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Natural light vs artificial light. Effects of light pollution on the bioluminescence of dinoflagellate *Pyrocystis lunula*

Luz natural vs. luz artificial. Efectos de la contaminación lumínica sobre la bioluminiscencia de dinoflagelado *Pyrocystis lunula*

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ABSTRACT

Although there are thousands of marine bioluminescent species, very little is known about the effects of artificial light at night (ALAN) on these organisms, particularly those living near the sea surface, such as dinoflagellates. These organisms have a circadian clock that influences their rhythmic physiology, including processes like photosynthesis and nitrogen metabolism, which help regulate marine carbon and nitrogen cycles, respectively. The purpose of this study is to partially address this knowledge gap and research the effects of light pollution on the bioluminescent dinoflagellate *Pyrocystis lunula* through a series of experiments aimed at verifying the consequences due to changes in the normal day-night circadian cycle and exposure to different types of light source, colors, and light intensities. The response variable was the Corrected Total Algal Bioluminescence, which was recorded with a digital camera and then calculated with the ImageJ software. Results show that dinoflagellates do not appear to be susceptible to slight changes in the light/dark cycle. However, a total absence of light and darkness leads to a drastic inhibition of their bioluminescence, particularly under white LED or incandescent artificial light and with a light intensity of 100 lux or higher.

Keywords*:* bioluminescence, color, intensity, light pollution, light source

RESUMEN

Aunque hay miles de especies bioluminiscentes marinas, se sabe muy poco sobre los efectos que la luz artificial durante la noche puede tener en estos organismos, especialmente en aquellos que viven cerca de la superficie del mar, como los dinoflagelados, cuyo reloj circadiano impulsa su fisiología rítmica, como la

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Recepción 23 julio 2024 • Corregido 23 octubre 2024 • Aceptado 31 octubre 2024
DOI: http://dx.doi.org/10.15359/revmar.16-2.5
Rev. Mar. Cost. Vol. 16 (2): 79-98, julio-diciembre 2024
ISSN: 1659-455X • e-ISSN: 1659-407X

fotosíntesis y el metabolismo del nitrógeno, que ayuda a regular el ciclo del carbono marino y el nitrógeno, respectivamente. El objetivo de esta investigación es llenar parcialmente, este vacío de conocimiento e investigar los efectos de la contaminación lumínica sobre el dinoflagelado bioluminiscente *Pyrocystis lunula* a través de una serie de experimentos destinados a verificar las consecuencias debidas a los cambios del ciclo día-noche (ciclo circadiano) y la exposición a diferentes tipos de fuente luminosa, colores e intensidades de luz. La variable de respuesta fue la Bioluminiscencia Total de Algas Corregida, grabada con una cámara digital y luego calculada con el *software* ImageJ. Los resultados muestran que los dinoflagelados no parecen ser susceptibles a ligeros cambios en el ciclo luz/oscuridad, pero una ausencia total de luz y oscuridad conduce a una inhibición drástica en su bioluminiscencia, especialmente bajo led blanco o luz artificial incandescente y con una intensidad de luz de 100 lux o mayor.

Palabras clave: bioluminiscencia, color, contaminación lumínica, fuente de luz, intensidad

INTRODUCTION

Bioluminescence is the light emission by living organisms, predominantly occurring in marine creatures, from bacteria to large deep-sea vertebrates (Haddock *et al*. 2010). Some of the most relevant bioluminescent organisms are dinoflagellates, which have a cosmopolitan distribution (Sherr & Sherr, 2007), are vital to marine ecosystems, significantly contribute to primary production, and serve as the foundation for numerous marine food networks (Cohen *et al*. 2021).

In particular, *Pyrocystis lunula* is a photoautotrophic dinoflagellate that serves as a model organism due to its bioluminescent ability, which is related to circadian rhythms (Fajardo *et al*. 2020) and diel vertical migration within the water column (Hastings, 2013). *P. lunula* spends the day in deeper waters where nitrogen, a fundamental nutrient for its growth, is more abundant (Bhovichitra & Swift, 1977), and it is in this phase when its photosynthetic activity reaches its peak (Hastings, 2013). It spends the night in shallower waters, and during this phase, its bioluminescence is more pronounced, emitting light predominantly at the blue wavelength (Morin, 1983). Bioluminescence in dinoflagellates may have an antipredatory purpose (Esaias & Curl, 1972): to scare predatory crustaceans such as copepods, warn predators of potential toxicity, and attract the attention of higher-order visual predators to the position of copepods (Hanley & Widder, 2017).

Almost all organisms, including humans, have evolved following a natural alternation between day and night, called circadian rhythm

Rev. Mar. Cost. Vol. 16 (2): 79-98, julio-diciembre 2024
ISSN: 1659-455X • e-ISSN: 1659-407X
DOI: http://dx.doi.org/10.15359/revmar.16-2.5

(Hölker *et al*. 2010b), which is also fundamental in the regulation of vital functions of dinoflagellates (Roenneberg & Morse, 1993). An endogenous circadian system represents a complex "internal clock" in the body, which keeps synchronized with the natural day and night cycle through stimuli such as sunlight or temperature (Vitaterna *et al*. 2001). Organisms can better capitalize on the available environmental resources by anticipating and preparing for regular environmental changes (Corfitsen, 1996).

Other various physicochemical factors can influence the bioluminescence of marine microalgae. The availability of nutrients, such as nitrogen and phosphorus, can affect the growth and bioluminescence of microalgae. Excess nutrients can lead to algal blooms, which may alter light production (Heisler *et al*. 2008). Water temperature can influence the reaction rates of metabolic pathways involved in bioluminescence. Optimal temperatures promote bioluminescence, while extreme temperatures can inhibit light production (Rabha *et al*. 2021). Water salinity can affect microalgae physiology, impacting their ability to emit light. Changes in salinity can alter cellular functions and metabolism (Park *et al*. 2024). Extreme pH levels influence chemical reactions within algal cells. An optimal pH is essential for the enzymatic activities involved in bioluminescence (Craig *et al*. 2003). Oxygen concentration in the water can influence bioluminescence since some biochemical reactions require oxygen. Hypoxic conditions can reduce bioluminescent activity (Lambrechts *et al*. 2014).

Artificial light at night (ALAN) can also affect bioluminescent marine bacteria, which play a crucial ecological role in marine ecosystems. Artificial light can suppress the expression of genes responsible for bioluminescence in some bacteria. This reduction in light emission is critical for communication and defense mechanisms (Love & Prescher, 2020). Many bioluminescent bacteria form symbiotic relationships with marine organisms, such as fish. Light pollution can interfere with these connections, affecting the growth and survival of species involved (Arroyo *et al*. 2024). Nighttime lighting can alter the population dynamics of bioluminescent bacteria, influencing their ecological interactions and their role in the marine carbon cycle (Wang, 2018). Artificial light can also induce environmental stress in bacteria, affecting their growth and metabolism. This stress can compromise their ability to compete with other microbial species (Sathish *et al*. 2023).

In the last decades, artificial light at night has grown globally (Davies & Smith, 2018) with an annual increase of 6% (Hölker *et al*. 2010b). More

than 80% of the world's population is affected by light pollution (Falchi *et al*. 2016), which represents the alteration of natural lighting levels in the nocturnal environment due to human introduction of light sources (Falchi *et al*. 2011). Artificial light at night has been introduced to prolong daylight into the dark hours, allowing for a broader range of human activities typically performed during the day (Gaston *et al*. 2015). In addition, as new population centers and infrastructures are built, outdoor lighting continues to grow. Nighttime lighting is utilized to illuminate roads, bridges, airports, commercial and industrial buildings, parking areas, sports facilities, and residential neighborhoods (Falchi *et al*. 2011). It is important to highlight that this has dramatically influenced our well-being, boosting the likelihood of engaging in productive work, recreational activities, socializing, and fostering community ties (Gaston *et al*. 2015).

However, artificial nighttime lighting exhibits disadvantages of immediate interest, such as economic drawbacks. Substantial quantities of energy are necessary to maintain ALAN, which also leads to significant carbon dioxide emissions and other greenhouse gases (Gaston *et al*. 2015). Grid-based electric lighting is estimated to represent 195 percent of electricity production, consuming energy that releases 1 900 Mt of CO_2 annually. The total cost of lighting each year is around 360 billion dollars, encompassing energy, labor, and equipment expenses (International Energy Agency, 2006).

Over time, humans have tried to find increasingly energy-efficient light sources. Specifically, after the global financial crisis and the intense pressure on public spending, local, regional, and national governments in many countries have tried to reduce the cost of public lighting (Gaston *et al*. 2015). Recently, there has been a considerable transition from narrow-spectrum light sources, such as High-Pressure Sodium (HPS) and Low-Pressure Sodium (LPS) lamps that mainly emit yellow or amber light, to broader-spectrum white sources like Metal Halide (MH) lamps and Light Emitting Diodes (LEDs) (Gaston *et al*. 2012). In particular, switching to LEDs has become increasingly appealing, as they are particularly suited to operate at variable brightness and can be turned off at times of low demand; in fact, they operate at full effectiveness without experiencing prolonged heating times (Gaston *et al*. 2012). Additionally, LEDs offer better control of night lighting through centralized control systems and the transition to whiter lighting with a consequent improvement in color rendering for human vision (Gaston *et al*. 2015).

The rise of LEDs has also resulted in many negative health

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consequences for humans and other organisms (Davies & Smith, 2018). In fact, in terrestrial environments, about 30% of vertebrates and 60% of invertebrates are nocturnal organisms (Hölker *et al*. 2010a), for which a wide variety of impacts caused by ALAN (biochemical, physiological, behavioral, intra– and interspecific interactions) have already been documented at the level of individual organisms, populations and communities belonging to the most disparate taxonomic groups (Gaston *et al*. 2015).

Light pollution also affects 22% of coastal marine areas (Davies *et al*. 2014) due to the presence of over 3 350 coastal cities globally (Depledge *et al*. 2010), which means that different types of light sources such as housing, walks, piers, ports, and lighthouses (Garratt *et al*. 2019) brightly illuminate long stretches of coastline (Luijendijk *et al*. 2018). Several adverse effects of ALAN pollution on coastal marine organisms are known: temporal desynchronization of gamete release in corals (Kaniewska *et al*. 2015), alteration of predation in gastropods (Manríquez *et al*. 2021) and mobility in sea urchins (Di Bari, 2023), reduction of growth rate in amphipods (Luarte *et al*. 2016) and reproductive success in fish (Fobert *et al*. 2019), fatal collisions in seabirds (Rodríguez *et al*. 2017) and disorientation of hatchlings of sea turtles (Price *et al*. 2018). However, while in the terrestrial environment, the effects of light pollution on bioluminescent organisms, such as fireflies, are known (Owens *et al*. 2022), influencing their reproduction (Costin & Boulton, 2016; Kivelä *et al*. 2023), development (Owens & Lewis, 2021), and predator-prey interactions (Firebaugh & Haynes, 2019), very little is known about the effects of ALAN on the coastal marine bioluminescent organisms.

This study aims to fill this knowledge gap and explore the effects of ALAN on the bioluminescent dinoflagellate *Pyrocystis lunula* through a series of experiments intended to verify the consequences of changes in the normal day-night circadian cycle and exposure to different types of light sources, colors, and light intensities.

MATERIALS AND METHODS

Cultures of the marine bioluminescent dinoflagellate *Pyrocystis lunula* were grown at a temperature of 21°C, a salinity of 31 PSU, a pH of 8.2, and f/2 Medium as the nutrient supplement (Guillard, 1975) under a natural 12h/12h light/dark cycle for a week (exponential phase) (Fajardo *et al*. 2020). Dinoflagellate *P. lunula* emitted short flashes of blue light of approximately 100 ms in duration (Latz & Rohr, 2005), with a wavelength of 474 nm (Watanabe & Tanaka, 2011), which were stimulated mechanically by fluid shear stress (Latz *et al*. 2004) using a vortex mixer (TX4 Digital Vortex

Mixer) at 2 000 rpm on 40 ml vials. Each experiment lasted ten nights (for a total period from January 10 to February 18, 2024) with a measurement per experimental condition each time, after 3 h into the dark phase when cells were fully dark-adapted and at the maximum bioluminescence capacity (Lindström *et al*. 2017).

A Canon PowerShot G7 X Mark II digital camera was used to take photos of algae bioluminescence, setting the ISO speed to 10 000 and having a wide aperture (f/2.8) to allow maximum light to hit the sensor (Fig. 1). Algal bioluminescence was quantified using ImageJ, a free and open-source software for processing and analyzing pixel intensity of scientific images. The response variable was the Corrected Total Algal Bioluminescence (CTAB), calculated with the following formula: CTAB = Integrated Algal Density - (Algal bioluminescence area · Background pixel intensity).

The first experiment aims to evaluate the possible effects of changes in the typical day-night circadian cycle on the bioluminescent dinoflagellate *Pyrocystis lunula* with different experimental conditions (12:12, 18:6, 6:18, 24:0, and 0:24 h of light/dark cycle), achieved either by extending the period of natural light with artificial light or by moving dinoflagellates into a dark room, to verify how high the stress level is induced and measured as inhibition of bioluminescent function.

The second experiment aims to evaluate differences in the possible

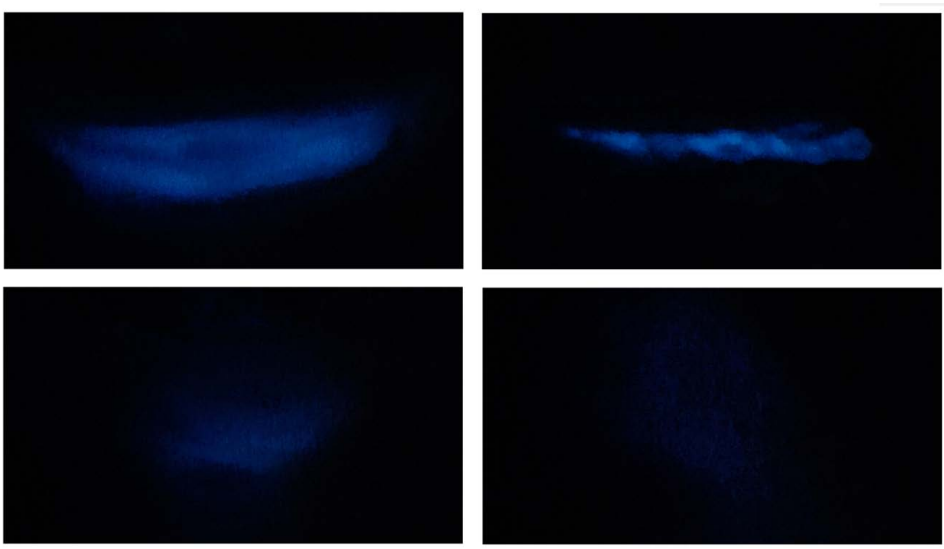

Fig. 1. Examples of dinoflagellate bioluminescence after mechanical stimulation
Fig. 1. Ejemplos de bioluminiscencia dinoflagelada tras estimulación mecánica

Rev. Mar. Cost. Vol. 16 (2): 79-98, julio-diciembre 2024
ISSN: 1659-455X • e-ISSN: 1659-407X
DOI: http://dx.doi.org/10.15359/revmar.16-2.5

effects of various types of light sources (light-emitting diode, incandescent, gas-discharge, and organic light-emitting diode) at the same light intensity (100 lux) to test for differences in bioluminescence inhibition during a 12 h night period.

The third experiment aims to evaluate differences in the possible effects of various light intensity values (0, 0.5, 5, 25, 100, and 500 lux), similar to those found in actual conditions under the same light source, an ordinary steady white LED lamp, to test for differences in bioluminescence inhibition during a 12 h night period.

The fourth experiment aims to evaluate differences in the possible effects of different colors (red, orange, yellow, green, blue, and white) with the same type of light source (steady LED lamp) and light intensity (100 lux) during a 12-hour night period. In addition, changes between a steady light source and a blinking light have also been tested using a white LED lamp.

The experimental design included a comparison between the experimental conditions using the Analysis of Variance (ANOVA) after verifying the observance of the assumptions (normality through the Shapiro–Wilk test and equality of variances through Levene's test). Data analysis and graph plotting were conducted using the statistical software R 4.2.3, considering $P < 0.05$.

RESULTS AND DISCUSSION

Circadian rhythm

The bioluminescence rhythm of many light-emitting dinoflagellate species is prominent (Valiadi & Iglesias-Rodríguez, 2013), is regulated at the translational level (Hastings, 2007), and shows no resemblance to any known model eukaryotic or prokaryotic clock architecture (Jadhav *et al*. 2022). For example, in *Lingulodinium polyedrum*, the regulation of bioluminescent activity is allowed by a daily synthesis and destruction of proteins (Dunlap & Hastings, 1981), while the amino acids from the degradation are conserved and used in the subsequent cycle (Johnson *et al*. 1984). Contrarily, in *Pyrocystis lunula,* there is no degradation and resynthesis of luciferase (Knaust *et al*. 1998); instead, the position of the luminous organelles emitting light varies from day to night (Colepicolo *et al*. 1993).

The results of the statistical analysis of the four experimental conditions of different light/dark cycles (Fig. 2), through the unidirectional ANOVA, show significant differences between at least two averages of the analyzed groups ($F = 594$). Subsequently, to know how many and which groups had statically different averages, unplanned multiple comparisons were conducted using a post–hoc test (Tukey–Kramer), which showed

significant differences between the following groups: $q_{0:24/6:18} = 39.71$, $q_{0:24/12:12} = 48.95$, $q_{0:24/18:6} = 35.73$, $q_{0:24/24:0} = 4.13$, $q_{6:18/12:12} = 9.23$, $q_{6:18/24:0} = 43.85$, $q_{12:12/18:6} = 13.22$, $q_{12:12/24:0} = 53.08$, $q_{18:6/24:0} = 39.86$.

In the extreme cases of the light/dark cycle (0:24 and 24:0) in this first experiment, a drastic reduction in bioluminescence production is observed. In contrast, differences in intermediate

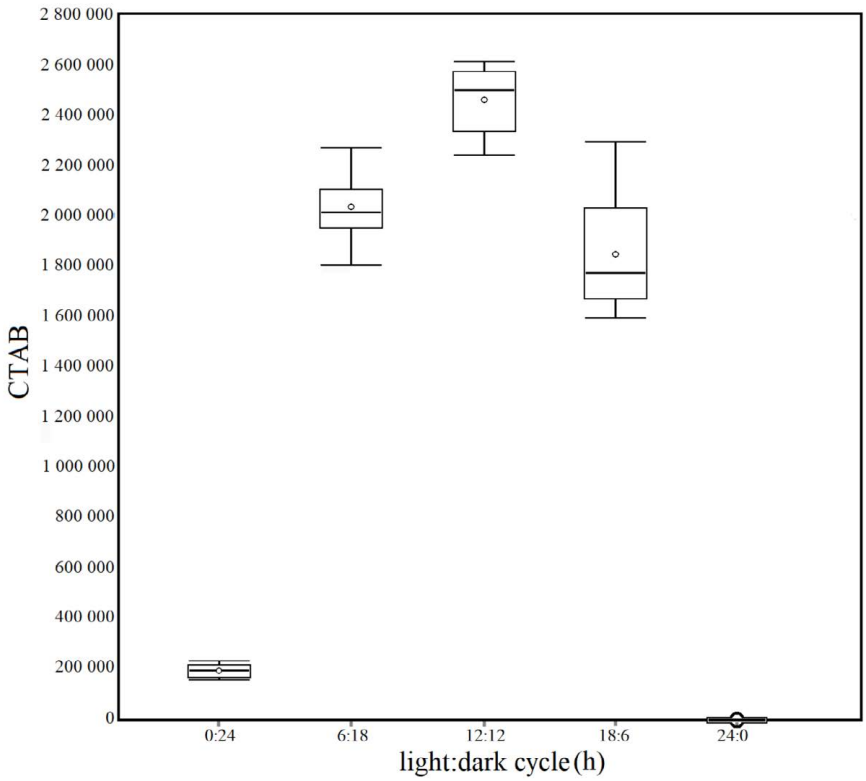

Fig. 2. Box plots of the Corrected Total Algal Bioluminescence (CTAB) in each light/dark cycle condition. Mean (± standard error): 0:24 = 191 600 ± 8 261; 6:18 = 2 032 889 ± 46 154; 12 :12 = 2 460 961 ± 43 597; 18 :6 = 1 847 988 ± 81 538; 24:0 = 0 ± 0

Fig. 2. Diagramas de caja de la bioluminiscencia total de algas corregida (CTAB, por sus siglas en inglés) en cada condición de ciclo de luz/oscuridad. Media (± error estándar): 0:24 = 191 600 ± 8 261; 6:18 = 2 032 889 ± 46 154; 12 :12 = 2 460 961 ± 43 597; 18 :6 = 1 847 988 ± 81 538; 24:0 = 0 ± 0

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DOI: http://dx.doi.org/10.15359/revmar.16-2.5

conditions (6:18, 12:12, and 18:6) decrease, potentially simulating natural seasonal variations in day and night cycles. These results confirm that the bioluminescent capacity of dinoflagellate *P. lunula* is significantly influenced by an alternation of light and dark according to a variable, but necessarily the circadian rhythm.

Light sources

Chemical reactions, heat, mass conversion, or a different frequency of electromagnetic energy are some energy sources that light sources use to produce photons (Nemade, 2023; Mandal *et al*. 2024). While some studies indicate that various light sources do not affect algal growth or the production of photosynthetic pigments (Bialevich *et al*. 2022), others claim that growth under fluorescent light outperformed growth under LED sources (Satthong *et al*. 2019). Even though conventional fluorescent lights based on older technology have a very irregular quantum spectrum, they proved to be better culture light sources for some algae than lighting based on LED technology (Ritchie & Sma-Air, 2023).

The results of the statistical analysis of the four experimental conditions of different light sources (Fig. 3), through the unidirectional ANOVA, show significant differences between at least two averages of the analyzed groups ($F = 237$). Subsequently, to know how many and which groups had statically different averages, unplanned multiple comparisons were conducted using a post–hoc test (Tukey–Kramer), which showed significant differences between the following groups: $q_{OLED/Gas\text{-}discharge} = 24.56$, $q_{OLED/LED} = 32.51$, $q_{OLED/Incandescent} = 32.51$, $q_{Gas\text{-}discharge/LED} = 7.95$, $q_{Gas\text{-}discharge/Incandescent} = 7.95$).

The differences in this study also show a higher CTAB under gas-discharge light sources than LEDs and incandescent lamps, probably due to the latter's lower luminosity, which has a reduced impact on these organisms. Finally, CTAB values were much higher in the organic light-emitting diode (OLED), mainly used to create digital displays in devices such as computers, phones, and television screens (Zou *et al*. 2020) than in any other experimental condition. OLEDs generate diffused light in various shades that are not glaring and lack harsh shadows. However, they are still underused because of the current high cost of their technology compared to other lighting systems.

Light intensity

Light intensity is one of the determinants of the depth within the water column at which phytoplankton are found (Enright, 1977) with modifications of diel vertical migration (Ludvigsen *et al*. 2018). In particular, artificial light inhibits division rate and cell size in phototrophic dinoflagellates at high levels (Swift & Meunier, 1976), while it stimulates photosynthetic

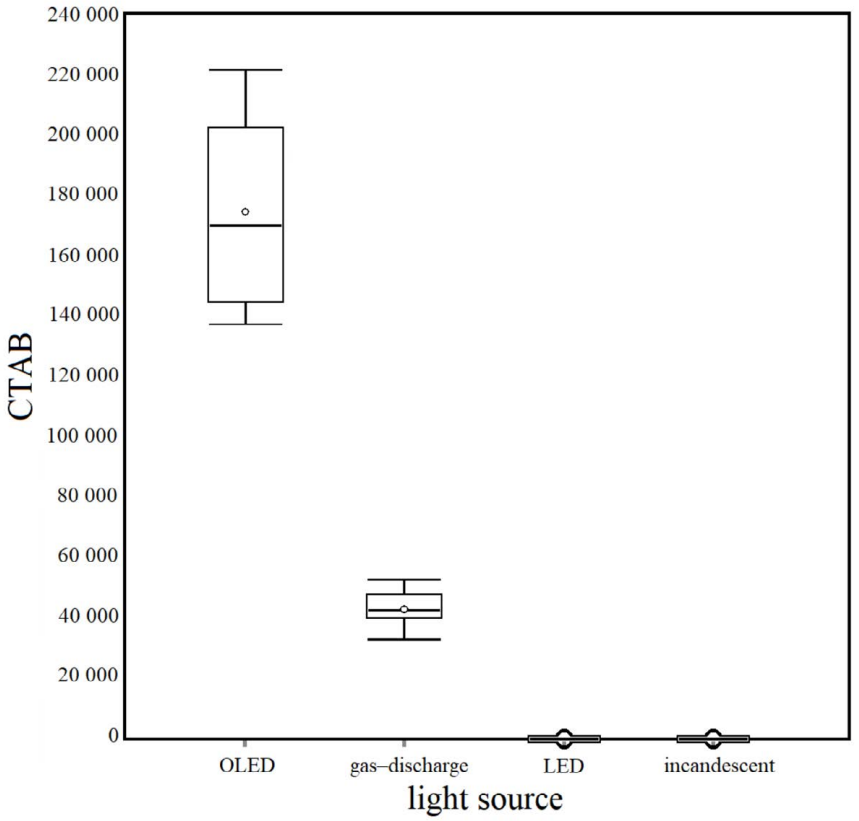

Fig. 3. Box plots of the Corrected Total Algal Bioluminescence (CTAB) in each light source condition. Mean (± standard error): Organic light-emitting diode (OLED) = 173 901 ± 10 496; Gas–discharge = 42 518 ± 2 068; Light-emitting diode (LED) = 0 ± 0; Incandescent = 0 ± 0

Fig. 3. Diagramas de la bioluminiscencia total de algas corregida (CTAB) en cada condición de fuente de luz. Media (± error estándar): diodo emisor de luz orgánico (OLED) = 173 901 ± 10 496; descarga de gas = 42 518 ± 2 068; diodo emisor de luz (LED) = 0 ± 0; incandescente = 0 ± 0

activity, growth, and ingestion rates at low levels (Jeong *et al*. 2018). Light intensity can also alter the consequences of fish predation on zooplankton and potentially, in turn, affect their population and community structure (Talanda *et al*. 2022).

The results of the statistical analysis of the control condition in the dark and the five experimental conditions of different light intensities (Fig. 4) through the unidirectional ANOVA show significant differences between at least two averages of

Rev. Mar. Cost. Vol. 16 (2): 79-98, julio-diciembre 2024
ISSN: 1659-455X • e-ISSN: 1659-407X
DOI: http://dx.doi.org/10.15359/revmar.16-2.5

the analyzed groups (F = 1 928). Subsequently, to know how many and which groups had statically different averages, unplanned multiple comparisons were conducted using a post–hoc test (Tukey-Kramer), which showed significant differences between the following groups: $q_{0/0.5\ lux} = 41.34$, $q_{0/5\ lux} = 44.63$, $q_{0/25\ lux} = 80.39$, $q_{0/100\ lux} = 110.85$, $q_{0/500\ lux} = 110.85$, $q_{0.5/25\ lux} = 39.05$, $q_{0.5/100\ lux} = 69.51$, $q_{0.5/500\ lux} = 69.51$, $q_{5/25\ lux} = 35.76$, $q_{5/100\ lux} = 66.22$, $q_{5/500\ lux} = 66.22$, $q_{25/100\ lux} = 30.46$, $q_{25/500\ lux} = 30.46$.

Artificial light emission interferes with the dinoflagellate's natural bioluminescence, completely inhibiting it at high intensities of 100 and 500 lux. However, low levels of artificial light intensity (0.5 and 5 lux) can

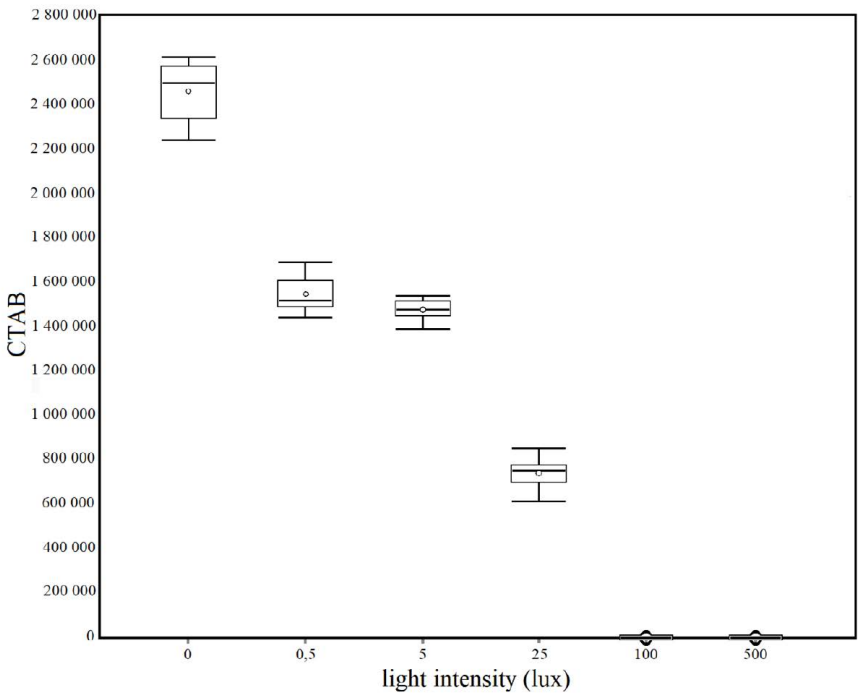

Fig. 4. Box plots of the Corrected Total Algal Bioluminescence (CTAB) in each light intensity condition. Mean (± standard error): 0 lux = 2 460 961 ± 43 597; 0.5 lux = 1 543 166 ± 25 181; 5 lux = 1 470 112 ± 16 406; 25 lux = 676 260 ± 12 385; 100 lux = 0 ± 0; 500 lux = 0 ± 0

Fig. 4. Diagramas de la bioluminiscencia total de algas corregida (CTAB) en cada condición de intensidad de luz. Media (± error estándar): 0 lux = 2 460 961 ± 43 597; 0.5 lux = 1 543 166 ± 25 181; 5 lux = 1 470 112 ± 16 406; 25 lux = 676 260 ± 12 385; 100 lux = 0 ± 0; 500 lux = 0 ± 0

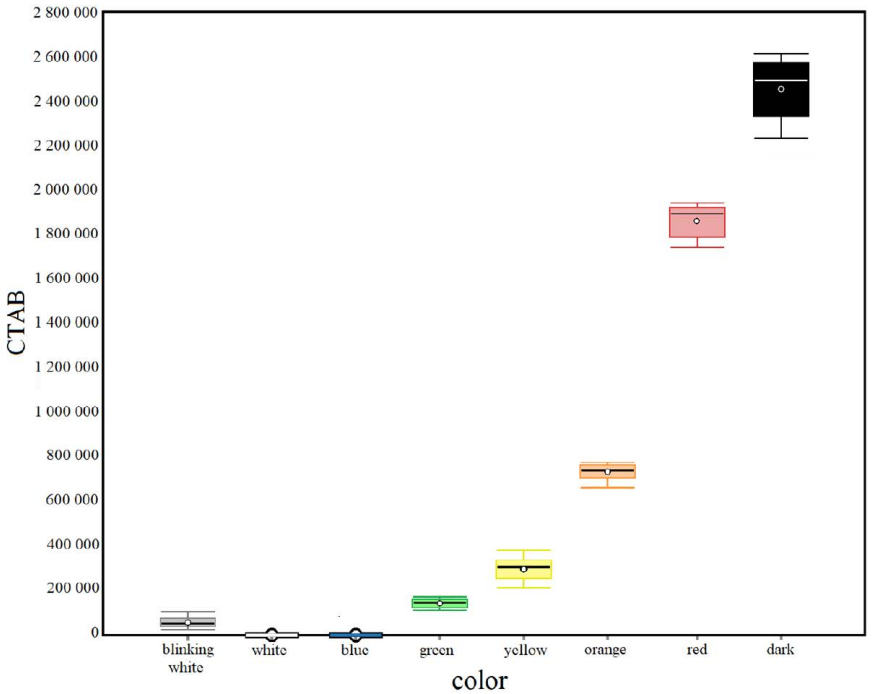

Fig. 5. Box plots of the Corrected Total Algal Bioluminescence (CTAB) in each light color condition. Mean (± standard error): Blinking White = 49 698 ± 6 307; White = 0 ± 0; Blue = 0 ± 0; Green = 149 415 ± 1 779; Yellow = 288 479 ± 17 789; Orange = 711 303 ± 9 373; Red = 1 858 968 ± 22 437; Dark = 2 460 961 ± 43 597

Fig. 5. Diagramas de la bioluminiscencia total de algas corregida (CTAB) en cada condición de color claro. Media (± error estándar): blanco intermitente = 49 698 ± 6 307; blanco = 0 ± 0; azul = 0 ± 0; verde = 149 415 ± 1 779; amarillo = 288 479 ± 17 789; naranja = 711 303 ± 9 373; rojo = 1 858 968 ± 22 437; oscuro = 2 460 961 ± 43 597

also affect this function, decreasing it by more than 40%, which shows their high sensitivity to this form of pollution. The threshold value of total inhibition of bioluminescent is between 25 and 100 lux.

Light color

The efficiency of the phytoplankton's photosynthetic activity is affected not only by light quantity, i.e., intensity but also by its quality, i.e., the availability of different wavelengths (Neun *et al*. 2022). Moreover, the available light quality is linked to

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the vertical distribution and coexistence of phytoplankton species within the water column (Stomp *et al*. 2008; Hickman *et al*. 2009), also affecting their biomass and diversity (Diamantopoulou *et al*. 2021).

The results of the statistical analysis of the control condition in the dark and the seven experimental conditions of different light colors (Fig. 5) through the unidirectional ANOVA show significant differences between at least two averages of the analyzed groups (F = 2 532). Subsequently, to know how many and which groups had statically different averages, unplanned multiple comparisons were conducted using a post–hoc test (Tukey-Kramer), which showed significant differences between the following groups: $q_{\text{blinking white/green}} = 5.28$, $q_{\text{blinking white/yellow}} = 12.65$, $q_{\text{blinking white/orange}} = 35.04$, $q_{\text{blinking white/red}} = 95.81$, $q_{\text{blinking white/dark}} = 127.69$, $q_{\text{white/green}} = 7.91$, $q_{\text{white/yellow}} = 15.28$, $q_{\text{white/orange}} = 37.67$, $q_{\text{white/red}} = 98.44$, $q_{\text{white/dark}} = 130.32$, $q_{\text{blue/green}} = 7.91$, $q_{\text{blue/yellow}} = 15.28$, $q_{\text{blue/orange}} = 37.67$, $q_{\text{blue/red}} = 98.44$, $q_{\text{blue/dark}} = 130.32$, $q_{\text{green/yellow}} = 7.36$, $q_{\text{green/orange}} = 29.76$, $q_{\text{green/red}} = 90.53$, $q_{\text{green/dark}} = 122.41$, $q_{\text{yellow/orange}} = 22.39$, $q_{\text{yellow/red}} = 83.17$, $q_{\text{yellow/dark}} = 115.05$, $q_{\text{orange/red}} = 60.78$, $q_{\text{orange/dark}} = 92.66$, $q_{\text{red/dark}} = 31.88$.

Various colors of artificial light cause a different alteration of bioluminescence with a decrease in light emitted by dinoflagellates in all experimental conditions compared to the natural condition of darkness. Results show a bioluminescence inhibition scale concerning the wave frequency of colors of the visible spectrum. In particular, a decrease in bioluminescence is observed as the wave frequency increases. Long-wavelength red light is quickly absorbed and extinguished by the upper layers of water, and most marine creatures cannot perceive red light. This is likely why red also has a limited impact on the bioluminescence of dinoflagellates.

CONCLUSIONS

To date, known marine bioluminescent organisms include 9 405 species, of which 2 781 are luminescent (Claes *et al*. 2024). The ability to emit light at night is essential for these organisms. It is equally crucial to know the effects that artificial light at night has on them, especially those living near the sea surface, such as the dinoflagellates whose circadian clock drives their rhythmic physiology, including their nitrogen metabolism and photosynthesis, which help them regulate the nitrogen cycle and marine carbon, respectively (Jadhav *et al*. 2022).

This paper is one of the first studies to analyze light pollution's effects on marine bioluminescent organisms by applying a fast, innovative, and inexpensive methodological

photography-based approach in this research field. Using a digital camera to measure the bioluminescence of microalgae offers several advantages compared to other techniques. First, digital cameras allow for the capture of visual images and the documenting of bioluminescent events in real time, providing both qualitative and quantitative data useful for analysis. The versatility of this type of camera enables the adjustment of settings such as ISO, aperture, and exposure time, making it easier to adapt to varying light conditions and specific experimental needs. Additionally, images can be easily analyzed and stored, simplifying the comparison of different experiments or samples. Finally, digital cameras are generally more accessible and cost-effective than specialized instruments like luminometers or spectrophotometers, making them an ideal choice for laboratories with budget constraints or for preliminary studies. This combination of practicality and functionality makes digital cameras a valuable tool in bioluminescence research.

The results of the four experiments show that dinoflagellates do not appear susceptible to slight changes in the light/dark cycle. However, a total absence of light and darkness leads to a drastic (if not total) decrease in bioluminescence, especially under white LED or incandescent artificial light and with a light intensity of 100 lux or higher. OLEDs represent a rapidly growing eco-friendly lighting source due to their extreme flexibility, mercury-free manufacture, excellent color quality, and wide viewing angle (Zou *et al*. 2020) and also appear to be the source with the least impact on bioluminescent organisms. However, they are still highly underused for external lighting systems in coastal areas.

This study could provide valuable insights to minimize the effects of artificial light on bioluminescent organisms and inspire further studies to enhance our understanding of this issue, particularly regarding marine species that remain partially unexplored. With the rise of urbanization and light pollution, it is essential to consider the impact of artificial light in coastal planning and conservation strategies, as these factors threaten marine biodiversity and ecosystem health. Despite progress in understanding these effects, further research is needed to quantify the impact of artificial light on various species and environmental conditions and develop effective mitigation strategies.

ACKNOWLEDGMENTS

The author wishes to thank the anonymous reviewers for their insightful comments that enhanced this document.

Rev. Mar. Cost. Vol. 16 (2): 79-98, julio-diciembre 2024
ISSN: 1659-455X • e-ISSN: 1659-407X
DOI: http://dx.doi.org/10.15359/revmar.16-2.5

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ISSN: 1659-455X • e-ISSN: 1659-407X
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ISSN: 1659-455X • e-ISSN: 1659-407X
DOI: http://dx.doi.org/10.15359/revmar.16-2.5