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Lethal effect of lightstick contents on gray shrimps *Litopenaeus vannamei*

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Lightsticks (LSs) are used as bait in surface longline fishing to capture swordfish. These sticks emit light as a result of a chemiluminescent reaction between two compounds originally separated by a glass ampoule; when the stick is bent, the glass ampoule breaks, mixing a trichlorosalicylate derivative with hydrogen peroxide. Light is emitted for about 48 hours, with fluorescent polycyclic aromatic hydrocarbons catalyzing the reaction in di-n-butyl phthalate, a highly viscous solvent (Stevani and Baader, 1999). After use in longline fishing by fishing vessels, LSs are discharged into the ocean, causing marine pollution with solid garbage and chemical compounds that eventually leak into the marine environment (Cesar-Ribeiro *et al*., 2017). LSs are used by commercial longline fishing vessels and are attached to each hook to attract fish, thus increasing bait efficiency. It has proven to be a successful method for commercial capture on an industrialized scale, but it contributes to bycatch, plastic litter and overfishing (Hazin *et al*., 2005; Thompson, 2013; Mills *et al*., 2014; Solomon and Ahmed, 2016; Detloff and Istel, 2016, Nguyen *et al*., 2017; Nguyen and Winger, 2019). No governmental regulations can be found that ban LSs (Nguyen and Winger, 2019); however, light fishing has been completely prohibited in the coastal waters of Ghana (Solomon and Ahmed, 2016). After wrongly being discarded in the ocean, some sticks rupture, dispersing their contents into the water or on shore (Cesar-Ribeiro and Palanch-Hans, 2010). Reports indicate that fishing communities collect LSs and use their contents as suntan lotion or to treat diseases such as rheumatism, vitiligo, and mycoses (Cesar-Ribeiro and Palanch-Hans, 2010, De Oliveira *et al.,* 2014; Araújo *et al*.,2015; Cesar-Ribeiro *et al*. 2017).

Samples collected from LSs discharged on the beaches of Costa dos Coqueiros, Bahia, Brazil, by the NGOs Global Gargabe and Capitães da Areia were analyzed by De Oliveria *et al*. (2014) in HPLC-UV-ESI-MS/MS, who identified the internal solution - hydrogen peroxide; rubrene (5, 6,11,12-tetraphenylnaphthacene); di-n-butyl phthalate ($[M+H]^+$ *m/z* 279; fragments *m/z* 149 as base peak, *m/z* 121, *m/z* 93, and *m/z* 59), bis (2,4,6-trichlorophenyl) oxalate (TCPO) ($[M+H]^+$ *m/z* 223/225/227/229) and 9,10- diphenylanthracene (DPA) ($[M+H]^+$ *m/z* 330; fragments m/z 252 as base peak and *m/z* 313) - and the external solution - dimethyl phthalate ($[M+H]^+$ *m/z* 195; fragments: *m/z* 163 as base peak, *m/z* 133, *m/z* 135, and *m/z* 105); and sodium salicylate.

There have been few studies regarding the effects of the chemical contents of LSs on marine life. Once these sticks are opened in the ocean, their chemical contents may cause deleterious effects on marine life. Table 1 compares the toxicity of the contents of LSs to different marine species. Pinho *et al.* (2009) found the LC50-24h for *Artemia sp*. nauplii to be 0.063 ml L^{-1}, while Cesar-Ribeiro and Palanch-Hans (2010) considered sticks containing liquid collected on the beaches to be toxic because of deleterious effects on embryo and larval development of the sea urchins *Echinometra lucunter* (EC50 effective concentration of the contaminant that causes an alteration in 50%

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Table 1. Comparison of lightstick toxicity evaluated in different marine invertebrates, methodologies, and fractions (acute and chronic toxicity).

Organism	Effect	Expression	Results	References	Fraction
Acute toxicity					
Artemia sp.	Mortality	24 h; LC50	0.22% (0.16-0.32)	Cesar-Ribeiro *et al*. (2017)	supernatant
		48h; LC50	0.10%		supernatant
		24 h; LC50	0.063 ml/L	Pinho *et al*. (2009)	lightstick
L. vannamei	**Mortality**	**24 h; LC50**	**0.0297%**	**This study**	**lightstick + ethanol**
Chronic toxicity					
Artemia sp.	hatchability	48h; LOEC	0.2 ml/L		lightstick
L. variegatus	fertilization	~40 min; EC50	0.011% (0.009-0.013)		supernatant
	embryo development	24 h; EC50	0.032% (0.026-0.038)	Cesar-Ribeiro *et al*. (2017)	supernatant
		24 h; EC50	0.00062%		lightstick + ethanol
		24 h; EC50	0.011%	Cesar-Ribeiro and Palanch-Hans (2010)	supernatant
E. lucunter		36 h; EC50	0.062%		supernatant
Crassostrea rhizophorae	embryo development	24 h; EC50	0.35%	Araújo *et al*. (2015)	lightstick

of exposed individuals; EC50-36h = 0.062%) and *Lytechinus variegatus* (EC50-24h = 0.0285%). Araújo *et al.* (2015) found an EC50-24h of 0.35% for embryo development of *Crassostrea rhizophorae*, and Cesar-Ribeiro *et al.* (2017) found an LC50-24h of 0.22% (0.16–0.32) and an LC50-48h of 0.10% for *Artemia* sp. Assays of fertilization and embryo and larval development for *L. variegatus* found EC50-40 min and EC50-24 h to be 0.011% (0.009–0.013) and 0.00062%, respectively.

The present study aimed to evaluate the toxic effects of the solution contained in orange light LSs collected on the beaches of Costa dos Coqueiros, state of Bahia, Brazil, on the mortality of juvenile gray shrimp, *Litopennaeus vannamei* (Boone, 1931). The gray shrimp is native to the eastern Pacific and was brought to Brazil in 1981 for commercial purposes. It currently represents 95% of marine shrimp production in Brazil, being cultivated in several states (Cassola *et al.,* 2004).

Costa dos Coqueiros (15º54'S, 38º20'W to 11º34'S, 37º47'W) comprises seven municipalities located north of Salvador, Bahia, Brazil. It comprises 200 kilometers of coastline, is one of the most sought-after tourist areas in Bahia and is home to one of the main spawning pockets of sea turtles in Brazil (Marcovaldi and Marcovaldi, 1999). With the support of the German NGO Global Garbage, a scientific hike was undertaken from 14 to 31 July 2007 along almost 200 km of the beaches of Costa dos Coqueiros (Figure 1 – upper panel). Among the 2,554 LSs collected by Cesar-Ribeiro and Palanch-Hans (2010), 34% were opened and 63% were still closed.

The orange (closed) LSs were taken to the laboratory, where they were opened and used to prepare a stock solution for toxicity tests (Cesar-Ribeiro and Palanch-Hans 2010; Cesar-Ribeiro *et al.*, 2017). The immiscible compounds were extracted through dissolution of 0.1 mL of stock solution in 100 mL of filtered seawater (salinity 35) with ethanol 0.5% (v/v) as a solvent [Stock Solution Ethanol, SSE 0.1%].

The effects of the chemical contents of the LSs on the mortality of marine crustaceans were evaluated using the exotic marine shrimp *L. vannamei.* The shrimp were brought from farms in Natal, state of Rio Grande do Norte, Brazil, and kept in tanks with filtered seawater (salinity 35, pH 8.1) under constant aeration and temperature (25 ± 2 ºC) until their use in the experiments. The average weight of the shrimp was 1.85 ± 0. 25 g, taken after the experiments to minimize handling stress.

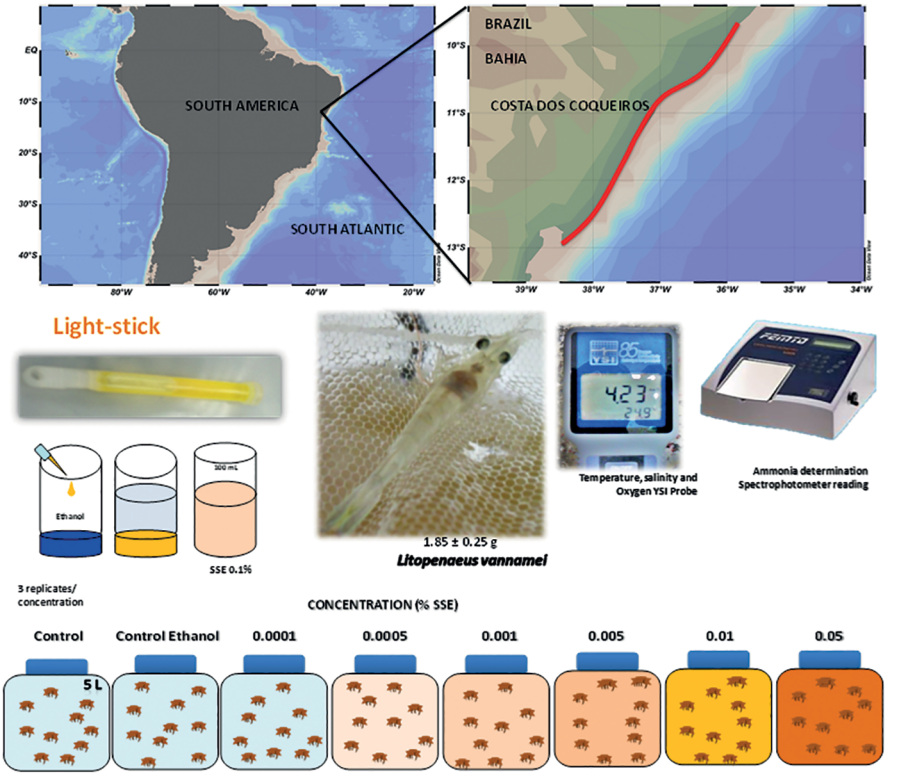

Figure 1. Map of South America where the NGO Global Garbage realized the 1st Scientific Hike from 14 to July 31, 2007, along almost 200 km of the Costa dos Coqueiros beaches – Bahia, Brazil (red line) collecting lightsticks (Ocean Data View ODV) - upper panel. Experimental graphical abstract from the LS extraction with Ethanol, mortality experiment with juvenile gray shrimp *L. vannamei* and parameters evaluated - lower panel.

Figure 1 (lower panel) is a graphical abstract representation of the acute ecotoxicology test conducted using different concentrations of SSE (0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05%) and controls (control and ethanol control 0.5% v/v) to determine LC50 (lethal concentration of the contaminant that causes mortality to 50% of the exposed population) after 24 h of exposure, with four replicates (5 L aquarium with ten individual) per treatment. The physical and chemical parameters of temperature, salinity, and oxygen were monitored with a YSI probe, pH with a digital pH meter, and ammonia with by the colorimetric method, according to Koroleff (1983). During testing, the animals were maintained under 25 ± 2 °C, with a salinity of 35, pH of 8.2, and a 12 h photoperiod (12 h light: 12 h dark). The number of surviving individuals was counted at the end of the test. All procedures followed the recommendations of the United States Environmental Protection Agency (EPA, 1985).

Data from the experiments were checked for normality and homoscedasticity and then submitted to an analysis of variance (ANOVA) followed by Tukey's test ($p<0.05$). The Kruskal-Wallis test was applied in BIOSTAT 5.0 software. The LC50 was assessed using the MLA "Quest Graph™ LC50 Calculator (2020) and APAAAT Bioquest, Inc. (2020).

The LC50-24 h for the gray shrimp was 0.0297% for lightsticks (Figure 2), with the regression shown as equation (1).

$$Y = 22.0442 + \frac{1206.5861 - 22.0442}{1 + \left(\frac{X}{0.0297}\right)^{-2.4424}} \quad (1)$$

Regression equation for determining LC50-24 h for the gray shrimp *L. vannamei*

The results confirmed the toxicity of the LS contents and that it may cause adverse effects to marine life with a LC50-24h of 0.0297% for *L. vannamei*. Once

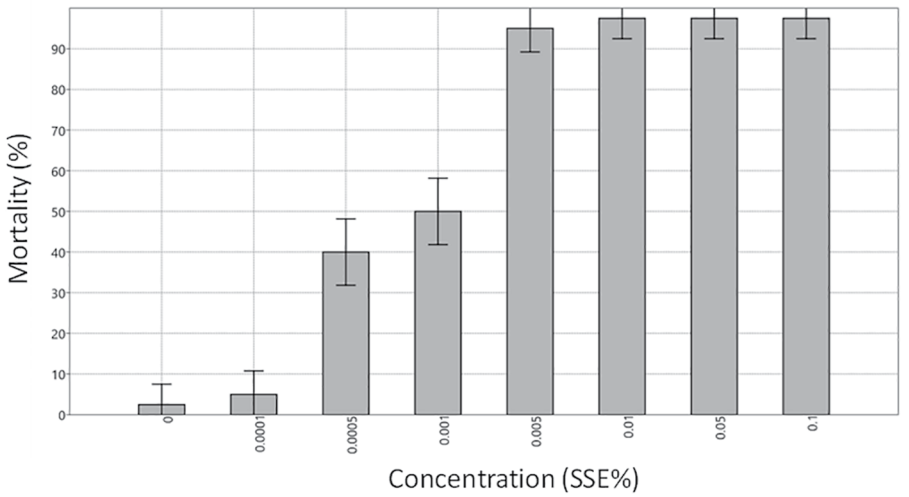

Figure 2. Lethal effect (% of mortality mean ± standard deviation) of chemical contents in lightsticks (SSE – Stock Solution Ethanol % of orange color lightsticks collected on Costa dos Coqueiros, Brazil) in gray shrimp *L. vannamei*.

in the environment, the concentrations of the contaminants from the LSs can decrease rapidly due to dispersion, marine currents, and wave action towards the coast. Upon reaching the coast, multiple factors can determine the persistence of the contaminants, such as properties of the contaminants themselves, sediment porosity, organisms, and wave activity (Bícego *et al.,* 2008).

The contents of the LSs includes ester-oxalates, 9,10 diphenyllanthracene, perylene, rubrene, di-n-butyl phthalate, dimethyl phthalate (DMP), diisobutyl phthalate (DIBP), butyl benzoate, butyl 2-ethylhexyl phthalate, diethyl phthalate (DEP), monomethyl phthalate, phthalic anhydride, 1-pentanol; tert-butyl isopropyl ether, n-butyl acetate, butyl butanoate, methyl benzoate, butyl methyl phthalate, benzenesulfonic acid 4-methyl butyl ester, t-butyl hydrogen phthalate, butyl cyclohexyl phthalate, mono-2-ethylhexyl phthalate and trichlorosalicylic acid (Araújo *et al.,* 2015). Each of these compounds has been shown to have high toxicity for marine crustaceans, mysids, branchiopod crustaceans, amphipods, harpacticoid copepods, and different benthic organisms (Mayer and Sanders, 1973; Linden *et al.,* 1979; Kolosnjaj *et al*., 2007).

The significant toxicity of the LS contents derives not only from 2- ethyl nitrophenyl acetate and hydrogen peroxide but also dimethyl phthalate (DMP) as a solvent. The toxicity is also affected by sodium thiosulfate and aeration, indicating that oxidizable compounds, such as hydrogen peroxide, are removed by sodium thiosulfate and volatiles, such as polycyclic aromatic hydrocarbons (PAHs), by aeration and that they may be responsible for the toxic effects (Cesar-Ribeiro *et al*., 2017). The chronic effect of hydrogen peroxide has already been evaluated for cladocerans (Meinertz *et al.,* 2008), with demonstrated xenobiotic activity.

The group of chemicals associated with LS toxicity is composed of PAHs, mainly rubrene (De Oliveira *et al*., 2014; Cesar-Ribeiro *et al*., 2017). PAHs comprise two or more benzene rings ordered in a linear, angular, or grouped manner, and are hydrophobic and quite resistant to microbiological biodegradation, giving them considerable persistence in the environment (Bidleman, 1988). Lee and Nicol (1978) suggest that once incorporated into cell membranes, PAHs can cause physical destabilization and changes to enzymatic processes and membrane transport. Sublethal concentrations of hydrocarbons may cause physiological disturbances and developmental alterations, resulting in premature death (Clark, 1986).

Among the substances identified by Araujó *et al.* (2015) are three of the 126 priority pollutants listed by the USEPA, namely DBP, DEP, DMP (USEPA, 2010), which are highly toxic. The first is very persistent, bioaccumulative, carcinogenic, and toxic (Burke, 2009), and both DBP and DMP have been classified as genotoxic to human cells. The toxic properties of DBP deserve more attention, considering its persistence

in the environment and its high potential for bioaccumulation in different organisms (Fardy and Yang, 2008). The presence of salicylic acid derivatives can also have a dangerous effect on the marine environment; this compound can be converted into an organochloride and chloride salicylic acid can act as a mutagen (Bagattini *et al.*, 2006).

In general, LSs have deleterious effects on several marine organisms (crustaceans, sea-urchins, bivalves) when they are opened. However, they also affect the human population that applies them as liquid-like medicines, mainly due to the reported carcinogenic effect of the internal solution, as shown with Winstar rats (Ivar do Sul *et al*., 2007), and results of cytotoxicity tests (Bagattini *et al.,* 2006). Among other kinds of ships, fishing vessels comply with the important International Convention for the Prevention of Pollution from Ships (MARPOL, 1978) Problems associated with marine litter have been reported globally and the garbage has broad effects on marine fauna. Two-hundred and sixty-seven species of marine animals from all over the world suffer from the presence of solid waste in oceans and on beaches, including 86% of all species of marine turtles, 44% of marine birds, 43% of marine mammals and many fish and crustacean species (Mascarenhas *et al*., 2004). Sea turtles can be killed by pelagic longlines equipped with LSs, which has been identified as a significant cause of mortality for sea turtle populations (Gless *et al*., 2008). International regulations established in the 1970s have prohibited garbage disposal at sea, but this is hard to control (Detloff and Istel, 2016). Light fishing has been completely banned from the coastal waters of Ghana (Solomon and Ahmed, 2016), although no government regulations can be found regarding use of LSs. Thus, international-scale regulations need to be discussed and adopted (Thompson, 2013; Mills *et al*., 2014; Solomon and Ahmed, 2016; Detloff and Istel, 2016, Nguyen *et al*., 2017; Nguyen and Winger, 2019).

The use of low-powered LED lights reduces the bycatch of small and juvenile fish and reduces the bycatch of turtles in South America (Melli *et al*., 2018; Nguyen and Winger, 2019). Ortiz *et al*. (2016) registered significant reductions in sea turtle bycatch, being by 60% in Mexico, 63.9% in Peru, and 85.7% in Ecuador, without affecting the catch rate of target species.

Environmental education projects directed at coastal human populations have been suggested as a way to prevent public health problems. Marine debris discharge must be monitored to avoid the release of toxic waste from ships and continental discharges in oceans, which have deleterious effects on marine life. Emphasis must be made on the importance of cleaning beaches and educating the public on the dangers of using the contents of discarded LSs. Thus, new mechanisms need to be created to inspect fishing vessels and prevent the improper disposal of lightsticks at sea and in common garbage. The actions suggested above are part of the continued fight to preserve the environment and ensure the survival and maintenance of diversity of coastal zone ecosystems of the Brazilian state of Bahia and the oceans of the world. Even at low concentrations, LSs contain some potentially toxic compounds (hydrogen peroxide, PAHs, phthalates, and others), that have lethal effects on decapods and marine life, as well as the fishing population.

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