was applied to each tank value at each time series, in order to analytically describe the periodic phenomenon, characterizing the main rhythmic parameters according to the single cosinor procedure.[18] Four rhythmic parameters were determined: MESOR (midline statistic of rhythm), amplitude, acrophase, and robustness. Robustness greater than 3% is above noise level and indicates statistically significant rhythmicity. A two-way for repeated measure analysis of variance (ANOVA) was applied to circadian parameters to establish the effect of experimental lighting and day of monitoring on the rhythmic parameters.

Bonferroni’s test was applied for post hoc comparison. P<0.05 was considered statistically significant. Statistical analysis was performed using the statistical software Prism v. 5.00 (Graphpad Software Ldt, Boston, MA, USA).

Results

Figure 1 shows the mean (seven days) waveform obtained in each experimental photoperiod.

The single cosinor procedure application showed a daily rhythm of swimming activity in all tanks, in all experimental conditions (Table 1). The application of two-way for repeated measure ANOVA showed a statistical effect of experimental lighting (p<0.0001) and day of monitoring (p<0.0001) on MESOR, amplitude, acrophase, and robustness. Results of Bonferroni’s post hoc comparison are reported in Figures 2 and 3. During the first white light schedule the highest MESOR value was observed. It decreased just on the first day of the red-light schedule to reach a value between 3.41 and 1.67 arbitrary unit during the three coloured schedules (red, green, and blue). During the second white light schedule the swimming activity did not reach the value recorded during the first white light schedule; even though it was higher than the coloured schedules (red, green, and blue). Similar conditions were observed for the amplitude values. Acrophase was diurnal during the first white light schedule, on the second day of red light it was shifted by about ten hours and was observed at the beginning of the light phase. During the green and blue schedules, acrophase was observed during the dark phase of the experimental photoperiod (ZT (Zeitgeber time) 16.34±1.96 green; ZT 13.49±2.10 blue). In the second white schedule, it shifted in the middle of the light phase (ZT 8.59±2.43). Robustness was variable among the different wavelengths lighting schedules and days of monitoring.

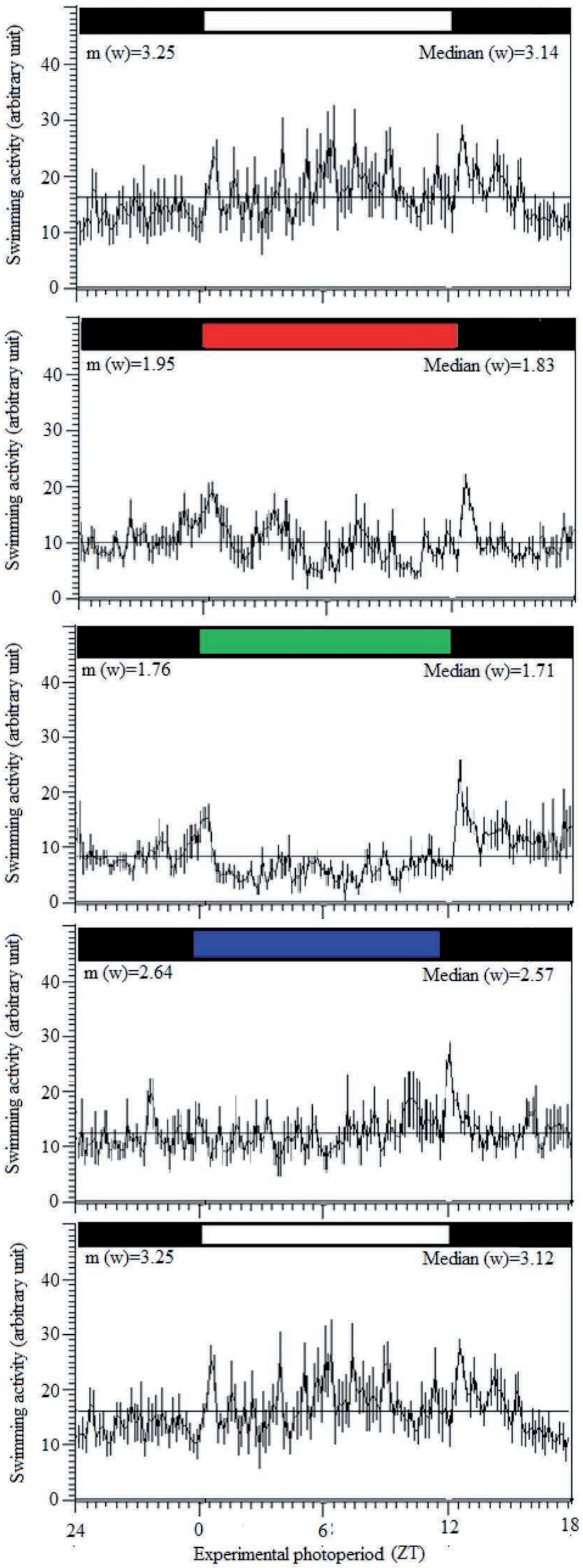

Figure 1. Mean (seven days) waveform obtained in each experimental photoperiod. The trend represents the mean (m), and the black line represents the median. Locomotor activity is expressed in its conventional unit (arbitrary unit).

Discussion

Many of the studies conducted have highlighted the need to investigate developmental stage-specific and species-specific lighting regimes during fish rearing.[1] The knowledge of stage-specific and species-specific light intensity sensitivity is scarce because of the lack of standardization in the unit of light measurement, the use of various light sources with different spectral compositions, the range of rearing systems, and husbandry protocols. For specific fish species, finding the ideal light conditions for fish welfare in aquaculture seems to be a difficult matter; experimental protocols should use narrow-bandwidth light sources with normalized intensities and avoid light contamination that could taint the results. Light is a complex environmental factor that depends on the lighting systems used, the photoperiod, water absorbance properties as well as the specific light sensitivities of the species being reared.[13] The response to light is species-specific and depends on fish ecology. Fishes living in the deep sea have a maximum photoreceptors sensitivity in the blue band, while fish living in coastal areas have maximized visual contrast in the green band.[19] The water column acts as a powerful colour filter. The blue wavelengths penetrate deeper reaching depths of up to 150 m. In coastal and continental waters, suspended particles and substances originating from the decay of organic matter affect the transparency and spectral absorbance of water by shifting the perceived spectral profile.[13]

Artificial lights differ markedly from the spectrum of the sun, particularly underwater. The ability of LEDs to emit only a specific wavelength of light has been investigated on fish. In particular, in flounders, it has been observed that green light reduces stress response in an unfavourable environment, reduces oxidative stress, increases immunity, and increases fish growth.[20] Adult sea bass is more sensitive to the blue end of the visual spectrum inducing a reduction of nocturnal plasma melatonin.[21,22] Differences in spectral sensitivity have been observed between species. In Sea bass and sole, increases in growth have been reported when larvae were exposed to the blue and white light/dark cycle, with respect to the red light/dark cycle.[23,24] In haddock reared under blue light high feeding and capture prey activity have been reported.[25]

Under the different wavelengths lighting schedules, a daily rhythm of swimming activity was observed on all days of monitoring. The MESOR, amplitude, acrophase, and robustness of rhythm changed significantly among the lighting schedules and days of monitoring.

Rhamdia quelen has been described as a nocturnal species, whereas when housed in tanks their activity has been described as diurnal.[26–28] In its ecological niche, swimming activity presented a nocturnal acrophase.[15] Our results indicated a diurnal daily rhythm of swimming activity during the two white-lighting schedules with the highest MESOR and amplitude values. During the second white light schedule the swimming activity did not show the same circadian parameters observed during the first white light schedule.

Probably, in *Rhamdia quelen* seven days are not enough to re-establish the daily rhythm of locomotor activity, contrary to that observed in goats in which only some days were necessary.[29] During the red lighting schedule, one day was necessary to induce a decrease in swimming activity. During this lighting schedule, swimming activity was not constant in its rhythmicity showing no constant acrophase among the days of monitoring. The reason for the instability of circadian parameters during the red lighting regimes is not clear, probably the lack of reproducibility of the activity in red light could be due to the use of a less intense light in the red. Previous studies reported that fish cannot detect red light, they per-

Table 1. Mean±standard deviation of rhythmic parameters (MESOR, amplitude, acrophase and robustness) obtained by the application of single Cosinor procedure, expressed in their conventional unit, recorded in the ten tanks during the different 12/12 light/dark cycles. ZT 0 indicates the time of turning on the light.

Experimental lighting	Day	Mesor (arbitrary unit)	Amplitude (arbitrary unit)	Acrophase (ZT)	Robustness (%)
White light (150 lux)	1	13.22±5.04	14.19±3.01	9.86±2.36	12.30±4.13
	2	12.38±6.93	13.1±2.85	9.42±3.90	12.10±2.80
	3	14.91±7.98	16.56±5.04	8.53±3.46	16.88±6.14
	4	15.67±2.19	9.71±2.86	9.06±2.26	15.75±6.57
	5	23.57±1.13	6.83±1.11	9.66±2.78	18.88±7.95
	6	23.08±3.04	15.30±4.81	8.26±2.70	10.31±4.52
	7	22.39±1.98	11.70±2.94	9.09±2.31	17.88±2.82
	Mean	*17.89±4.92*	*12.48±2.94*	*9.00±0.52*	*14.65±3.10*
Red light (12 lux)	1	7.40±1.42	5.70±0.39	8.53±5.52	17.34±5.90
	2	3.41±1.63	1.63±0.79	21.51±2.06	11.10±4.35
	3	2.94±1.03	1.46±0.52	0.55±3.25	9.78±4.22
	4	2.88±0.85	1.57±0.16	4.27±4.76	11.36±2.26
	5	2.95±1.00	1.75±0.37	3.55±5.12	13.75±4.87
	6	3.09±0.71	1.43±0.63	1.93±3.45	9.85±7.66
	7	2.91±0.54	1.43±0.68	20.57±1.24	12.33±1.70
	Mean	*3.65±1.66*	*2.14±1.57*	*8.70±8.78*	*12.21±2.64*
Green light (180 lux)	1	1.63±0.73	1.37±0.68	16.67±4.01	12.68±5.12
	2	2.06±0.51	1.34±0.50	15.56±6.02	10.60±4.31
	3	1.71±0.88	1.22±0.68	18.86±1.75	9.95±4.59
	4	1.77±0.28	1.95±0.86	16.06±3.15	20.50±3.31
	5	2.35±0.91	1.51±0.59	12.94±4.65	12.01±6.76
	6	2.62±0.58	1.90±0.56	15.86±4.29	11.90±6.27
	7	2.25±0.73	*2.96*±0.81	18.42±0.71	14.73±6.80
	Mean	*2.06±0.37*	*1.69±0.62*	*16.34±1.96*	*13.36±3.56*
Blue light (50 lux)	1	1.67±0.45	1.06±0.54	14.61±3.85	7.91±2.97
	2	3.50±0.81	3.64±1.26	16.19±6.45	23.87±6.47
	3	2.01±0.28	2.44±0.21	13.63±2.15	17.83±5.19
	4	4.66±0.30	4.98±2.97	10.44±1.27	18.80±5.99
	5	3.30±0.80	1.66±0.61	11.84±4.17	12.72±5.30
	6	4.53±0.84	2.79±1.11	12.13±5.15	11.61±4.59
	7	3.41±0.58	2.47±0.96	15.56±4.98	15.56±7.93
	Mean	*3.30±1.13*	*2.72±1.28*	*13.49±2.10*	*15.47±5.27*
White light (150 lux)	1	6.60±2.52	7.42±2.83	9.70±1.47	14.68±5.60
	2	7.8±4.84	5.88±3.17	9.03±4.99	12.90±4.16
	3	8.28±3.42	9.51±5.17	10.03±3.07	17.21±5.61
	4	10.89±1.39	7.67±2.09	4.73±5.16	22.00±6.08
	5	12.09±3.61	10.20±4.66	11.60±2.77	21.97±5.75
	6	12.56±2.98	7.16±2.98	9.24±2.69	10.35±4.57
	7	12.69±2.58	4.50±1.58	5.82±3.48	21.72±8.61
	Mean	*10.13±2.52*	*7.48±1.96*	*8.59±2.43*	*17.26±4.79*

ceive the ambient as darker than other LED spectra, having higher plasma melatonin levels.[30,31] During the green lighting schedules, stability in MESOR, amplitude, and acrophase was observed. Different light wavelengths had an influence on the amplitude and median of the daily rhythm of swimming activity.

Also, in diurnal and nocturnal mammals, a change in daily activity has been observed when animals have been exposed to different light wavelengths. Similarly, in mammals, the red light seems to affect the circadian system in an opposite way comparing diurnal and nocturnal species, in any cases there was an inversion of locomotor activity phase, from diurnal to nocturnal, and *vice versa*. It has been supposed that the effect of light on the locomotor activity depends on the property of light and on the species temporal organization.[32,33]

Rhamdia quelen lives in the deep regions of rivers, this could be the reason because the green lighting creates a reasonable environment that does not disturb the biological clock driving the swimming activity in this fish species. In sea bass, it is well established that feed and photoperiod are synchronizers of the daily rhythm of locomotor activity, even though photoperiod appeared to be the principal synchronizer;[34] in the present study, food was provided once a day, at irregular intervals, to avoid any possible food anticipatory activity. Anyway, on the basis of the experimental conditions, it was not possible to establish if the modification in activity rhythm observed dur-

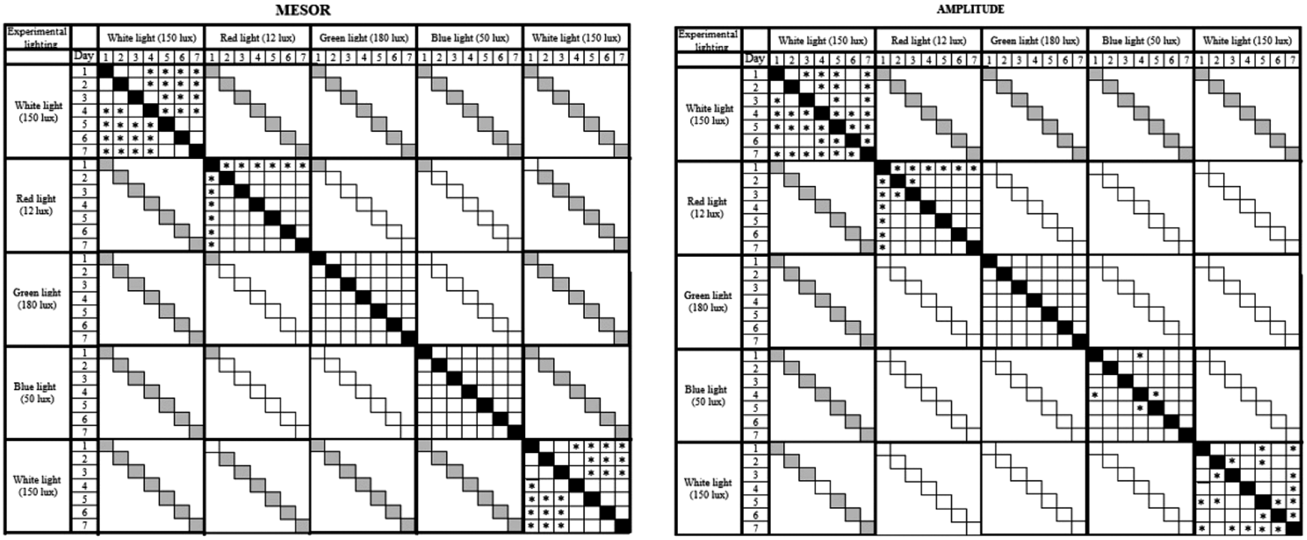

Figure 2. Results of Bonferroni's post hoc comparison applied to MESOR and amplitude recorded during the different lighting schedules. * indicates the statistically significant effect of the day of monitoring within the same lighting schedule. The grey area indicates the statistically significant effect of the different lighting schedules.

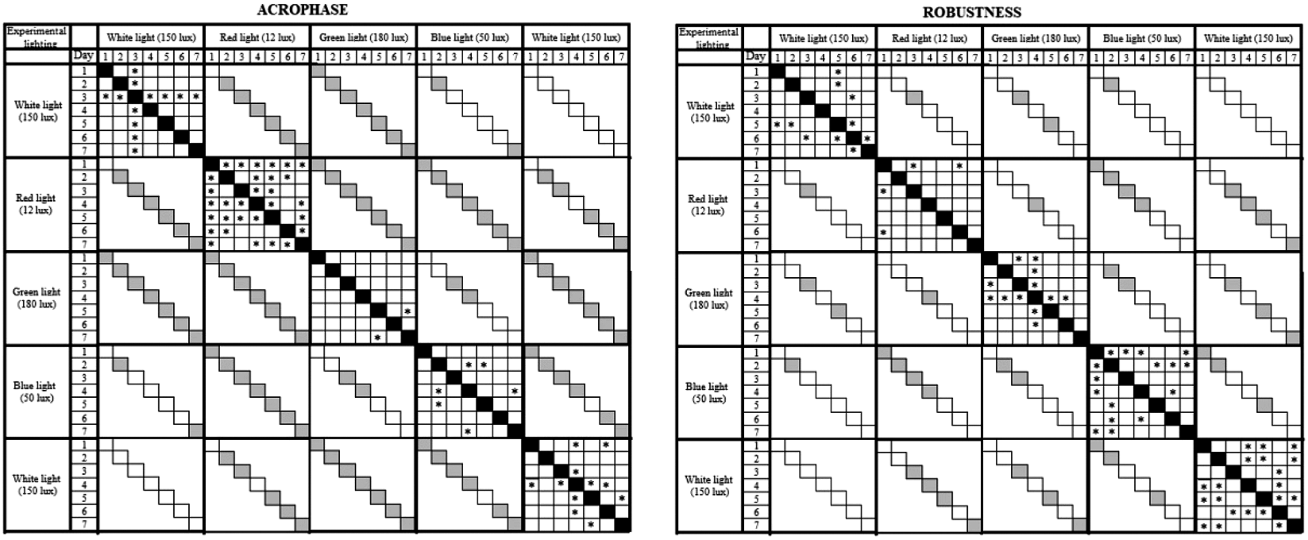

Figure 3. Results of Bonferroni's post hoc comparison applied to acrophase and robustness of rhythm recorded during the different lighting schedules. * indicates the statistically significant effect of the day of monitoring within the same lighting schedule. The grey area indicates the statistically significant effect of the different lighting schedules.

ing the different lighting regimes was due to an inhibition of expressed rhythms or a masking effect that did not involve the pacemaker itself.

Conclusions

For *Rhamdia quelen* the use of white and red light should be avoided since these wavelengths determine behaviour that does not reflect the behaviour of this fish in nature; the daily rhythm of swimming activity is more similar to that observed in nature when fish are maintained with light emissions with shorter wavelengths (green and blue). Identifying wavelengths promoting swimming activity daily rhythm more similar to that observed in nature can deepen the knowledge of the fish's biological rhythm useful in aquaculture to improve fish welfare, reduce costs, and increase productivity.

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