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# Ecotoxicological Assessment of Surfactant Pollution Using Pyrocystis lunula Bioluminescence

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# Ecotoxicological Assessment of Surfactant Pollution Using *Pyrocystis lunula* Bioluminescence

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## Abstract

Bioluminescence-based bioassays have emerged as sensitive tools for assessing the ecotoxicological effects of marine contaminants. Among marine microplankton, bioluminescent dinoflagellates offer ecologically relevant responses due to their position at the base of the food web and their sensitivity to membrane-active compounds. In this study, the toxic effects of the anionic surfactants linear alkylbenzene sulfonate (LAS) and sodium dodecyl sulfate (SDS), widely used in detergents and frequently detected in coastal waters, were evaluated using the bioluminescent dinoflagellate *Pyrocystis lunula*. Cultures in the stationary growth phase were exposed for 24 and 48 h to increasing concentrations of LAS (0.1–50.0 mg $L^{-1}$) and SDS (5–50.0 mg $L^{-1}$), following standardized ecotoxicological guidelines. Bioluminescence inhibition was quantified at the nocturnal emission peak using a Tecan Infinite® M200 Pro Luminometer, and complemented by cell density and viability assessments. LAS induced a clear concentration-dependent inhibition of bioluminescence, with an estimated $IC_{50}$ of 4.01 mg $L^{-1}$ after 24 h, while SDS exhibited higher toxicity, particularly at concentrations ≥25 mg $L^{-1}$. Combined exposure to LAS and SDS resulted in stronger inhibitory effects than single-compound treatments. Notably, bioluminescence proved more sensitive than cell density in detecting early sublethal effects, indicating disruptions in membrane integrity and metabolic activity prior to cell death. These results highlight the suitability of *P. lunula* bioluminescence as a rapid, cost-effective, and ecologically relevant tool for monitoring surfactant contamination in marine environments and supporting coastal water-quality assessment.

**Keywords:** Toxicology bioassay; Anionic surfactants; Marine pollution; Dinoflagellate; Linear alkylbenzene sulfonate; Sodium dodecyl sulfate

## 1. INTRODUCTION

Surfactants are among the most widely used chemical compounds worldwide, driven by their extensive application in household detergents, personal care products, industrial cleaners and agrochemicals. In 2022, global surfactant production was valued at approximately USD 43.2 billion, with projections indicating growth to nearly USD 55.2 billion by 2030 (GMI, 2023). The largest consumers include China, the United States, India, Japan, and Brazil (The Brainly Insights, 2023).

Surfactants reach marine ecosystems through both direct and indirect pathways. Direct inputs include untreated sewage discharges, submarine outfalls, beach pollution, and residues from maritime activities, whereas indirect sources involve fluvial and pluvial runoff, particularly in urbanized regions. Although wastewater treatment plants can reduce surfactant loads, a substantial fraction is still released into aquatic systems (Jackson *et al*., 2015). Between the most commonly used anionic surfactants are linear alkylbenzene sulfonate (LAS, typically represented by sodium dodecylbenzenesulfonate) and sodium dodecyl sulfate (SDS).

In coastal waters and estuaries influenced by wastewater discharges, LAS concentrations typically range from 0.01 to 0.15 mg $L^{-1}$, but can exceed 1.0 mg $L^{-1}$ near untreated outfalls (Ying, 2006; Olkowska *et al*., 2014; Badmus et al., 2021). It was also reported as up to 1.24–2.45 mg $L^{-1}$ in polluted river sites (Abd El-Gawad, 2014). In contrast, SDS undergoes rapid biodegradation and is usually detected at much lower trace levels (µg $L^{-1}$) in surface waters, though it can reach higher concentrations (up to several mg $L^{-1}$) in raw municipal effluents (Cserháti *et al*., 2002; Cruz de Carvalho *et al*., 2022; Wu *et al*., 2023). Thus, both surfactants may occur in the same broad aquatic concentration domain, especially in wastewater-impacted systems (Wu *et al*., 2023).

In order to mitigate these environmental risks, robust international and national guidelines have been established. In Europe, the Human and Environmental Risk Assessment (HERA) defines a Predicted No-Effect Concentration (PNEC) of 0.25 mg $L^{-1}$ specifically for LAS in aquatic ecosystems (HERA, 2013). Furthermore, environmental agencies frequently regulate anionic surfactants collectively as Methylene Blue Active Substances (MBAS). The United States Environmental Protection Agency (USEPA) sets a secondary maximum contaminant level of 0.5 mg $L^{-1}$ for foaming agents to prevent aesthetic and ecological degradation, while the World Health Organization (WHO) recommends a stricter 0.2 mg $L^{-1}$ limit for water supplies. Additionally, national frameworks, such as Japan's Water Supply Act, strictly regulate total anionic surfactants and LAS homologues to ensure water safety. Similarly, in Brazil, CONAMA Resolution No. 357 establishes a maximum concentration of 0.2 mg $L^{-1}$ for surfactants (reacting as MBAS) in Class I saline waters intended for primary contact recreation, aquaculture, and fisheries (Brasil, 2005).

Despite these regulations, environmental monitoring frequently reports that these legal limits are exceeded in both Brazilian and international coastal waters, particularly in areas receiving untreated municipal effluents (Ying, 2006; Badmus *et al*., 2021). For instance, surfactant concentrations have been reported up to 1.43 mg $L^{-1}$ in Brazilian estuaries, above the PNEC threshold in Malaysian rivers, and as high as 23.17 mg $L^{-1}$ in Peruvian Andean micro-basins during the dry season (Choque-Quispe *et al.*, 2021). Conversely, in cleaner or effectively treated environments, concentrations remain well below toxic thresholds. Seasonal averages in Egyptian coastal waters have been recorded at 0.12–0.13 mg $L^{-1}$ (Okbah *et al*., 2013), and treated effluents in Germany report average LAS concentrations as low as 14.4 µg $L^{-1}$ (Freeling *et al.*, 2019), leading to a high probability of minimal environmental risk in regions with robust wastewater infrastructure, such as Japan (Shiode *et al*., 2020).

Beyond their environmental concentrations, both LAS and SDS pose distinct ecotoxicological challenges based on their chemical structures. LAS contains an aromatic benzene ring, a planar structure associated with greater chemical stability, environmental persistence, and specific toxicological effects in aquatic ecosystems (Eganhouse et al., 1984; Kocal et al., 2001; Badmus et al., 2021). Conversely, SDS is an aliphatic straight-chain alkyl sulfate. Although it lacks the stable aromatic ring and degrades more easily, its strong amphiphilic nature allows it to rapidly penetrate and disrupt biological lipid membranes, often exerting severe acute toxicity even before degradation occurs (Lewis, 1990; Cruz de Carvalho *et al*., 2022; Singh *et al*., 2024).

At the cellular level, surfactants can disrupt biological membranes even at low concentrations, altering permeability and inducing protein and lipid denaturation, loss of cellular bioactivity, and, ultimately, cell lysis (Braga, 2000; Cruz de Carvalho *et al*., 2022). Due to these effects, monitoring surfactant toxicity in marine environments is essential for water quality assessment and ecosystem protection. Ecotoxicological evaluations increasingly rely on bioassays as complementary tools to chemical analyses. Among these, bioluminescence-based assays stand out for their sensitivity, rapid response, and cost-effectiveness.

Bioluminescence in marine dinoflagellates is mediated by a luciferin–luciferase reaction occurring in specialized lipid-bound organelles known as scintillons, and is regulated by endogenous circadian rhythms (Hastings & Dunlap, 1986; Shimomura, 2006; Valiadi & Iglesias-Rodriguez, 2013). Because scintillons depend on transmembrane proton fluxes to trigger light emission, the destructive action of amphiphilic surfactants on lipid bilayers directly and rapidly uncouples these essential pH gradients. Consequently, surfactant-induced membrane disruption causes a measurable drop in bioluminescence well before widespread cellular lysis occurs, making light emission an exceptionally

sensitive indicator of early cellular stress (Craig *et al*., 2003; Valiadi & Iglesias-Rodriguez, 2013; Cruz de Carvalho *et al*., 2022).

As unicellular eukaryotic primary producers, dinoflagellates represent an ecologically relevant component of marine phytoplankton and may provide toxicity responses more representative of higher organisms than standard bacterial models like *Aliivibrio fischeri* (Taylor, 1987; Hildenbrand *et al*., 2015; Girotti *et al*., 2008). Previous studies have demonstrated the applicability of bioluminescent dinoflagellates as sensitive bioindicators of marine pollution, including exposure to organic contaminants and metals (Lapota *et al*., 1993; Okamoto *et al*., 1999; Heimann *et al*., 2002; Craig *et al*., 2003; Mirjani *et al*., 2021; Perin *et al*., 2022). However, while the ecotoxicity of surfactants has been extensively studied in bacteria and microcrustaceans, their specific impact on marine bioluminescent eukaryotes remains underexplored. Specifically, the use of *Pyrocystis lunula* as a model to assess the individual and combined toxicological effects of LAS and SDS represents a significant scientific gap.

Given the widespread presence of surfactants in marine environments and their potential biological effects, the use of sensitive and ecologically relevant tools to evaluate surfactant toxicity is essential. This study aims to fill this gap by assessing the effects of the anionic surfactants LAS and SDS, which enter marine ecosystems primarily through domestic and industrial effluents, using ecotoxicological assays based on the bioluminescence of the marine dinoflagellate *Pyrocystis lunula* (Fig. 1). Additionally, the study evaluates the suitability of this bioluminescence-based bioassay as a rapid tool for monitoring marine surfactant contamination.

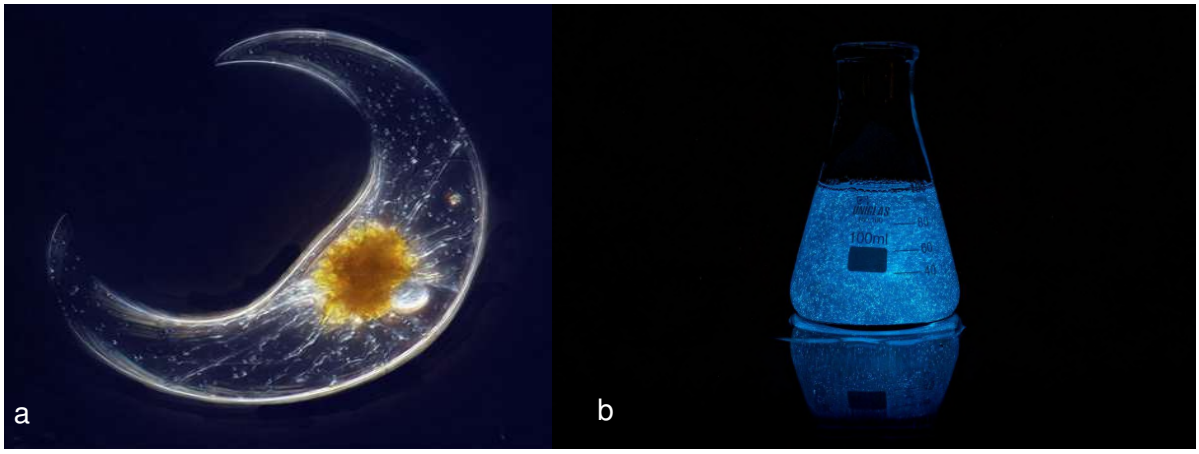



**Figure 1**. *Pyrocystis lunula* vegetative cell by John Dolan (a) and its bioluminescence in culture by Andrade, G.F. (b).

## 2. MATERIAL AND METHODS

### 2.1. Surfactants

The experiments were conducted using linear alkylbenzene sulfonate (LAS) — represented by sodium dodecylbenzenesulfonate ($(CH_{12})_{11}C_6H_4SO_3Na$ – LAS; 50.8% active ingredient; Neon) — and sodium dodecyl sulfate ($C_{12}H_{25}OSO_3Na$ – SDS; 95% purity; Mallinckrodt OR). Both were selected to represent anionic surfactants commonly found in detergents that frequently reach marine environments.

The surfactant concentrations tested were chosen to encompass environmentally relevant levels —which typically range from 0.01 to 0.15 mg $L^{-1}$ in coastal waters but can exceed 1.0 mg $L^{-1}$ near untreated sewage outfalls (Ying, 2006; Olkowska *et al*., 2014)— and to compare with the Brazilian CONAMA Resolution No. 357, which limits surfactants to 0.2 mg $L^{-1}$ in Class I saline waters (Brasil, 2005). The maximum tested concentrations (up to 50 mg $L^{-1}$) were selected not to reflect baseline environmental levels, but rather to ensure a complete suppression of bioluminescence, allowing for the establishment of a full concentration-response curve necessary to accurately calculate the $IC_{50}$.

To optimize dilution and prevent the addition of large volumes that could destabilize the pH and salinity of the culture water, stock solutions were prepared in sterile distilled water. For both LAS and SDS, two stock solutions were prepared: Stock A (500 mg $L^{-1}$) and Stock B (5000 mg $L^{-1}$). For the lower test concentrations (0.1, 0.2, 0.5, and 1.0 mg $L^{-1}$), volumes ranging from 6.0 to 60.0 µL of Stock A were added. For the higher concentrations (5.0, 10.0, 25.0, and 50.0 mg $L^{-1}$), volumes ranging from 30.0 to 300.0 µL of Stock B were used. This dual-stock approach ensured that the maximum volume of surfactant solution added to the experimental units never exceeded 300 µL. Because the added volumes were extremely low, the pH and salinity of the water were not checked after the addition of the surfactants.

The appropriate volumes of surfactant for each target concentration (ranging from 6.0 to 300.0 µL) were pipetted directly into the experimental units. To ensure utmost accuracy and consistency across all treatments, each unit was meticulously adjusted to a final volume of exactly 100 mL (or 30 mL in the second experiment) with an initial cell density of 3,000 cells $mL^{-1}$. First, the necessary volume of the mother culture was calculated and added based on its prior cell count. Then, the remaining volume was completed using sterile Guillard F/2 medium, strictly discounting the specific micrometric volume of the added surfactant. This adjustment ensured that neither the total volume nor the nutrient availability was altered by the surfactant spikes.

### 2.2. Culture of the test organism – *Pyrocystis lunula*

The stock culture of the dinoflagellate *Pyrocystis lunula* (strain BMAK 264) was obtained from the Aidar and Kutner Microorganism Bank (BMAK) of the Oceanographic Institute of the University of São Paulo (IO-USP). The species was cultivated in Guillard F/2 medium (Guillard, 1975), prepared

with autoclaved seawater with salinity adjusted to 35. Cultures were acclimated to the experimental conditions since the end of June 2023 and maintained in a Caltech® BOD incubator at a constant temperature of 21°C and 12-hour light/dark cycles with artificial LED light (124 μE $m^{-2}$ $s^{-1}$). Because light emission is nocturnal, a photoperiod inversion was applied in the incubator (dark during the day and light at night), as the peak light emission for *P. lunula* occurs 4 hours after the onset of the dark phase (Sweeney, 1982).

Bioassays were performed at the stationary phase of culture growth (30-40 days of cultivation). While standard algal growth inhibition assays typically utilize cells in the exponential growth phase, the stationary phase was selected for this bioluminescence-based bioassay. This approach minimizes the confounding effects of rapid cell division during the short-term exposure (24–48 h), ensuring that the observed declines in light emission are primarily driven by the toxicological response and membrane disruption, rather than variations in cell population dynamics.

### 2.3. Experimental organization

This study evaluated the survival and recovery viability of the dinoflagellate *P. lunula* exposed to surfactants diluted in culture medium. The experimental design was guided by the recommendations of the Brazilian Standard NBR 16181 (ABNT, 2013) and international guidelines for ecotoxicological assays. Mother cultures were maintained in batch conditions for 30–40 days to ensure cellular stability and reproducibility. For all bioassays, Erlenmeyer flasks were prepared with Guillard's F/2 culture medium and inoculated to ensure a constant initial cell density of 3,000 cells $mL^{-1}$ (Fig. 2).

Two independent experiments were conducted. The first experiment aimed to compare the effects of single and combined surfactant exposures. In this phase, 250 mL Erlenmeyer flasks containing a final volume of 100 mL were used. Three treatments were evaluated: *(i)* exposure to SDS, *(ii)* exposure to LAS, and *(iii)* combined exposure to SDS and LAS (1:1 ratio). Due to the limited availability of experimental flasks at this stage, four test concentrations plus a control were used (0, 5.0, 10.0, 25.0, and 50.0 mg $L^{-1}$ for single treatments; and 5+5, 10+10, 25+25, 50+50 mg $L^{-1}$ for the mixture). Consequently, the total nominal surfactant load in the combined treatments was double that of the individual treatments (e.g., the highest mixture resulted in a total surfactant concentration of 100 mg $L^{-1}$). This additive design choice was intended to evaluate the combined presence of both pollutants at their respective target concentrations, though it inherently implies a higher total chemical load.

A second experiment was subsequently conducted to test a broader concentration gradient in full compliance with the ABNT guidelines, which recommend a minimum of five test concentrations. Because LAS demonstrated higher toxicity in the initial assessments, and due to experimental time constraints, this expanded assay focused solely on LAS. To accommodate the larger number of

treatments (eight concentrations plus a control) while managing the limited availability of the *P. lunula* mother culture, the final exposure volume was reduced to 30 mL per flask. Despite this volume reduction, the initial cell density was kept identical to the first experiment (3,000 cells $mL^{-1}$). The final LAS concentrations tested were 0, 0.1, 0.2, 0.5, 1.0, 5.0, 10.0, 25.0, and 50.0 mg $L^{-1}$.

All treatments in both experiments were performed in triplicate and maintained at 21 °C for a total duration of 48 h. After 24 and 48 h of exposure, aliquots of 100 µL were collected in triplicate from each flask for bioluminescence measurements, along with a 1 mL aliquot for cell counting. Sampling was performed approximately 4 h after the onset of the dark phase under low-light conditions in a laminar flow hood.

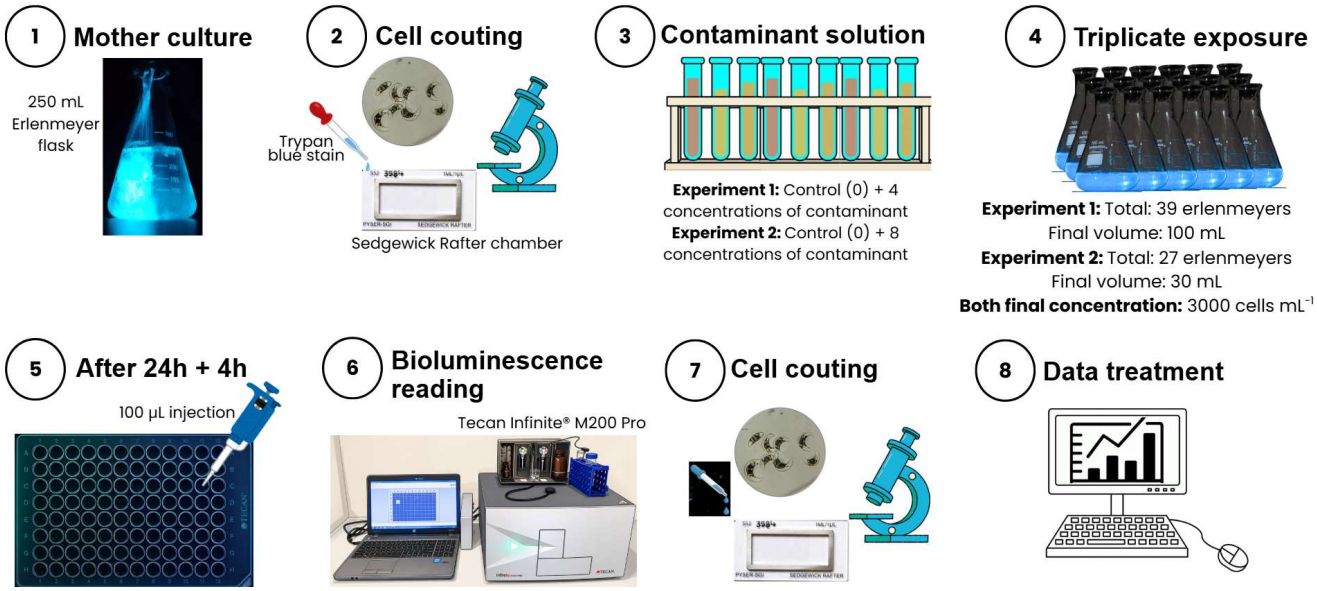



**Figure 2.** Experimental design of bioassays for exposing *Pyrocystis lunula* to different surfactant concentrations.

### 2.4. Light Emission Measurements

According to Stauber *et al.* (2008), bioluminescence readings should be taken 4 hours after the onset of the dark phase to ensure peak emission, resulting in an optimal and consistent light response. Bioluminescence was measured using a Tecan Infinite® M200 Pro luminometer. Standard black 96-well microplates for luminescence assays were used, and 100 µL of each sample were manually dispensed into the wells in triplicate. During the measurement, the instrument automatically injected 100 µL of Guillard's culture medium into each well, providing a mechanical stimulus that induces bioluminescence emission by *Pyrocystis lunula*. The emitted light was quantified by the luminometer and expressed as relative light units per second (RLU $s^{-1}$) (Costa *et al.*, 2004).

### 2.5. Determination of cell density

Samples were homogenized and transferred to a Sedgewick-Rafter chamber (1 mL). Cell counting was performed under a Leica® DME optical microscope at 100× magnification until at least 400 cells were recorded. The cell density was then calculated as the number of cells counted per chamber area.

### 2.6. Trypan Blue Stain

Cell viability was identified using Trypan Blue Stain (0.4%) as a dye for dead cells. A 1:100 ratio of dye to sample was applied by adding 25 µL of the dye solution to 2.5 mL of the culture sample. After homogenization, a 1.0 mL aliquot of the mixture was placed on a chamber for analysis under an optical microscope. Viable and non-viable cells are distinguished by color: viable cells do not absorb the dye due to membrane selectivity and integrity, whereas dead cells, with permeable membranes, are filled by the dye and appear blue.

### 2.7. Data treatment

The results were expressed as the percentage of luminescence relative to the respective control for each exposure time, according to the following equation:

$$P = \frac{\text{mean}}{\text{mean}_{control}} \times 100$$

Data processing, normalization, and graphical representations were performed using Microsoft Excel® and R software (version 4.5.1; R Core Team). Outliers were identified and removed from the bioluminescence datasets based on the interquartile range (IQR) method. Because bioluminescence measurements comprised 9 analytical replicates per treatment (3 experimental flasks × 3 well aliquots), the first (Q1) and third (Q3) quartiles could be reliably determined. The IQR was calculated (IQR = Q3 – Q1), and upper (UL) and lower (LL) limits were defined as UL = Q3 + 1.5×IQR and LL = Q1 – 1.5×IQR. Values outside this range were excluded prior to computing the means (Vinutha *et al*., 2018). It is important to note that this outlier exclusion was not applied to the cell density data, as the sample size for cell counting ($n = 3$) is statistically insufficient for robust quartile derivation.

Statistical analyses were primarily descriptive, with variability among replicates expressed using the interquartile range (IQR) to provide a robust measure of dispersion. Although inferential statistical tests (e.g., ANOVA) were not applied to compare individual treatments, a comprehensive quantitative dose-response analysis was conducted. The half-maximal inhibitory concentration ($IC_{50}$) for LAS was estimated by fitting a four-parameter log-logistic model (LL.4) to the normalized bioluminescence data. To ensure biological relevance, the model's upper response limit was fixed at 100% (representing the uninhibited control) and the lower limit was fixed at 0%. All model fitting and

parameter estimations, including the derivation of 95% confidence intervals using the delta method, were performed using the drc package in R software. Finally, data visualization was conducted using the ggplot2 and ggtext packages to apply consistent aesthetic parameters across all generated figures.

## 3. RESULTS AND DISCUSSION

### 3.1. Experiment 1: Comparative toxicity and combined exposure

Within the tested range, SDS exposure produced more pronounced toxic effects than LAS, particularly at concentrations ≥ 25 mg $L^{-1}$. After 24 h, low SDS concentrations (5–10 mg $L^{-1}$) transiently stimulated bioluminescence, whereas higher concentrations strongly suppressed light emission (Fig. 3a). Such stimulation at low doses is consistent with early, mild membrane disruption of scintillons, leading to uncontrolled light release, followed by severe inhibition as cellular damage progresses. After 48 h, bioluminescence decreased across all SDS treatments, indicating sustained metabolic impairment. Cell density also declined in SDS treatments compared to controls (Fig. 3b), especially at 25 mg $L^{-1}$, demonstrating that SDS exerts both rapid metabolic effects and pronounced cytotoxicity, consistent with its interaction with lipid membranes (Lewis, 1990; Cruz de Carvalho *et al*., 2022).

In contrast, while the lowest concentration of LAS (5 mg $L^{-1}$) also provoked a transient stimulation of bioluminescence similar to SDS, higher concentrations of LAS severely inhibited it (Fig. 3c). The population density remained relatively constant and stable across all LAS treatments over the 48-h period (Fig. 3d). This uncoupling between reduced light emission and stable cell survival suggests the hypothesis that LAS might interfere with the metabolic or cellular processes involved in light production —such as the disturbance of pH regulation, mechanosensitivity, or signaling pathways linked to bioluminescence, rather than generalized cell death— prior to causing widespread cell mortality (Chen *et al*., 2007, Stires & Latz, 2018, Kamerlin *et al*., 2020). However, because direct biochemical mechanisms were not evaluated in this study, this interpretation remains speculative and highlights the need for further specific mechanistic investigations to fully understand LAS toxicity pathways in *P. lunula*.

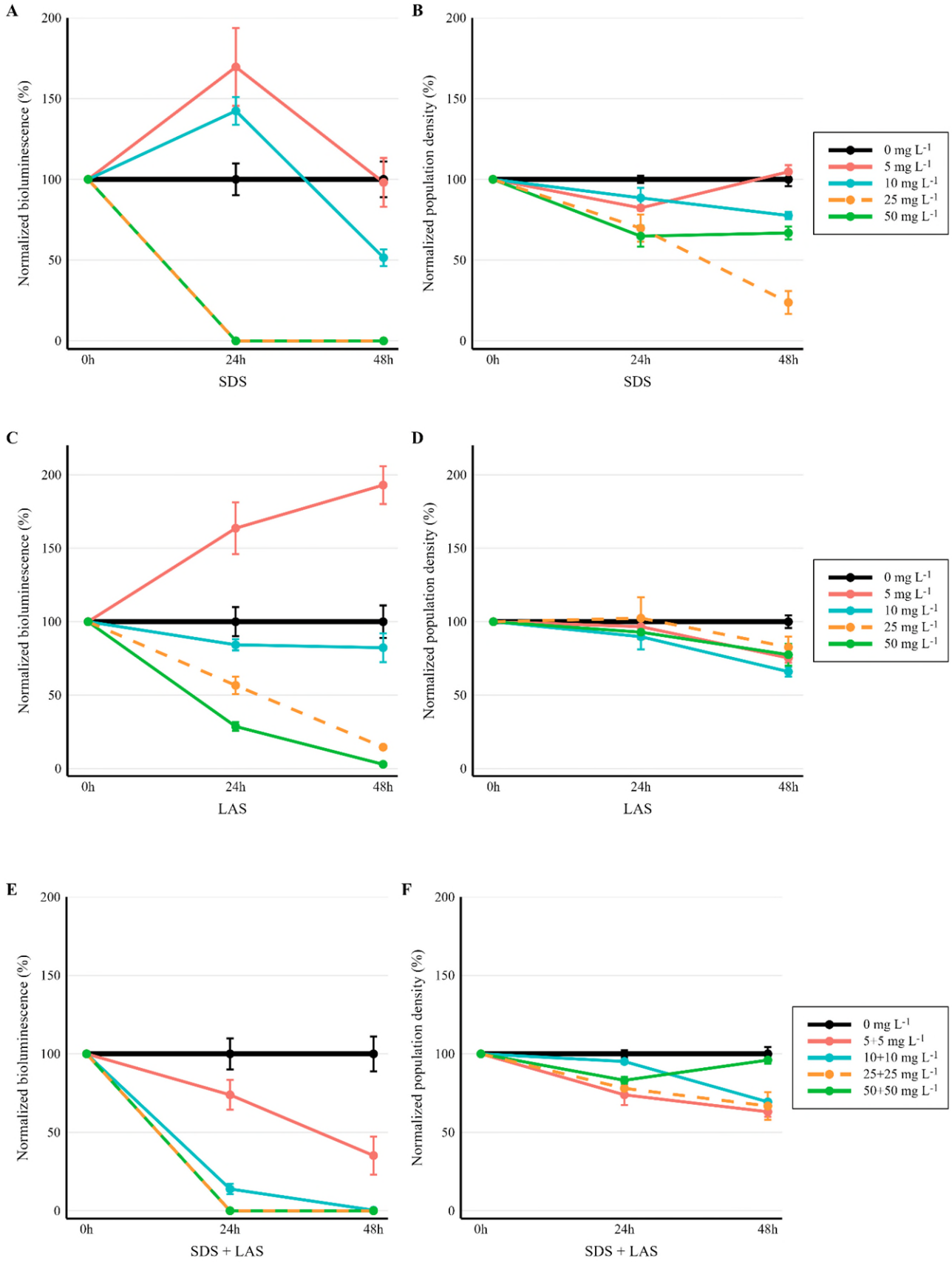



**Figure 3.** Bioluminescence values (a, c and e) and cell density values (b, d and f) considering 24 and 48h exposure of *P. lunula* to surfactants.

The observation that LAS severely suppresses bioluminescence prior to causing clear reductions in cell density has ecological and physiological implications for *P. lunula*. While the exact mechanisms remain hypothesized —potentially involving early sub-lethal disruptions of the scintillon membranes or metabolic uncoupling rather than immediate structural collapse— the loss of light emission significantly compromises the organism's fitness (Chen *et al*., 2007; Latz *et al.*, 2008; Lapointe & Morse, 2008; Ebenezer & Ki, 2013; Stires & Latz, 2018; Kamerlin *et al*., 2020, Perin *et al*., 2022). In dinoflagellates, bioluminescence functions as a primary anti-predatory defense mechanism, often described as the "burglar alarm" effect, which startles grazers or attracts secondary predators to reduce grazing pressure (Mensinger & Case, 1992; Abrahams & Townsend, 1993). If exposure to LAS and other surfactants chemically "blinds" this defense system at sub-lethal concentrations, *P. lunula* populations become highly vulnerable to predation in their natural habitat. Because reduced luminescence may weaken this anti-predator defense, sublethal surfactant exposure can have severe ecological consequences even when the cells remain physically present and viable (Haddock *et al*., 2010; Cusick & Widder, 2014; Lindström *et al*., 2017, Di Bari, 2025). Consequently, relying solely on traditional viability or cell-density endpoints would severely underestimate the ecological risks of surfactant pollution in coastal environments, reinforcing the value of bioluminescence as a highly sensitive, ecologically relevant biomarker (Craig *et al*., 2003).

The distinct toxicological profiles of these surfactants can be attributed to their chemical structures. LAS is structurally characterized by a stable, planar benzene ring combined with a long carbon chain, effectively classifying it as a hydrocarbon-related pollutant (Solomons & Fryhle, 2016). This hydrocarbon nature may influence its specific interactions with, and affinity for, the lipid bilayers of cell membranes, a characteristic often associated with its specific toxicity in aquatic organisms (Rosety *et al*., 2001). In contrast, SDS is an aliphatic straight-chain alkyl sulfate that, while lacking the aromatic ring, exhibits a strong amphiphilic nature that allows it to rapidly penetrate and disrupt selective biological lipid membranes, leading to the severe acute toxicity observed even at lower concentrations (Lewis, 1990; Warne & Schifko, 1999).

Combined exposure to LAS and SDS resulted in the most severe effects among the treatments, with no transient stimulation of bioluminescence at low concentrations and a sharp decline in light emission as concentrations increased (Fig. 3e). Cell densities were consistently lower than in single-compound treatments (Fig. 3f), indicating intensified cellular stress. Because formal mixture-toxicity models (such as concentration addition or independent action) were not applied in this study, these results suggest intensified effects driven primarily by the increased total surfactant load, rather than a quantifiable additive or synergistic interaction — a complexity previously described for surfactant mixtures (Warne & Schifko, 1999; Cedergreen, 2014).

It is important to clearly acknowledge the logistical constraints that guided this experimental design, which precluded an equivalent concentration-response evaluation for all treatments. Ideally, an expanded experimental setup should have been conducted for LAS, SDS, and their mixture. However, due to the limited volume of the *P. lunula* mother culture, a restricted number of experimental flasks, and the availability of only a single incubator at the time, it was not possible to perform the broader assay for all compounds simultaneously. Consequently, the expanded concentration-response experiment (ranging from 0.1 to 50.0 mg $L^{-1}$) was conducted exclusively for LAS, prioritized due to its widespread environmental relevance. Because an $IC_{50}$ was calculated solely for LAS in this second phase, comparisons between LAS, SDS, and their combination are based exclusively on the initial exploratory range (5.0–50.0 mg $L^{-1}$). To avoid overinterpreting these differences, the comparative effects among the surfactants are discussed cautiously as descriptive comparative estimates rather than robust equivalent toxicological metrics.

### 3.2. Experiment 2: Effects of LAS exposure

Exposure of *Pyrocystis lunula* to increasing concentrations of LAS resulted in a concentration-dependent inhibition of bioluminescence. Normalized bioluminescence declined progressively across the tested range (0.1–50 mg $L^{-1}$) after 24 h, with no complete suppression even at the highest concentration (Fig. 4). The fitted four-parameter log-logistic concentration-response curve yielded an $IC_{50}$ of 4.01 mg $L^{-1}$, indicating a measurable sensitivity of *P. lunula* bioluminescence to LAS (Fig. 5). Although a transient stimulation at 5 mg $L^{-1}$ was observed in the initial exploratory assay (Experiment 1), this effect was absent in this expanded evaluation. Such variability at low concentrations is common in ecotoxicological bioassays and likely reflects minor shifts in the physiological state or baseline sensitivity of the different *P. lunula* culture batches used across the non-simultaneous experiments.

These results support the hypothesis that LAS interferes with cellular processes involved in light production before causing widespread cell mortality, which may indicate a rapid uncoupling of transmembrane pH gradients or altered mechanosensitivity in the scintillons (Chen *et al*., 2007; Stires & Latz, 2018; Kamerlin *et al*., 2020).

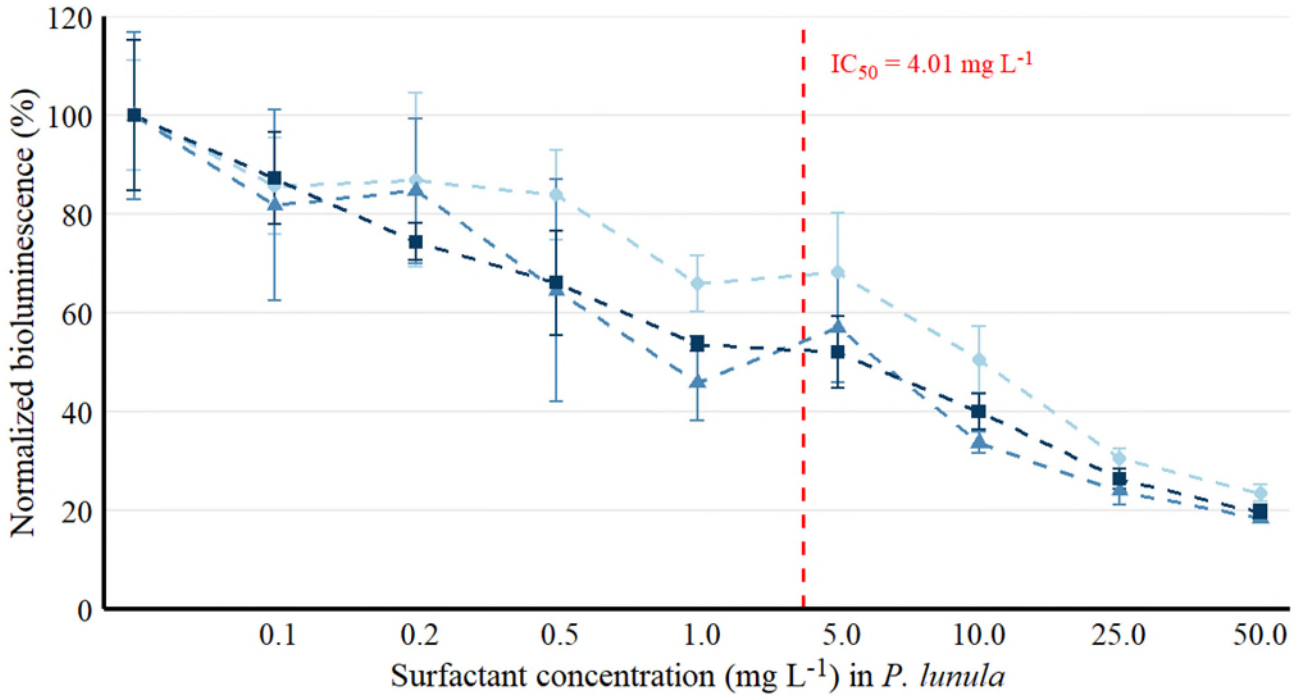



**Figure 4.** Bioluminescence response of *P. lunula* exposed to LAS, normalized by the control and obtained in three different experiments. Error bars represent standard error (n = 9).

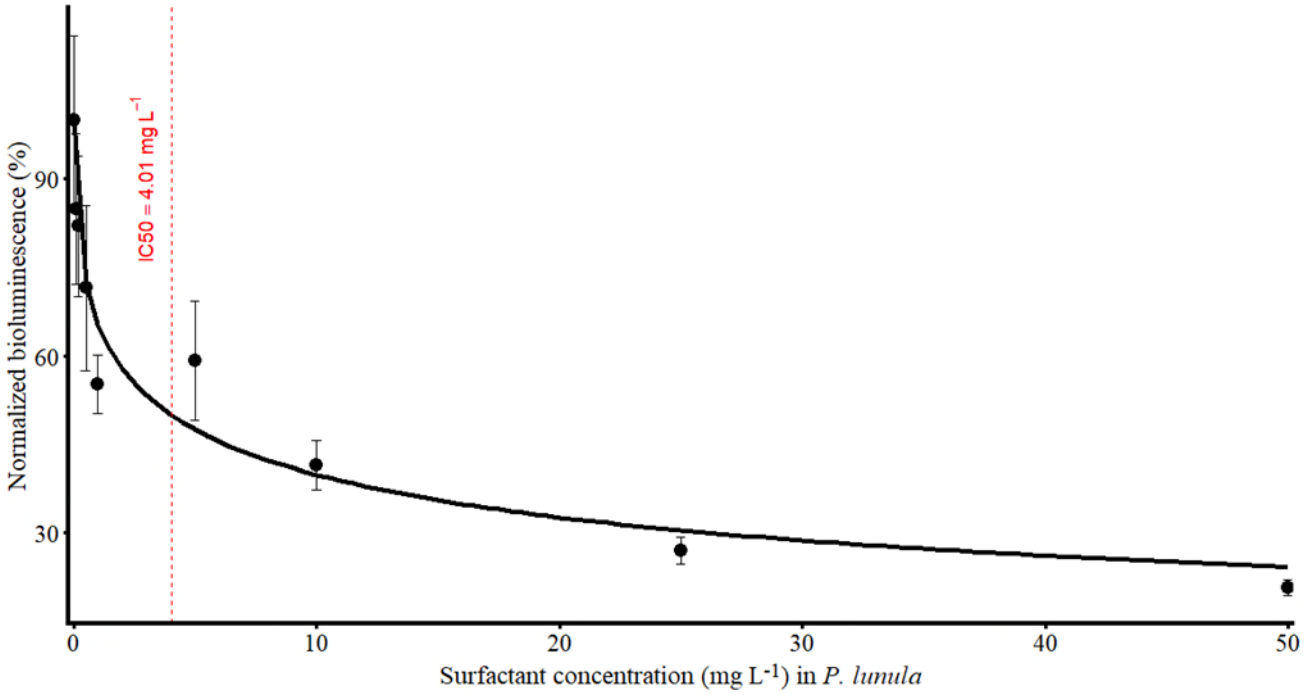


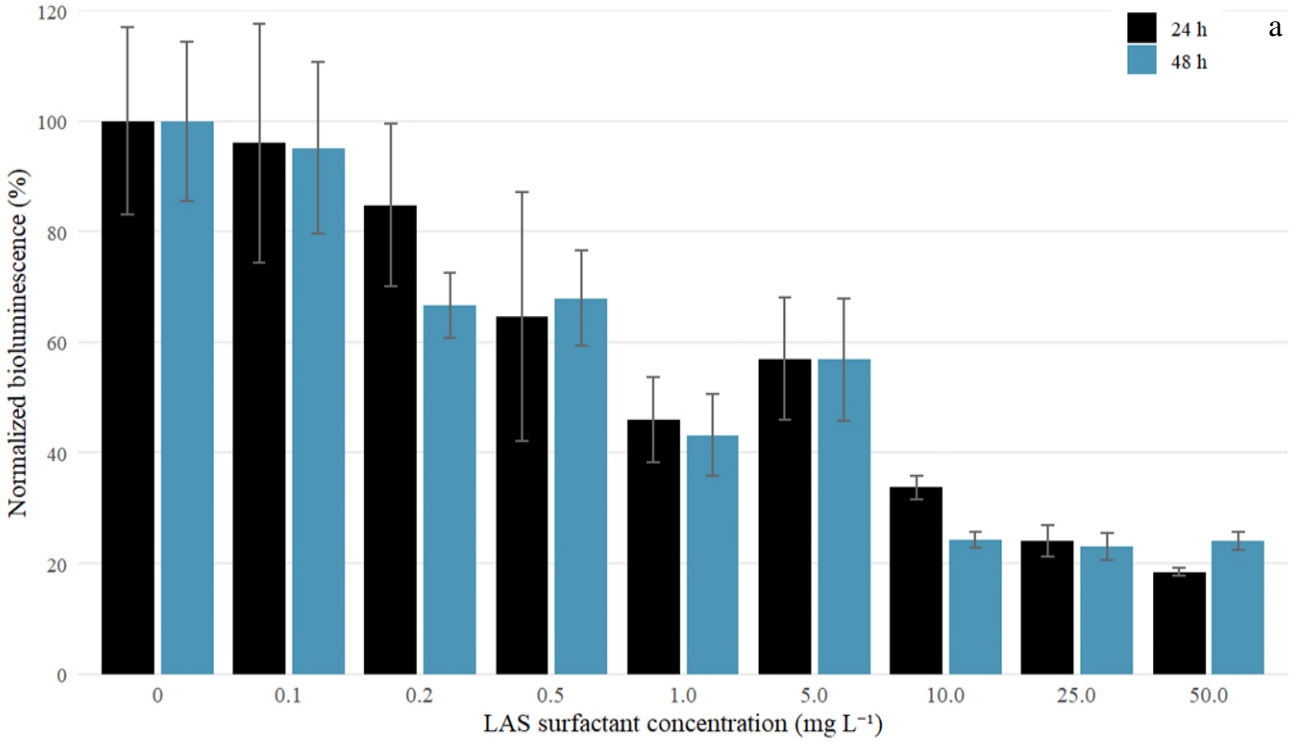

**Figure 5.** Fitted concentration–bioluminescence response curve and $IC_{50}$ for LAS (mean of exposures). Error bars represent standard error (n = 9).

After 48 h of exposure, LAS-induced inhibition of bioluminescence became qualitatively more pronounced at the highest concentrations, with values dropping below 30% of the control (Fig. 6a). Descriptively, the overall concentration-response pattern remained consistent between the 24 h and 48

h periods, suggesting that the onset of the primary toxicological effects affecting light emission occurs within the first 24 h of exposure.

In contrast to the marked reduction in bioluminescence, cell density did not exhibit an immediate concentration-dependent decline. Although all treatments were inoculated with an initial density of 3,000 cells $mL^{-1}$, the control and lower concentration treatments exhibited a decrease in cell density to approximately 1,900–2,100 cells $mL^{-1}$ after 24 h. This initial reduction can be attributed to handling stress during inoculation or the rapid settling and encystment of cells (as supported by our qualitative observations), which may temporarily reduce their availability in the sampled water column. Interestingly, at the highest concentration (50.0 mg $L^{-1}$), cell densities remained closer to the initial inoculum (approximately 3,000–3,300 cells $mL^{-1}$). This likely reflects a rapid acute toxicity that physically disrupted or "fixed" the cells in suspension before normal encystment and settling behaviors could occur, rather than indicating population growth. After 48 h, cell density showed limited variation compared to the 24 h period (Fig. 6b).

This apparent decoupling between light emission and population growth reinforces that population metrics alone may underestimate surfactant toxicity during short-term exposures. Instead, these findings emphasize the early sensitivity of luminescence as an endpoint that may detect sublethal stress before cell-density changes become obvious (Latz *et al*., 2008; Perin *et al*., 2022). Because bioluminescent dinoflagellates are widely used as toxicity indicators, this rapid bioluminescent response to LAS demonstrates strong value as an early warning signal for bioassays. Although qualitative viability observations indicated the presence of non-viable cells at concentrations ≥10 mg $L^{-1}$ and limited encystment, these findings suggest that LAS may impair membrane integrity and metabolic activity, potentially altering bioluminescence without immediately suppressing cell division (Lapointe & Morse, 2008; Ebenezer & Ki, 2013; Stires & Latz, 2018; Kamerlin *et al*., 2020).



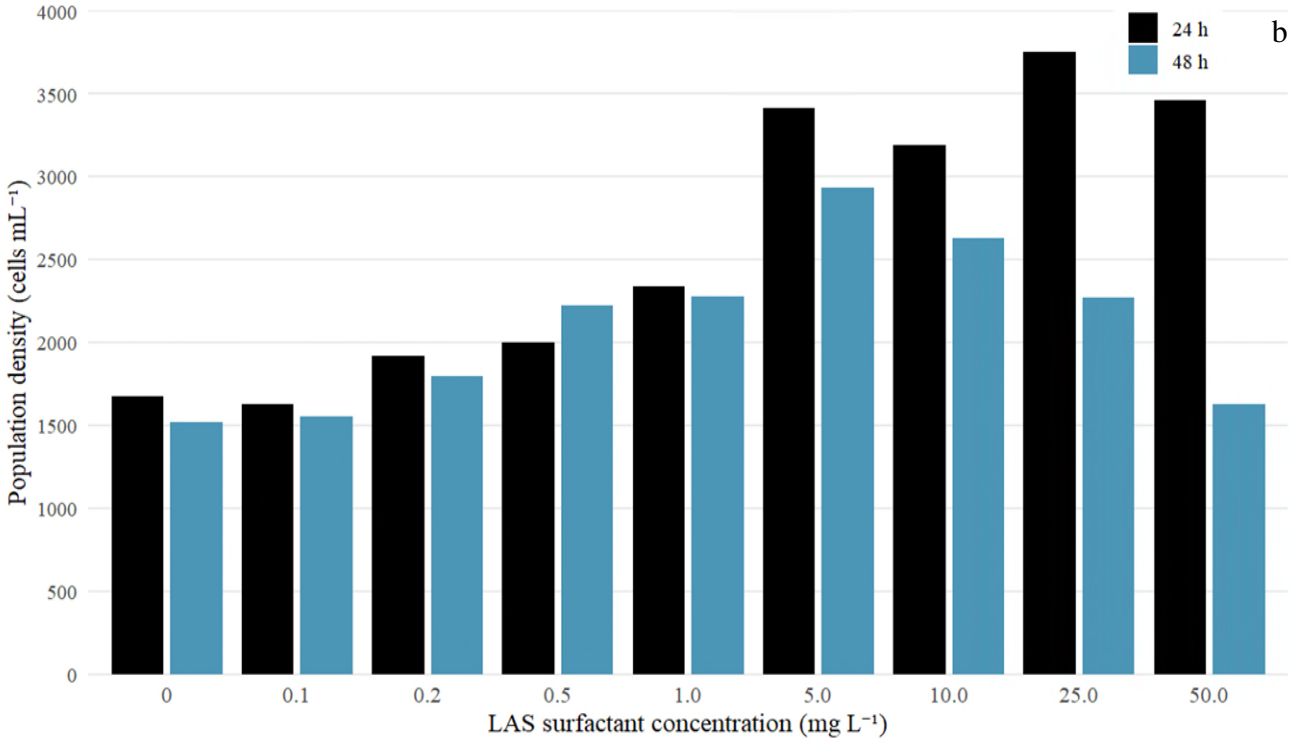




**Figure 6.** Bioluminescence (RLU) (a) and Population density (cells mL⁻¹) (b) of *P. lunula* exposed to increasing concentrations of LAS. Error bars represent the standard error. Bioluminescence shows a clearer concentration-dependent response than population density.

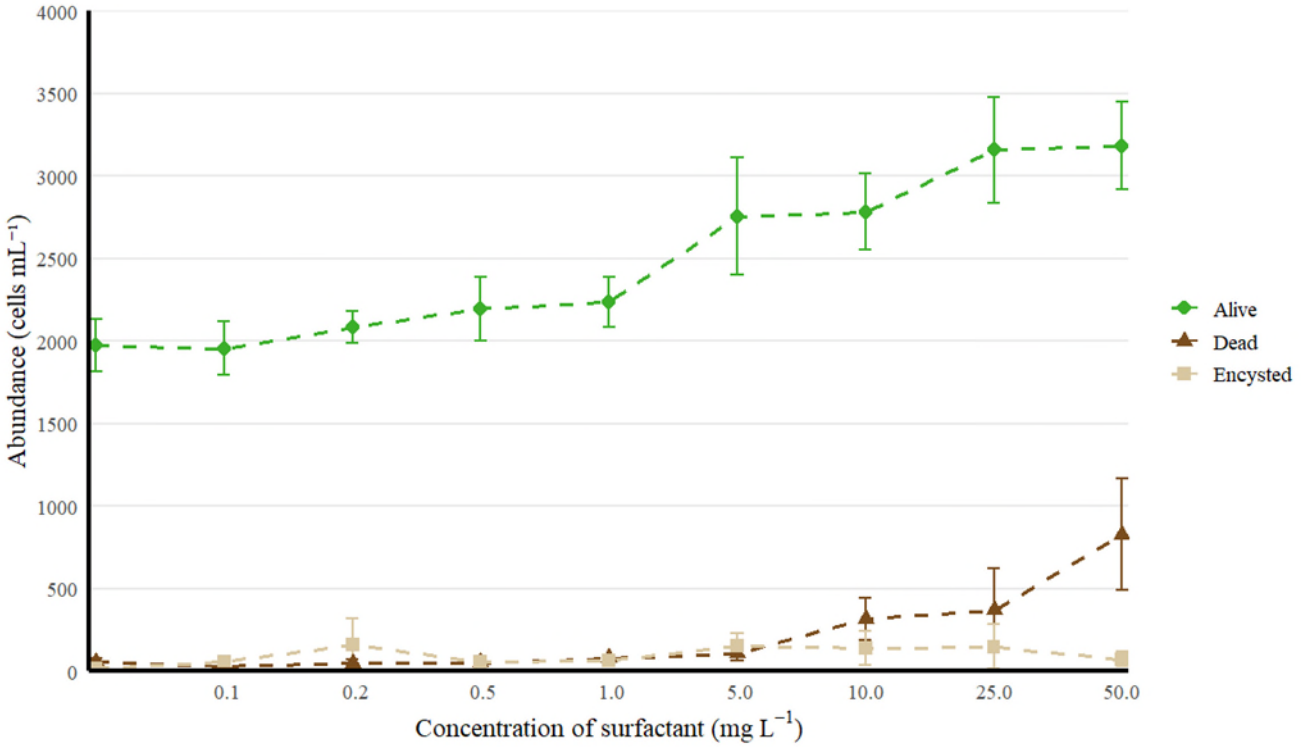



**Figure 7.** Abundance of *P. lunula* cells classified as live, dead, and encysted across different LAS concentrations. Error bars represent standard error (n = 3).

### 3.3. Cell morphology

Trypan Blue staining provided qualitative evidence indicating an increase in membrane-compromised cells with rising surfactant concentrations (Fig. 8). Even some cells that appeared morphologically intact under optical microscopy were permeable to the dye, suggesting a loss of viability prior to visible structural collapse. At lower concentrations (0.1–5.0 mg $L^{-1}$), qualitative observations noted the occurrence of encystment. At higher concentrations, qualitative observations noted the occurrence of membrane rupture and nuclear extrusion. These visual assessments, while descriptive, corroborate the observed reductions in bioluminescence and further support the hypothesis that surfactants primarily target and compromise cellular lipid membranes.

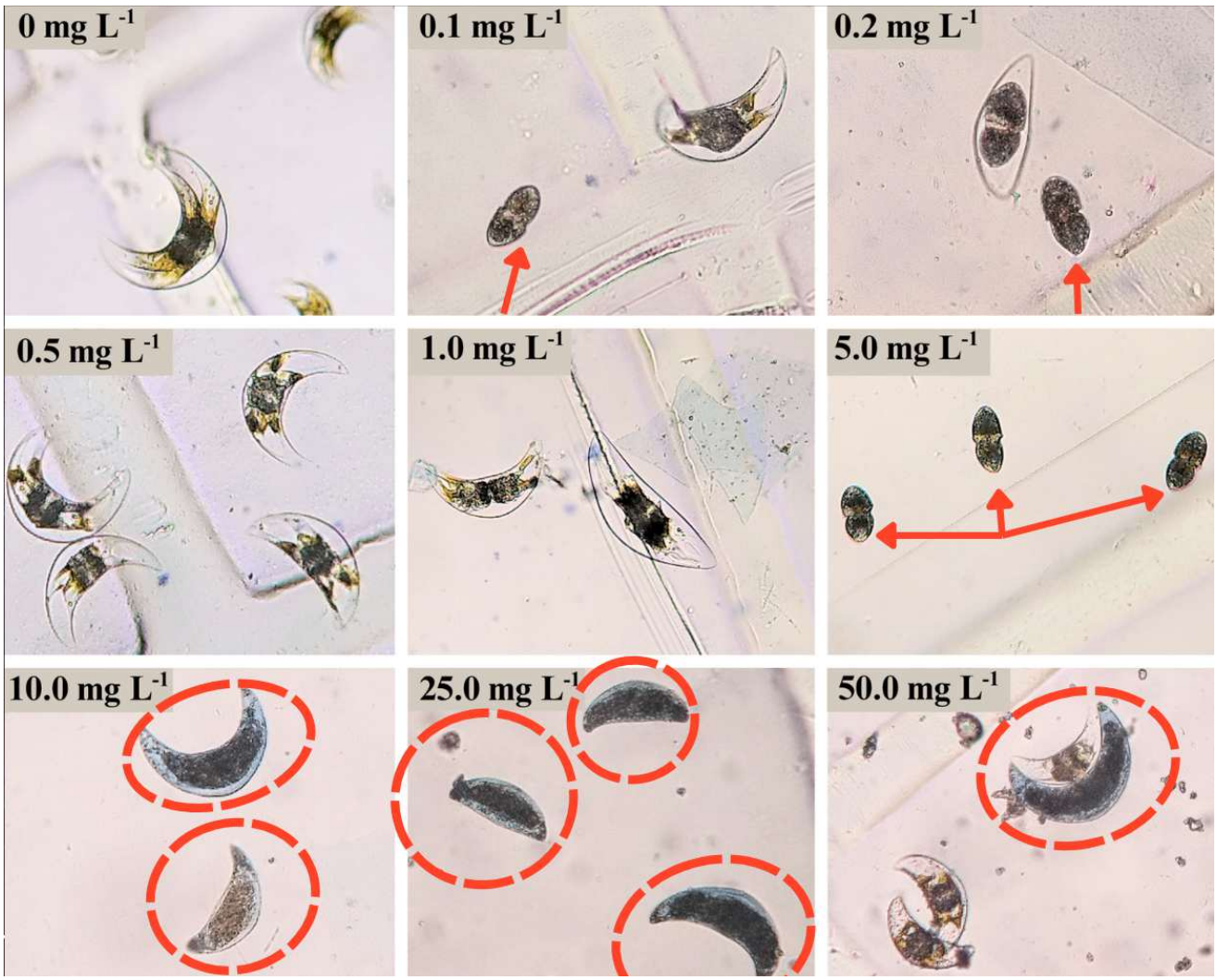



**Figure 8.** Example of cell count using Trypan blue dye. Photo by: Andrade, G. F.

## 4. CONCLUSION

This study demonstrates that the anionic surfactants LAS and SDS exert inhibitory effects on the bioluminescence of the marine dinoflagellate *Pyrocystis lunula*. Based on descriptive comparisons within the commonly evaluated concentration ranges, SDS elicited a more pronounced toxicological response. Combined exposures resulted in adverse effects compared to single treatments, primarily driven by the increased total chemical load inherent to the additive experimental design. Bioluminescence inhibition proved to be a more sensitive endpoint than cell density for detecting early surfactant-induced stress. This sensitivity suggests the occurrence of sublethal membrane and metabolic disruptions prior to widespread cell death, a hypothesis supported by our qualitative morphological observations. Overall, these findings support the application of *P. lunula* bioluminescence as a rapid, cost-effective, and ecologically relevant bioindicator for assessing surfactant contamination in marine environments. The results highlight the importance of incorporating sensitive, early-warning bioassays into monitoring programs to better evaluate the ecological risks associated with surfactant-derived pollutants in coastal waters.

## 5. CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Gabriella Ferreira de Andrade: Writing – original draft, Validation, Data curation, Methodology, Investigation, Project administration, Formal analysis, Software, Visualization, Conceptualization. Anderson Garbuglio de Oliveira: Writing – review & editing, Visualization, Validation, Methodology, Supervision, Investigation, Formal analysis, Conceptualization. Elisabete de Santis Braga: Writing – review & editing, Resources, Validation, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

## 6. 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.

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## 8. REFERENCES

ABD EL-GAWAD, H. S. Aquatic environmental monitoring and removal efficiency of detergents. *Water Science*, 2014, vol. 28, no 1, p. 51-64.

ABNT – ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. NBR 16181: Ecotoxicologia aquática — Toxicidade crônica — Método de ensaio com microalgas marinhas. Rio de Janeiro, 2013. 27 p.

ABRAHAMS, Mark V.; TOWNSEND, Linda D. Bioluminescence in dinoflagellates: A test of the burgular alarm hypothesis. *Ecology*, 1993, vol. 74, no 1, p. 258-260.

AIDAR, E.; SIGAUD-KUTNER, T. C.; NISHIHARA, L.; SCHINKE, K. P.; BRAGA, M. C. C.; FARAH, R. E.; KUTNER, M. B. Marine phytoplankton assays: effects of detergents. *Marine Environmental Research*, *43*(1-2), 55-68, 1997.

BADMUS, S. O., AMUSA, H. K., OYEHAN, T. A., & SALEH, T. A. Environmental risks and toxicity of surfactants: overview of analysis, assessment, and remediation techniques. *Environmental Science and Pollution Research*, *28*(44), 62085-62104, 2021.

425 BAKER, A.; ROBBINS, I.; MOLINE, M. A.; IGLESIAS-RODRÍGUEZ, M. D. Oligonucleotide
426 primers for the detection of bioluminescent dinoflagellates reveal novel luciferase sequences and
427 information on the molecular evolution of this gene. *Journal of Phycology*, v. 44, n. 2, p. 419–428,
428 2008.

429 BRAGA, E. S. Bioquímica marinha e efeitos da poluição nos processos bioquímicos. São Paulo:
430 FUNDESPA, 2000. 108 p.

431 BRASIL. Conselho Nacional do Meio Ambiente (CONAMA). Resolução nº 357, de 17 de março de
432 2005. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu
433 enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes. Diário
434 Oficial da União, Brasília, DF, 18 mar. 2005.

435 CEDERGREEN, N. Quantifying synergy: a systematic review of mixture toxicity studies within
436 environmental toxicology. *PloS one*, *9*(5), e96580, 2014.

437 CHEN, A. K., LATZ, M. I., SOBOLEWSKI, P., & FRANGOS, J. A. Evidence for the role of G-
438 proteins in flow stimulation of dinoflagellate bioluminescence. *American Journal of Physiology-*
439 *Regulatory, Integrative and Comparative Physiology*, 2007, vol. 292, no 5, p. R2020-R2027.

440 CHOQUE-QUISPE, D., MASCO-ARRIOLA, M. L., RAMOS-PACHECO, B. S., LIGARDA-
441 SAMANEZ, C. A., SOLANO-REYNOSO, A. M., CHOQUE-QUISPE, Y., & ALONZO-LANADO,
442 J. F. Study of the pollution by surfactants in a river of a high Andean micro basin. *Dyna*, 2021, *88*(217),
443 9-12.

444 COSTA, P. D.; ANDRADE, N. J.; PASSOS, F. J. V.; BRANDÃO, S. C. C.; RODRIGUES, C. G. F.
445 ATP-bioluminescence as a technique to evaluate the microbiological quality of water in food industry.
446 *Brazilian Archives of Biology and Technology*, v. 47, p. 399–405, 2004.

447 CRAIG, J. M.; KLERKS, P. L.; HEIMANN, K.; WAITS, J. L. Effects of salinity, pH and temperature
448 on the re-establishment of bioluminescence and copper or SDS toxicity in the marine dinoflagellate
449 *Pyrocystis lunula*. *Environmental Pollution*, v. 125, n. 2, p. 267–275, 2003.

450 CRUZ DE CARVALHO, R.; FEIJÃO, E.; MATOS, A. R.; CABRITA, M. T.; UTKIN, A. B.;
451 NOVAIS, S. C.; DUARTE, B. Ecotoxicological effects of the anionic surfactant Sodium Dodecyl
452 Sulfate (SDS) in two marine primary producers: *Phaeodactylum tricornutum* and *Ulva lactuca*. *Toxics*,
453 v. 10, n. 12, p. 780, 2022.

454 CSERHÁTI, T., FORGÁCS, E., & OROS, G. Biological activity and environmental impact of anionic
455 surfactants. *Environment international*, 2002, *28*(5), 337-348.

456 CUSICK, K. D.; WIDDER, E. A. Intensity differences in bioluminescent dinoflagellates impact
457 foraging efficiency in a nocturnal predator. *Bulletin of Marine Science*, 2014, vol. 90, no 3, p. 797-811.

458 DA SILVA COELHO, K.; ROCHA, O. Assessment of the potential toxicity of a linear alkylbenzene
459 sulfonate (LAS) to freshwater animal life by means of cladoceran bioassays. *Ecotoxicology*, *19*(4),
460 812-818, 2010.

461 DI BARI, D. The role of dinoflagellate bioluminescence in deterring copepod grazing. *Revista Ciencias*
462 *Marinas y Costeras*, 2025, p. 39-54.

463 EBENEZER, V., KI, J. Physiological and biochemical responses of the marine dinoflagellate
464 Prorocentrum minimum exposed to the oxidizing biocide chlorine. *Ecotoxicology and environmental*
465 *safety*, 2013, vol. 92, p. 129-134.

466 EGANHOUSE, R. P., BLUMFIELD, D. L., & KAPLAN, I. R. Long-chain alkylbenzenes as molecular
467 tracers of domestic wastes in the marine environment. *Environmental science & technology*, *17*(9),
468 523-530, 1983.

469 FREELING, F., ALYGIZAKIS, N. A., VON DER OHE, P. C., SLOBODNIK, J., OSWALD, P.,
470 AALIZADEH, R., & SCHEURER, M. Occurrence and potential environmental risk of surfactants and
471 their transformation products discharged by wastewater treatment plants. *Science of the total*
472 *environment*, 2019, vol. 681, p. 475-487.

473 GIROTTI, S.; FERRI, E. N.; FUMO, M. G.; MAIOLINI, E. Monitoring of environmental pollutants
474 by bioluminescent bacteria. *Analytica Chimica Acta*, v. 608, n. 1, p. 2–29, 2008.

475 GMI RESEARCH. Global Surfactants Market Report. 2023. Disponível em:
476 https://www.gmiresearch.com/report/global-surfactants-market/. Acesso em: 13 set. 2024.

477 GÓMEZ, F. A quantitative review of the lifestyle, habitat and trophic diversity of dinoflagellates
478 (Dinoflagellata, Alveolata). *Systematics and Biodiversity*, *10*(3), 267-275, 2012.

479 GUILLARD, R. R. L. Culture of phytoplankton for feeding marine invertebrates. In: SMITH, W. L.;
480 CHANLEY, M. H. (eds.). *Culture of Marine Invertebrate Animals*. New York: Plenum Press, 1975. p.
481 29–60.

HASTINGS, J. W.; DUNLAP, J. C. Cell-free components in dinoflagellate bioluminescence. In: *Methods in Enzymology*. New York: Academic Press, 1986. p. 307–327.

HEIMANN, K.; KLERKS, P. L.; HASENSTEIN, K. H. Involvement of actin and microtubules in regulation of bioluminescence and chloroplast translocation in *Pyrocystis lunula*. *Botanica Marina*, v. 52, p. 170–177, 2009.

HEIMANN, K.; MATUSZEWSKI, J. M.; KLERKS, P. L. Effects of metals and organic contaminants on the recovery of bioluminescence in *Pyrocystis lunula* (Dinophyceae). *Journal of Phycology*, v. 38, n. 3, p. 482–492, 2002.

HERA Project, Human and Environmental Risk Assessment on Ingredients of Household Cleaning Products. *LAS, Linear Alkylbenezene Sulphonate (CAS No. 68411-30-3)*. 2013. Available online: https://www.heraproject.com/wp-content/uploads/2025/08/HERA-LAS-revised-April-2013-Final1.pdf (accessed on 4 October 2024).

HILDENBRAND, Z. L.; OSORIO, A.; CARLTON JR, D. D.; FONTENOT, B. E.; WALTON, J. L.; HUNT, L. R.; SCHUG, K. A. Rapid analysis of eukaryotic bioluminescence to assess potential groundwater contamination events. *Journal of Chemistry*, 2015(1), 957608, 2015.

JACKSON, S. A.; BORCHERT, E.; O'GARA, F.; DOBSON, A. D. Metagenomics for the discovery of novel biosurfactants of environmental interest from marine ecosystems. *Current Opinion in Biotechnology*, v. 33, p. 176–182, 2015.

KAMERLIN, N., DELCEY, M. G., LINDH, R. Unravelling the mechanism of pH-regulation in dinoflagellate luciferase. *International journal of biological macromolecules*, 2020, vol. 164, p. 2671-2680.

KOCAL, J. A., VORA, B. V., & IMAI, T. Production of linear alkylbenzenes. *Applied Catalysis A: General*, *221*(1-2), 295-301, 2001.

KORTENKAMP, A., BACKHAUS, T., & FAUST, M. State of the Art Report on Mixture Toxicity—Final Report, Executive Summary. *University of London School of Pharmacy, London, UK*, 2009.

LAPOINTE, M., MORSE, D. Reassessing the role of a 3′-UTR-binding translational inhibitor in regulation of circadian bioluminescence rhythm in the dinoflagellate Gonyaulax. *Biological chemistry*, 2008, vol. 389, no 1, p. 13.

LAPOTA, D.; MOSKOWITZ, G. J.; ROSENBERGER, D. E.; GROVHOUG, J. G. The use of stimulable bioluminescence from marine dinoflagellates as a means of detecting toxicity in the marine environment. In: *Environmental Toxicology and Risk Assessment*. Philadelphia: ASTM, 1993. p. 3–18.

LAPOTA, D.; OSORIO, A. R.; LIAO, C.; BJORNDAL, B. The use of bioluminescent dinoflagellates as an environmental risk assessment tool. *Marine Pollution Bulletin*, *54*(12), 1857-1867, 2007.

LATZ, M. I., BOVARD, M., VANDELINDER, V., SEGRE, E., ROHR, J., & GROISMAN, A. Bioluminescent response of individual dinoflagellate cells to hydrodynamic stress measured with millisecond resolution in a microfluidic device. *Journal of Experimental Biology*, 2008, vol. 211, no 17, p. 2865-2875.

LEWIS, M. A. Chronic toxicities of surfactants and detergent builders to algae: a review and risk assessment. *Ecotoxicology and environmental safety*, *20*(2), 123-140, 1990.

LINDSTRÖM, J., GREBNER, W., RIGBY, K., & SELANDER, E. Effects of predator lipids on dinoflagellate defence mechanisms-increased bioluminescence capacity. *Scientific Reports*, 2017, vol. 7, no 1, p. 13104.

MESINGER, A. F.; CASE, J. F. Dinoflagellate luminescence increases susceptibility of zooplankton to teleost predation. *Marine Biology*, 1992, vol. 112, no 2, p. 207-210.

MIRJANI, M.; SOLEIMANI, M.; SALARI, V. Toxicity assessment of total petroleum hydrocarbons in aquatic environments using the bioluminescent bacterium *Aliivibrio fischeri*. *Ecotoxicology and Environmental Safety*, v. 207, p. 111554, 2021.

OKAMOTO, O. K., SHAO, L., HASTINGS, J. W., & COLEPICOLO, P. Acute and chronic effects of toxic metals on viability, encystment and bioluminescence in the dinoflagellate Gonyaulax polyedra. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, *123*(1), 75-83, 1999.

OKBAH, M. A.; IBRAHIM, A. M. A.; GAMAL, M. N. M. Environmental monitoring of linear alkylbenzene sulfonates and physicochemical characteristics of seawater in El-Mex Bay (Alexandria, Egypt). *Environmental monitoring and assessment*, 2013, vol. 185, no 4, p. 3103-3115.

OLKOWSKA, E., RUMAN, M., & POLKOWSKA, Ż. Occurrence of surface active agents in the environment. *Journal of analytical methods in chemistry*, *2014*(1), 769708.

PERIN, L. S., MORAES, G. V., GALEAZZO, G. A., & OLIVEIRA, A. G. (2022). Bioluminescent dinoflagellates as a bioassay for toxicity assessment. *International Journal of Molecular Sciences*, 23(21), 13012.

ROSETY, M.; ORDONEZ, F. J.; RODRÍGUEZ, M. R.; ROSETY, J. M.; CARRASCO, C.; RIBELLES, A. Acute toxicity of anionic surfactants sodium dodecyl sulphate (SDS) and linear alkylbenzene sulphonate (LAS) on the fertilizing capability of gilthead (Sparus aurata L.) sperm. *Histology and histopathology*, *16*(3), 839-843, 2001.

SINGH, N.; HU, X. H.; KUMAR, V.; SOLANKI, M. K.; KAUSHIK, A.; SINGH, V. K.; KUMAR, A. Microbially derived surfactants: an ecofriendly, innovative, and effective approach for managing environmental contaminants. *Frontiers in Bioengineering and Biotechnology*, *12*, 1398210, 2024.

SHIMOMURA, O. Bioluminescence: chemical principles and methods. Singapore: World Scientific, 2006. 470 p.

SHIODE, S., MCDONOUGH, K., BELANGER, S. E., & CARR, G. J. Probabilistic environmental risk assessment for linear alkyl benzene sulfonate (LAS) in Japan reduces assessment uncertainty. *Journal of Water and Environment Technology*, 2020, vol. 18, no 2, p. 80-94.

SOLOMONS, T. W. G.; FRYHLE, C. B.; SNYDER, S. A. Organic Chemistry (12th ed.). Wiley, 2016.

STAUBER, J. L.; BINET, M. T.; BAO, V. W.; BOGE, J.; ZHANG, A. Q.; LEUNG, K. M.; ADAMS, M. S. Comparison of the Qwiklite™ algal bioluminescence test with marine algal growth rate inhibition bioassays. *Environmental Toxicology: An International Journal*, *23*(5), 617-625, 2008.

STIRES, J. C.; LATZ, M. I. Contribution of the cytoskeleton to mechanosensitivity reported by dinoflagellate bioluminescence. *Cytoskeleton*, 2018, vol. 75, no 1, p. 12-21.

SWEENEY, B. M. Interaction of circadian and cell cycles in *Pyrocystis fusiformis*. *Plant Physiology*, v. 70, n. 1, p. 272–276, 1982.

TAYLOR, Frank John Rupert. The biology of Dinoflagellates (Botanical Monographs, Volume 21). Oxford: Blackwell Scientific Publications, 1987.

THE BRAINLY INSIGHTS. Surfactants Market Report. 2023. Disponível em: https://www.thebrainyinsights.com/report/surfactants-market-13030. Acesso em: 13 set. 2024.

USEPA – UNITED STATES ENVIRONMENTAL PROTECTION AGENCY. National Secondary Drinking Water Regulations: Guidance for Nuisance Chemicals. Code of Federal Regulations, Title

40, Part 143. Washington, D.C.: U.S. Government Printing Office, 2014. Disponível em: https://www.epa.gov/sdwa/secondary-drinking-water-standards-guidance-nuisance-chemicals.

VALIADI, M.; IGLESIAS-RODRIGUEZ, D. Understanding bioluminescence in dinoflagellates—how far have we come?. *Microorganisms*, v. 1, n. 1, p. 3–25, 2013.

VINUTHA, H. P., POORNIMA, B., & SAGAR, B. M. Detection of outliers using interquartile range technique from intrusion dataset. In *Information and decision sciences: Proceedings of the 6th international conference on ficta* (pp. 511-518). Singapore: Springer Singapore, 2018.

WARNE, M. S. J.; SCHIFKO, A. D. Toxicity of laundry detergent components to a freshwater cladoceran and their contribution to detergent toxicity. *Ecotoxicology and environmental safety*, *44*(2), 196-206, 1999.

WU, Z., LOU, F., TANG, Y., DONG, H., & QIANG, Z. Accelerated transformation of sodium dodecylbenzene sulfonate surfactant in the UV/chlorine process: Kinetics and formation of chlorinated disinfection by-products. *Chemosphere*, *310*, 136820, 2023.

YING, G. G. Fate, behavior and effects of surfactants and their degradation products in the environment. *Environment international*, 2006, *32*(3), 417-431.