Marine Pollution Bulletin 223 (2026) 119001

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Glowing hazards: Toxicological effects of festive glowsticks

Pedro Henrique Paixão [a], Felipe Teixeira Santana [a], Murilo Vieira Guimarães [c], João Vitor de Castro [a], Vinicius Gonçalves Pereira [a], Camila Prieto [a], Lilly Cristine Cunha De Oliveira [a], Vitória Nogueira Soares [a], Otto Müller Patrão de Oliveira [c], Caio Cesar-Ribeiro [a,b,c,*]

[a] *São Paulo State University—UNESP, Coastal Campus, Department of Biological and Environmental Sciences, São Vicente, 11380-900, SP, Brazil*
[b] *Centro de Ciências do Mar do Algarve (CCMAR/CIMAR-LA), Campus de Gambelas, Universidade do Algarve, 8005-139 Faro, Portugal*
[c] *Centro de Ciências Naturais e Humanas da Universidade Federal do ABC and Central de Equipamentos Multidisciplinar (CEM), Universidade Federal do ABC UFABC, São Bernardo do Campo, 09850-910, SP, Brazil*

ARTICLE INFO

Keywords:
Glowsticks
Marine invertebrates
PAHs
Embryo-larval toxicity
Reactive oxygen species
Coastal pollution

ABSTRACT

The widespread use and improper disposal of glowsticks (GS), especially during coastal festivities and in industrial fishing, raise ecotoxicological concerns for marine ecosystems. These devices contain complex chemical mixtures, including oxalate esters, hydrogen peroxide, phthalates, and polycyclic aromatic hydrocarbons (PAHs), many of which can generate reactive oxygen species (ROS) and cause toxic effects. This study assessed the acute toxicity of four GS colors (green, red, yellow, and blue) using embryo-larval development and mortality assays on three marine invertebrates: the sea urchin *Echinometra lucunter*, the sand dollar *Mellita quinquiesperforata*, and the brine shrimp *Artemia salina*. All colors caused developmental or lethal effects, with green GS consistently showing the highest toxicity. In *E. lucunter*, green GS fully inhibited larval development at all tested concentrations; EC50 values for yellow, red, and blue were 0.00072, 0.00877, and 0.02156 $\mathrm{mL \cdot L^{-1}}$, respectively. For *M. quinquiesperforata*, EC50s were 0.00538 (green), 0.05471 (red), and 0.0000732 $\mathrm{mL \cdot L^{-1}}$ (blue); yellow GS caused total mortality, precluding EC50 determination. In *A. salina*, LC50s were 0.00410 (yellow), 0.00583 (blue), and 0.01193 $\mathrm{mL \cdot L^{-1}}$ (red); green GS had a NOEC of 0.0001 and LOEC of 0.001 $\mathrm{mL \cdot L^{-1}}$, with no definable LC50. Results revealed species- and color-dependent sensitivity, implicating dye composition as a key toxicity driver. This is the first report of GS-derived toxicity in sand dollars and the first to provide comparative profiles across marine taxa. Findings highlight the need for regulation and environmental oversight of chemiluminescent product disposal.

1. Introduction

Lightsticks (LS) are chemiluminescent devices commonly employed in industrial pelagic fisheries, particularly as bait for species such as swordfish and tuna (De Oliveira et al., 2014b). These devices typically consist of a plastic tube housing oxalate esters and hydrogen peroxide, physically separated by a glass ampoule (Stevani and Baader, 1999). When the stick is bent, the ampoule breaks, initiating a chemiluminescent reaction (Koo and Schuster, 1977; Araujo et al., 2015). Although originally designed for professional use, LS have become increasingly prevalent in recreational contexts, including festivals and consumer products marketed to children—such as lollipop handles and party accessories—under the form of glowsticks (GS), which differ in shape but share the same chemical basis (Hoffman et al., 2002; Fact.MR, 2023; Cairns et al., 2018).

The growing popularity and improper disposal of these luminous products have raised significant environmental concerns, particularly regarding their impact on coastal and marine ecosystems. Numerous studies have documented the toxicity of LS-derived chemicals in a wide range of organisms, including marine invertebrates, fish, mammals, and even human cells (Hoffman et al., 2002; Pinho et al., 2009; Do Sul et al., 2009; Cesar-Ribeiro and Palanch-Hans, 2010; de Araujo et al., 2015; De Oliveira et al., 2014b; Cesar-Ribeiro et al., 2017; Cesar-Ribeiro, 2021b). These effects encompass embryotoxicity, oxidative stress, immunotoxicity, and genotoxicity, indicating that the risks are neither restricted to aquatic invertebrates nor to short-term exposures.

---

[*] Corresponding author at: Centro de Ciências do Mar do Algarve (CCMAR/CIMAR-LA), Campus de Gambelas, Universidade do Algarve, 8005-139 Faro, Portugal.
E-mail address: ccribeiro@ualg.pt (C. Cesar-Ribeiro).

https://doi.org/10.1016/j.marpolbul.2025.119001
Received 3 August 2025; Received in revised form 4 November 2025; Accepted 12 November 2025

The chemical formulations of LS and GS include various additives that enhance brightness and color, most notably polycyclic aromatic hydrocarbons (PAHs), oxalate esters, and phthalates such as dibutyl phthalate (DBP) and dimethyl phthalate (DMP) (Araujo et al., 2015; Coleman, 2009). These compounds are of particular concern due to their persistence and potential to generate reactive oxygen species (ROS) upon interaction with hydrogen peroxide, leading to molecular damage including DNA strand breaks. The dyes used to achieve specific glowstick colors vary in polarity and structure—factors that influence their environmental behavior and toxicity. For example, blue and green hues typically derive from 9,10-diphenylanthracene (DPA) and bis(phenylethynyl)anthracene (BPEA), respectively, while red and yellow tones involve more halogenated or complex aromatic compounds (Hanhela and Paul, 1981a, 1981b, 1981c; Coleman, 2009).

As billions of glowsticks are produced and discarded annually, their contribution to marine chemical pollution is increasingly relevant (Fact. MR, 2023). Once discarded, often on beaches, in estuarine zones, or directly into the sea, these devices may release toxic constituents into the water column and sediments, threatening local biota. Marine organisms, especially early life stages of invertebrates, are particularly vulnerable to such contamination, which may disrupt development, impair physiological functions, and alter population dynamics (Cesar-Ribeiro et al., 2017). There are also no environmental investigations focusing on the detection of chemical residues from LS or GS up to the date of submission of this article.

Despite growing awareness, there is a lack of regulatory frameworks addressing the environmental fate and effects of glowstick-derived pollutants. This study aims to fill part of this knowledge gap by assessing the acute toxicity of four glowstick colors (green, red, yellow, and blue) in three ecologically distinct marine invertebrates: the sea urchin *Echinometra lucunter*, the sand dollar *Mellita quinquiesperforata*, and the brine shrimp *Artemia salina*. We hypothesize that glowsticks of different colors, owing to their distinct chemical compositions, exhibit variable toxicological profiles but all represent a significant ecotoxicological threat. Understanding these effects is essential to inform waste management strategies and mitigate the environmental burden of chemiluminescent pollution in coastal environments.

2. Material and methods

The glowstick product evaluated in this study was *Pulseiras Neon* (Proplastik®), commercially available in four color variants: green, red, yellow, and blue. Acute toxicity tests were conducted on three marine invertebrate species representative of distinct ecological and taxonomic groups: the sea urchin *Echinometra lucunter*, the sand dollar *Mellita quinquiesperforata*, and the brine shrimp *Artemia salina*. Standardized protocols from regulatory and peer-reviewed sources were followed, with adaptations as needed for species-specific biology and laboratory conditions.

2.1. Sea urchin (Echinometra lucunter) embryo-larval development test

The testing procedure was adapted from ABNT NBR 15350 (2012). Approximately 20 adult specimens were collected by snorkeling from Ilha das Palmas, Guarujá (SP, Brazil), and maintained in aerated aquaria with filtered seawater under controlled temperature (25 ± 2 °C), salinity (35), and photoperiod (12 h light/12 h dark). Gametes were extracted from three females and three males via injection of 1 mL of 0.5 M KCl into the coelomic cavity. As external sexual dimorphism is absent, gametes were sexed based on color: orange eggs (females) and white sperm (males). Eggs were collected by placing females aboral side down in beakers with filtered seawater. The egg suspension was filtered through a 350 μm mesh and washed thrice with filtered seawater. Sperm was aspirated directly from the gonopores and diluted (0.5 mL in 24.5 mL seawater) to prepare the fertilizing solution. Fertilization was initiated by adding 2 mL of the diluted sperm to the egg suspension. Fertilization success (>80 %) was confirmed under an optical microscope. Test chambers received ~500 fertilized eggs per replicate and were incubated at 25 ± 2 °C until the pluteus stage was reached (36–42 h).

2.2. Sand dollar (Mellita quinquiesperforata) embryo-larval development test

Following ABNT NBR 15350 (2012) and dos Santos Laitano et al. (2008), approximately 100 adult individuals were collected from Itararé Beach, São Vicente (SP, Brazil) and maintained under controlled laboratory conditions. Gamete extraction and fertilization followed the same procedure described for *E. lucunter*. Embryos were exposed immediately after fertilization, and development was monitored microscopically after 36 to 42 h. Normal versus abnormal development was classified based on morphology and symmetry.

2.3. Brine shrimp (Artemia salina) lethality bioassay

Toxicity tests were adapted from Vanhaecke and Persoone (1984). *A. salina* cysts were hatched in filtered seawater at 25 ± 2 °C under continuous aeration and a 12 h/12 h photoperiod. After 24–36 h, actively swimming nauplii at instar II were collected and transferred to test chambers (10 nauplii per replicate). After 24 and 48 h of exposure, mortality was recorded by visual assessment. Individuals unresponsive to mechanical stimulation were considered dead. LC_{50} values were estimated using probit analysis.

2.4. Exposure conditions and test solutions

All exposures were performed in 10 mL glass tubes (four replicates per treatment). Test solutions were prepared by solubilizing the glowstick content (each color) in absolute ethanol, followed by dilution in reconstituted synthetic seawater (Red Sea®; salinity 35). Concentrations were tested ranging 0.0001 and 1 $mL \cdot L^{-1}$, with 0.01 % ethanol (*v*/v) used as a solubilizing agent across all treatments, following Cesar-Ribeiro et al. (2017); Cesar-Ribeiro (2021a). Control groups consisted of seawater with the same volume of ethanol (solvent control). Physicochemical parameters (temperature, salinity, dissolved oxygen, and ammonia) were monitored throughout the tests.

At the end of each test, embryos from *E. lucunter*, *M. quinquiesperforata*, and *P. perna* were fixed in borax-buffered formaldehyde and microscopically examined. For each replicate, 100 embryos were evaluated. Individuals were classified as normally developed (pluteus, D-larvae, or symmetrical blastula) or abnormal (arrested development, malformed structures).

2.5. Reference substance validation

To validate test sensitivity and reproducibility, an independent assay was conducted using sodium dodecyl sulfate (SDS) as a reference substance. A stock solution of 1000 $mg \cdot L^{-1}$ was prepared and serially diluted to five test concentrations (0.2, 0.5, 1.0, 2.0, and 5.0 $mg \cdot L^{-1}$). The same bioassay protocols were applied for quality assurance.

2.6. GC–MS analysis

Chemical characterization of the glowstick solutions was performed using a Varian CP-3800 gas chromatograph coupled to a mass spectrometer with an ion trap analyzer (Varian Saturn 2000) in CEM UFABC. The system allows detection of ions in both positive and negative modes and is capable of operating in MS/MS mode. The injector was operated in splitless mode at 275 °C. A CP-8400 autosampler handled up to 100 samples. The column used was a Zebron ZB-5MS (30 m × 0.25 mm × 0.25 μm film thickness). The oven temperature was programmed from 40 °C to 320 °C at a rate of 20 °C/min. Spectra were acquired in selected

Table 1
Chemical composition of glowsticks of different colors (blue, green, yellow, and red), quantified by GC–MS. Values are expressed in mg/mL of glowstick liquid. ND = Not detected.

Compound	Blue (mg/mL)	Green (mg/mL)	Yellow (mg/mL)	Red (mg/mL)
Dimethyl phthalate (DMP)	489	482	ND	ND
Dibutyl phthalate (DBP)	ND	ND	495	490
Oxalate ester	115	120	130	128
Hydrogen peroxide	15	13	11	12
9,10-Diphenylanthracene	0.55	ND	ND	ND
9,10-Bis(phenylethynyl) anthracene	ND	0.61	ND	ND
1,8-Dichloro-9,10-bis (phenylethynyl)anthracene	ND	ND	0.43	ND
Rhodamine B / Rubrene	ND	ND	ND	0.49

ion monitoring (SIM) mode for *m/z* 40–290.

For identification, authentic analytical standards were used for all target compounds, including dimethyl phthalate (DMP), dibutyl phthalate (DBP), oxalate ester, and hydrogen peroxide. Retention times and fragmentation spectra were compared to both standards and the NIST MS library. Deuterated dimethyl phthalate ($DMP\text{-}d_6$) was used as an internal standard to correct for matrix effects and instrumental variation.

Method validation included linearity assessment with calibration curves prepared at five concentration levels. Repeatability was verified by six replicate injections of diluted glowstick samples, yielding a relative standard deviation (RSD) of 11 %. A recovery test using DMP revealed recovery efficiency of 104 %, respectively, indicating high accuracy and reproducibility of the method.

2.7. Statistical analysis

All data were analyzed using GraphPad Prism 9.0 (GraphPad Software, CA, USA). Results were expressed as mean ± standard deviation (SD) from four replicates ($n = 4$). Normality of the data was assessed using the Shapiro-Wilk test, followed by one-way analysis of variance (ANOVA). When significant differences were detected, Dunnett's post hoc test was applied to compare each treatment to the control. Median lethal concentration (LC_{50}), as well as NOEC (No Observed Effect Concentration) and LOEC (Lowest Observed Effect Concentration) values, were calculated using non-linear regression with a 95 % confidence interval. Differences were considered statistically significant at $p < 0.05$.

3. Results

The GC–MS quantitative analysis revealed color-specific compositions of glowsticks, with solvents and fluorophores varying markedly among formulations (Table 1). Blue and green glowsticks presented high concentrations of dimethyl phthalate (DMP), reaching 489 mg/mL and 482 mg/mL, respectively. In contrast, yellow and red glowsticks were primarily composed of dibutyl phthalate (DBP), with concentrations of 495 mg/mL and 490 mg/mL. Oxalate esters, the core chemiluminescent reagents, ranged from 115 to 130 mg/mL across all colors, being highest in the yellow (130 mg/mL) and red (128 mg/mL) variants. Hydrogen peroxide was present in all formulations between 11 and 15 mg/mL, with the highest value in the blue variant.

Regarding the fluorescent dyes responsible for glowstick coloration, 9,10-diphenylanthracene was identified exclusively in the blue

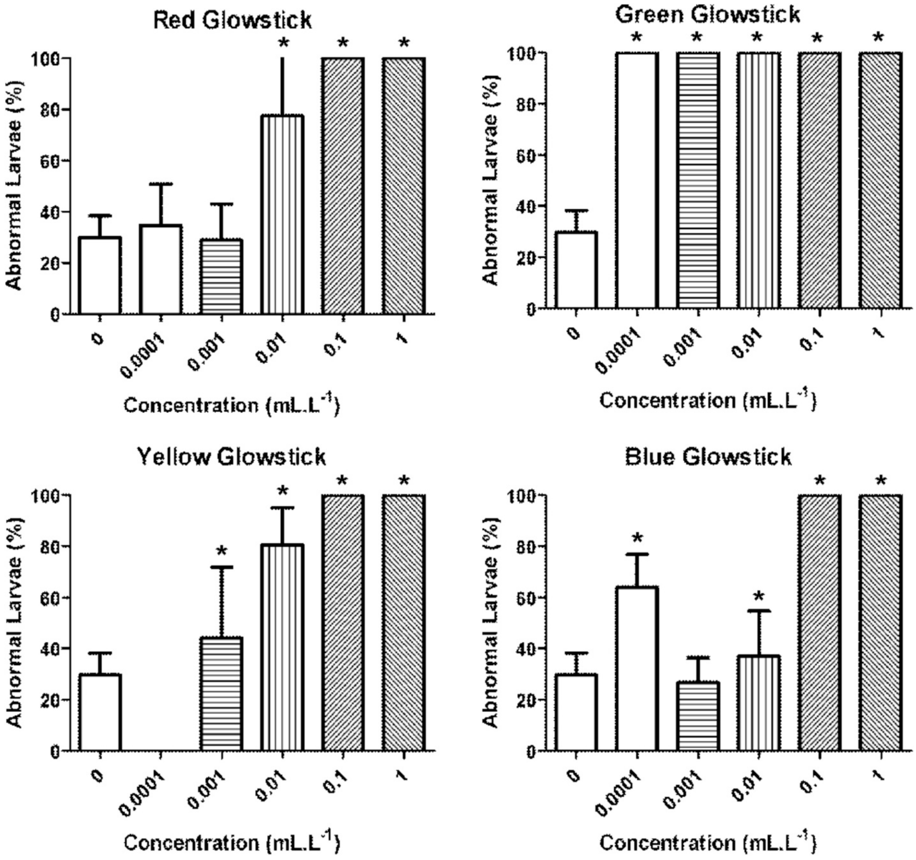

Fig. 1. Percentage of abnormally developed *Echinometra lucunter* larvae (pluteus stage) after 36 h of exposure to red, green, yellow, and blue glowstick (GS) extracts at concentrations ranging from 0 to 1.0 mL·L^{-1}. Data are presented as mean ± standard deviation ($n = 4$). Asterisks (*) indicate statistically significant differences compared to the control group ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

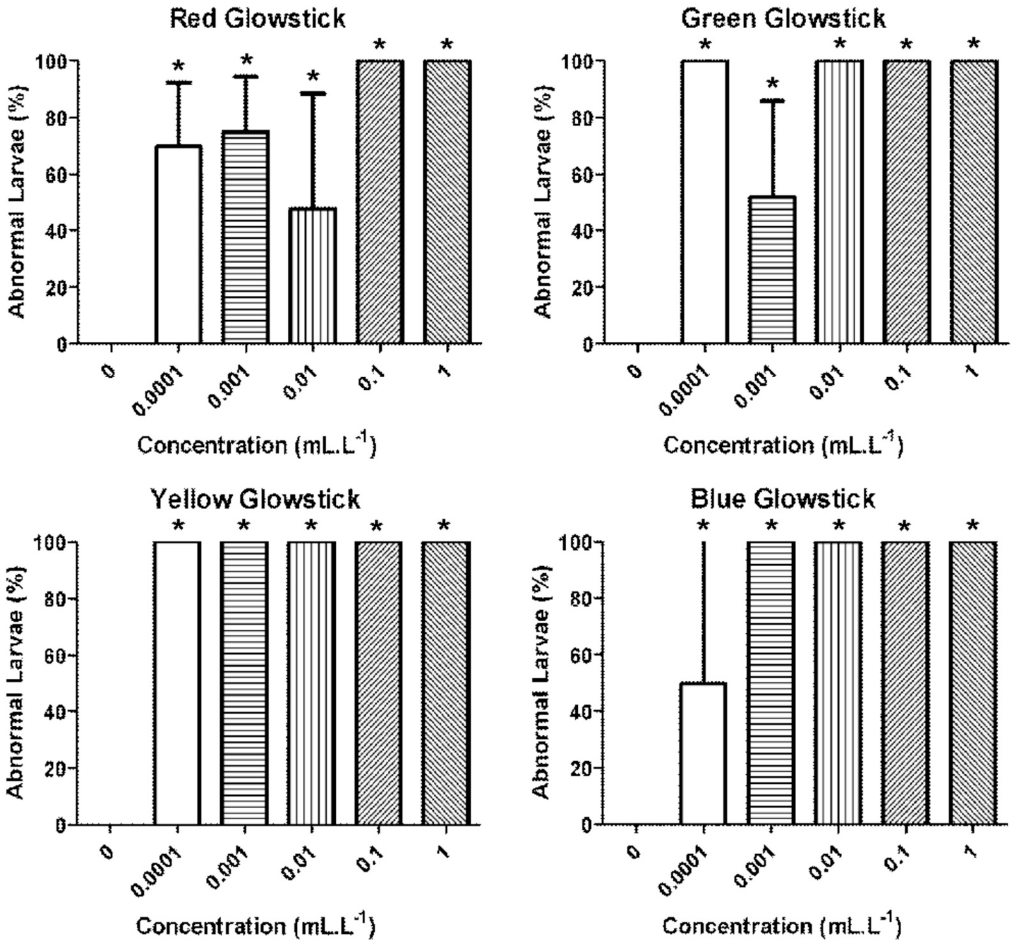

Fig. 2. Percentage of normally developed *Mellita quinquiesperforata* larvae after 42 h of exposure to glowstick (GS) extracts of four different colors (0–1.0 mL·L^{-1}). Data represent mean ± standard deviation ($n = 4$). Asterisks (*) indicate statistically significant differences relative to the control ($p < 0.05$).

formulation (0.55 mg/mL), while 9,10-bis(phenylethynyl)anthracene, the green-emitting fluorophore, was detected only in the green glowstick at 0.61 mg/mL. The yellow stick contained 1,8-dichloro-9,10-bis (phenylethynyl)anthracene (0.43 mg/mL), and the red variant contained Rhodamine B or Rubrene at 0.49 mg/mL. These aromatic compounds are structurally distinct and potentially linked to the differential ecotoxicological effects observed across colors and species.

Exposure to glowstick (GS) extracts resulted in color- and species-specific toxicological effects across all tested marine invertebrates. In *Echinometra lucunter*, a marked decrease in the percentage of normally developed larvae (pluteus stage) was observed for all colors. Green GS completely inhibited larval development at all tested concentrations, precluding CE_{50} estimation. The calculated CE_{50} values after 36 h of exposure were 0.008776 mL·L^{-1} (Red, 95 % CI: N.C.), 0.0007234 (Yellow, 95 % CI: 0.00005081–0.01030), and 0.02156 (Blue, 95 % CI: N. C.). Statistically significant effects ($p < 0.05$) were detected for all treatments from 0.001 mL·L^{-1} onwards (Fig. 1).

In *Mellita quinquiesperforata*, GS exposure for 42 h also led to high developmental toxicity. Yellow GS caused complete inhibition of larval development across all concentrations tested, preventing CE_{50} calculation. Blue GS elicited the highest sensitivity, with a CE_{50} of 0.0007321 mL·L^{-1} (95 % CI: N.C.), followed by green (0.005383, 95 % CI: N.C.) and red (0.05471, 95 % CI: N.C.). These results demonstrate severe sensitivity of *M. quinquiesperforata* embryos to GS-derived contaminants, especially those containing yellow dyes, following blue and green (Fig. 2).

In *Artemia salina*, 48 h exposure revealed a dose-dependent increase in mortality for all glowstick treatments. Yellow GS was the most toxic, with a CL_{50} of 0.004103 mL·L^{-1} (95 % CI: 0.002019–0.008337), followed by blue (0.005828, 95 % CI: 0.000901–0.037521) and red (0.01193, 95 % CI: 0.008070–0.01764). Although no CL_{50} was calculated for green GS, it displayed evident toxicity with a NOEC of 0.0001 mL·L^{-1} and a LOEC of 0.001 mL·L^{-1}. The absence of a determinable CL_{50} for green GS was due to high mortality across all concentrations tested (Fig. 3).

Together, these data confirm that glowstick formulations vary markedly in their toxicity profiles, with green and yellow products consistently showing the most pronounced effects across both developmental and mortality endpoints. The strong interspecific differences observed also underscore the relevance of testing multiple taxa in ecotoxicological assessments of consumer-derived chemical mixtures.

4. Discussion

The elevated concentrations of DMP and DBP confirmed via GC–MS analyses are consistent with previous surveys by Jacobsen et al. (2013), which reported solvent fractions exceeding 80 % in most glowstick formulations. These phthalates are well-known for their ecotoxicological potential, exerting both acute and chronic effects on marine organisms. DMP has been implicated in endocrine disruption and oxidative stress responses in marine invertebrates, while DBP is particularly embryotoxic, especially to echinoderms and mollusks.

The detection of oxalate esters and hydrogen peroxide adds further concern due to their role in the generation of reactive oxygen species (ROS), which compromise cellular integrity, induce lipid peroxidation, and impair larval development. Additionally, the selective presence of

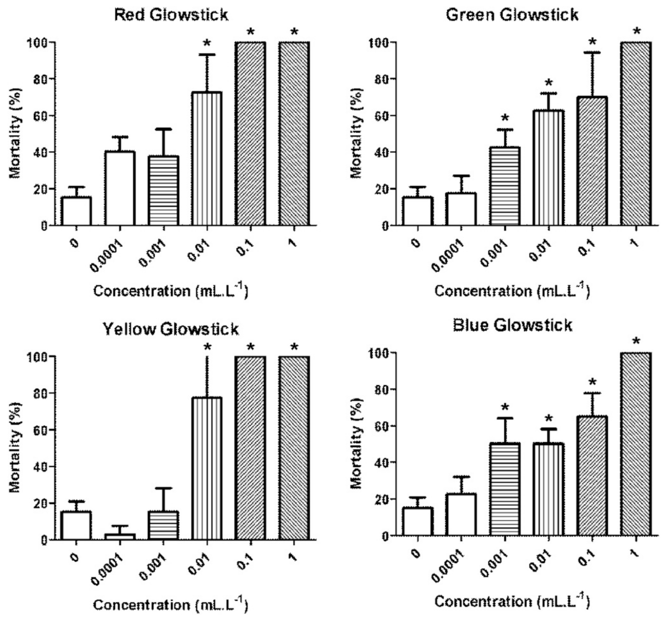

Fig. 3. Mortality (%) of *Artemia salina* nauplii after 48 h of exposure to glowstick (GS) extracts of red, green, yellow, and blue at concentrations from 0 to 1.0 mL·L^{-1}. Data are expressed as mean ± standard deviation (n = 4). Asterisks (*) denote statistically significant differences compared to the control ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

fluorophores such as 9,10-diphenylanthracene (DPA) and 9,10-bis(phenylethynyl)anthracene (BPEA) in blue and green glowsticks introduces further risks owing to their aromaticity, photoreactivity, and potential to undergo redox cycling.

The chemical profiles identified in this study validate the concern raised by Jacobsen et al. (2013), reinforcing the urgent need for regulatory attention regarding glowstick disposal, particularly in marine and coastal environments. When considered alongside the ecotoxicological outcomes observed in *Echinometra lucunter*, *Mellita quinquiesperforata*, and *Artemia salina*, the compositional data highlight the risk these products pose to sensitive marine life. The complete inhibition of larval development in *E. lucunter* and *M. quinquiesperforata* upon exposure to green and yellow glowsticks demonstrates the acute hazard posed by these formulations.

The CE_{50} and LC_{50} values obtained in this study were markedly lower than those reported for several common marine toxicants. In particular, green glowsticks induced significant mortality in *A. salina* at concentrations as low as 0.0001 mL·L^{-1}, with defined NOEC and LOEC values pointing to high acute toxicity. These findings are comparable or even more severe than previously reported EC_{50} values for lightstick (LS) leachates in sea urchins and crustaceans (Pinho et al., 2009; Cesar-Ribeiro, 2021b) (Table 2). Similarly, de Araujo et al. (2015) demonstrated that soluble fractions of LS induced shell deformities in oyster larvae at EC_{50} values ranging from 0.35 % to 0.65 %. Our results extend these concerns to a broader diversity of GS colors and species, confirming their harmful potential even at environmentally realistic exposures.

A central toxicological feature of glowsticks lies in their composite nature, consisting of oxalate esters, hydrogen peroxide, phthalates (DMP, DBP), and color-specific dyes primarily based on polycyclic aromatic hydrocarbons (PAHs) (Araujo et al., 2015; Coleman, 2009). These constituents, acting individually or synergistically, contribute to the observed biological damage. PAHs are particularly concerning due to their genotoxic, phototoxic, and redox-active behavior, which is intensified in the presence of H_2O_2, resulting in elevated ROS levels capable of damaging proteins, membranes, and DNA. Indeed, oxidative stress and histopathological alterations have been described in vertebrate models exposed to LS components (De Oliveira et al., 2014b; Do Sul et al., 2009).

Additionally, color-specific formulations confer variable toxicological profiles, reflecting both the chemical nature of the dyes and their solvent carriers, as previously described by Hanhela and Paul (1981a, 1981b, 1981c). The coloration in GS results from the selective solubilization of PAH-based dyes: green and blue hues are typically derived from BPEA and DPA in DMP solvents. The toxicological profile of BPEA

Table 2
Toxicity thresholds (EC_{50}, LC_{50}, NOEC, LOEC) of lightstick (LS) water-soluble fractions reported for marine and estuarine organisms across various endpoints. Values are expressed either as percentage of LS aqueous fraction (% *v*/v) or in mL.L^{-1}, as indicated. ND = Not determined.

Reference	Organism / Cell type	Endpoint	EC50 / LC50	LOEC / NOEC	Main observation
Cesar-Ribeiro and Palanch-Hans (2010)	*Echinometra lucunter*	Larval deformity	EC50: 0.062 %	ND	Even small amounts cause significant deformities
Cesar-Ribeiro et al. (2017)	*Lytechinus variegatus*	Larval deformity	EC50: 0.11 %	ND	Even small amounts cause significant deformities
de Araujo et al. (2015)	*Crassostrea rhizophorae*	Development (24 h)	EC50: 0.35 % (new tubes); 0.65 % (old tubes)	ND	Shell deformities
Pinho et al. *(2009)*	*Artemia salina*	Mortality (24 h)	LC50: 0.063 mL·L^{-1}	0.04 mL.L-1 / ND	Affects mortality
Pinho et al. *(2009)*	*Artemia salina*	Hatchability (48 h)	ND	0.2 mL.L-1 / ND	Affects cyst hatching
Cesar-Ribeiro (2021a)	*Promysis atlantica*	Mortality (3 h)	LC50: 0.001 %	ND	High mortality rates and behavioral changes
Cesar-Ribeiro (2021a)	*Promysis atlantica*	Behavior (3 h)	ND	0.005 % / ND	High mortality rates and behavioral changes
Cesar-Ribeiro (2021b)	*Pachygrapsus transverses*	Ammonia excretion	EC50: 0,0007 % (juvenile); 0,005 % (adults)	ND	Juveniles more sensitive than adults
Cesar-Ribeiro (2021b)	*Pachygrapsus transversus*	Oxygen consumption	EC50: 0.0004 % (juvenile); 0.0046 % (adults)	ND	Juveniles more sensitive than adults
Cesar-Ribeiro (2021b)	*Litopenaeus vannamei*	Ammonia excretion	EC50: 0.0006 %	ND	Changes in excretion and respiration
Cesar-Ribeiro (2021b))	*Litopenaeus vannamei*	Oxygen consumption	EC50: 0.072 %	ND	Changes in excretion and respiration
De Oliveira et al. *(2014b)*	HepG2 and NHDF (human cell's)	Cell viability	ND	ND	Oxidative stress and DNA damage
Do Sul et al. *(2009)*	Winstar rats (skin)	Dermatotoxicity	ND	ND	Severe dermatological effects; risk of cancer

remains unexplored in the literature; nevertheless, its potential toxicity is thought to arise from its lipophilic nature, which promotes bioaccumulation, and its ability to generate reactive oxygen species (ROS). Both processes can induce oxidative stress, damage to cellular membranes, and DNA strand breaks, likely resulting in substantial physiological effects in organisms exposed to this compound (Hanhela and Paul, 1981a, 1981b, 1981c).

In particular, Rhodamine B has been shown to induce acute toxic effects in aquatic organisms (Skjolding et al., 2021). These effects may involve the generation of reactive oxygen species (ROS), interference with enzymatic and endocrine processes, and cytotoxicity, as supported by subsequent studies. Sudarshan et al. (2022) demonstrated that Rhodamine B impairs growth and photosynthetic pigments in freshwater microalgae (*Chlorella vulgaris*), indicating oxidative stress and enzyme disruption. Priya et al. (2024) reported reproductive toxicity and teratogenic effects in zebrafish, highlighting impacts on early development and potential endocrine interference. Sharma et al. (2022) further observed alterations in antioxidant systems and biomarkers of cytotoxicity in aquatic organisms, reinforcing the role of ROS generation and cellular damage.

Importantly, this is the first report documenting ecotoxicological responses to GS exposure in *Mellita quinquiesperforata*, expanding the toolkit of echinoderm models for marine risk assessment. As sediment-associated organisms with critical roles in benthic-pelagic coupling, sand dollars particularly at early developmental stages are highly susceptible to waterborne contaminants. The extreme sensitivity of this species to blue GS ($CE_{50} = 0.00007321$ mL·L^{-1}) underscores the need to incorporate such taxa into standard ecotoxicological testing frameworks.

Given the prevalence of GS in fisheries and recreational contexts, often followed by improper disposal—the risk they pose to marine ecosystems is far from negligible. Our data support the implementation of stricter regulations regarding GS production, labeling, and post-use management. The development of lower-toxicity alternatives, such as peroxyoxalate systems for bioluminescent applications in plants (Tachapermpon et al., 2025), offers a promising path to mitigate their ecological footprint.

5. Conclusion

This study provides comprehensive chemical and ecotoxicological evidence that glowsticks, commonly used in recreational and fishing activities, pose a significant risk to marine ecosystems. GC–MS analyses confirmed that glowstick formulations contain high concentrations of hazardous solvents such as dimethyl phthalate (DMP) and dibutyl phthalate (DBP), along with reactive oxalate esters, hydrogen peroxide, and polycyclic aromatic hydrocarbon (PAH)-based dyes. These compounds, individually and synergistically, induce oxidative stress, developmental arrest, and mortality in a range of marine invertebrates.

Color-specific patterns of toxicity were evident, with green and yellow glowsticks exerting the most severe effects across multiple species. The extremely low EC_{50} and LC_{50} values observed—often in the μL·L^{-1} range—underscore the high potency of glowstick constituents, particularly when released into coastal environments. Notably, the inclusion of *Mellita quinquiesperforata* as a test organism broadens the ecotoxicological relevance of these findings and highlights the vulnerability of understudied benthic species.

Taken together, the results reinforce previous concerns about the environmental impact of glowsticks (Jacobsen et al., 2013) and emphasize the urgent need for regulatory measures governing their formulation, usage, and disposal. Furthermore, the development of safer chemiluminescent alternatives should be prioritized to reduce the ecological burden associated with these widely distributed products.

CRediT authorship contribution statement

Pedro Henrique Paixão: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Felipe Teixeira Santana:** Writing – original draft, Investigation. **Murilo Vieira Guimarães:** Investigation. **João Vitor de Castro:** Investigation. **Vinicius Gonçalves Pereira:** Investigation. **Camila Prieto:** Investigation. **Lilly Cristine Cunha De Oliveira:** Investigation. **Vitória Nogueira Soares:** Investigation. **Otto Müller Patrão de Oliveira:** Supervision. **Caio Cesar-Ribeiro:** Writing – review & editing, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study received Portuguese national funds from FCT – Foundation for Science and Technology through the projects UIDB/04326/2020 (DOI:10.54499/UIDB/04326/2020) and LA/P/0101/2020 (DOI:10.54499/LA/P/0101/2020) and CEECINSTLA/00028/2022/CP2990/CT0002 (https://doi.org/10.54499/CEECINSTLA/00028/2022/CP2990/CT0002). Caio Cesar Ribeiro also acknowledges support from the São Paulo Research Foundation (FAPESP) through a postdoctoral fellowship (grant #2022/06164-4). We thank Denis Abessa and the NEPEA group for providing equipment and materials. We also acknowledge the support of CEM – Central Multiuser Equipment Facility at the Federal University of ABC (UFABC) for the chemical analyses.

Data availability

Data will be made available on request.

References

ABNT – Associação Brasileira de Normas Técnicas, 2012. Ecotoxicologia aquática – Toxicidade crônica de curta duração – Método de ensaio com ouriço-do-mar (Echinodermata: Echinoidea) (NBR 15350). ABNT, Rio de Janeiro.

Araujo, M.M.S., Menezes Filho, A., Nascimento, I.A., Pereira, P.A.P., 2015. Lightsticks content toxicity: effects of the water soluble fraction on the oyster embryonic development. Chemosphere 139, 73–80.

Cairns, R., Brown, J.A., Dawson, A.H., Davis, W., Buckley, N.A., 2018. Carols by glow sticks: a retrospective analysis of poisons information Centre data. Med. J. Aust. 209 (11), 505–508.

Cesar-Ribeiro, C., 2021a. Lightsticks cause adverse effects on behavior and mortality of marine mysids Promysis atlantica. Lat. Am. J. Aquat. Res. 49 (4), 632–639.

Cesar-Ribeiro, C., 2021b. Chemical contents of disposed light sticks affect the physiology of rocky crab Pachygrapsus transversus and gray shrimps Litopenaeus vannamei. Bull. Environ. Contam. Toxicol. 107 (2), 370–377.

Cesar-Ribeiro, C., Palanch-Hans, M.F., 2010. Teste de toxicidade crônica com ouriço-do-mar Echinometra lucunter e Lytechinus variegatus (Echinodermata: Echinoidea), expostos ao bastão de luz—bandeira paternoster usado para pesca de espinhel de superfície. Braz. J. Oceanogr. 58, 71–75.

Cesar-Ribeiro, C., Rosa, H.C., Rocha, D.O., Dos Reis, C.G.B., Prado, T.S., Muniz, D.H.C., Palanch-Hans, M.F., 2017. Light-stick: a problem of marine pollution in Brazil. Mar. Pollut. Bull. 117 (1–2), 118–123.

Coleman, W.F., 2009. Molecular models of compounds in lightsticks. J. Chem. Educ. 86 (1), 128.

de Araujo, Menezes Filho, A., Nascimento, I.A., Pereira, P.A.P., 2015. Lightsticks content toxicity: Effects of the water soluble fraction on the oyster embryonic development. Chemosphere 139, 73–80.

De Oliveira, T.F., Da Silva, A.L.M., De Moura, R.A., Bagattini, R., De Oliveira, A.A.F., De Medeiros, M.H.G., De Melo Loureiro, A.P., 2014b. Luminescent threat: toxicity of light stick attractors used in pelagic fishery. Sci. Rep. 4 (1), 5359.

Do Sul, J.A.I., Oliveira, T.F., Da Silva, A.L.M., De Moura, R.A., Bagattini, R., De Oliveira, A.A.F., De Medeiros, M.H.G., De Melo Loureiro, A.P., 2009. Skin irritation and histopathologic alterations in rats exposed to lightstick contents, UV radiation and seawater. Ecotoxicol. Environ. Saf. 72 (7), 2020–2024.

dos Santos Laitano, K., Gonçalves, C., Resgalla Jr., C., 2008. Viability of the Use of the Sand Dollar Mellita quinquiesperforata as a Test Organism. Ecotoxicol. Environ. Contam. 3 (1), 9–14.

Fact.MR, 2023. Glow sticks market: Global industry analysis, size, share, growth, trends, and forecast, 2023–2033. https://www.factmr.com/report/4204/glow-sticks-market.

Hanhela, P.J., Paul, D.B., 1981a. Synthesis and evaluation of fluorescent materials for colour control of peroxyoxalate chemiluminescence. I. The phenylethynylation of anthraquinone. Aust. J. Chem. 34 (8), 1669–1685.

Hanhela, P.J., Paul, D.B., 1981b. Synthesis and evaluation of fluorescent materials for colour control of peroxyoxalate chemiluminescence. II. Violet and blue emitters. Aust. J. Chem. 34 (8), 1687–1700.

Hanhela, P.J., Paul, D.B., 1981c. Synthesis and evaluation of fluorescent materials for colour control of peroxyoxalate chemiluminescence. III. Yellow and red fluorescent emitters. Aust. J. Chem. 34 (8), 1701–1717.

Hoffman, R.J., Nelson, L.S., Hoffman, R.S., 2002. Pediatric and young adult exposure to chemiluminescent glow sticks. Arch. Pediatr. Adolesc. Med. 156 (9), 901–904.

Jacobsen, E., Tønning, K., Poulsen, L.F., Elmegaard, N., 2013. Survey and health assessment of glow sticks.

Koo, J.Y., Schuster, G.B., 1977. Chemically initiated electron exchange luminescence. A new chemiluminescent reaction path for organic peroxides. J. Am. Chem. Soc. 99 (18), 6107–6109.

Pinho, G.L.L., Ihara, P.M., Fillmann, G., 2009. Does light-stick content pose any threat to marine organisms? Environ. Toxicol. Pharmacol. 27 (1), 155–157.

Priya, P.S., Nandhini, P.P., Vaishnavi, S., Pavithra, V., Almutairi, M.H., Almutairi, B.O., Arockiaraj, J., 2024. Rhodamine B, an organic environmental pollutant induces reproductive toxicity in parental and teratogenicity in F1 generation in vivo. Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol. 280, 109898.

Sharma, J., Sharma, S., Bhatt, U., Soni, V., 2022. Toxic effects of Rhodamine B on antioxidant system and photosynthesis of Hydrilla verticillata. J. Hazard. Mater. Lett. 3, 100069.

Skjolding, L.M., Dyhr, K.S., Köppl, C.J., McKnight, U.S., Bauer-Gottwein, P., Mayer, P., Baun, A., 2021. Assessing the aquatic toxicity and environmental safety of tracer compounds Rhodamine B and Rhodamine WT. Water Res. 197, 117109.

Stevani, C.V., Baader, W.J., 1999. O sistema quimiluminescente peróxi-oxalato. Quim. Nova 22 (5), 715–723.

Sudarshan, S., Bharti, V.S., Harikrishnan, S., Shukla, S.P., RathiBhuvaneswari, G., 2022. Eco-toxicological effect of a commercial dye Rhodamine B on freshwater microalgae Chlorella vulgaris. Arch. Microbiol. 204 (10), 658.

Tachapermpon, Y., Hematulin, S., Treesubsuntorn, C., 2025. The development of a low-toxic peroxyoxalate chemiluminescent system for light-emitting plants. Environ. Sci. Pollut. Res. 1–11.

Vanhaecke, P., Persoone, G., 1984. The ARC-test: A standardized short-term routine toxicity test with Artemia nauplii: Methodology and evaluation.