Can Encapsulation of the Biocide DCOIT Affect the Anti-Fouling Efficacy and Toxicity on Tropical Bivalves?

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Featured Application: The present study can foster nanoecotoxicology research on tropical environments and can be also applied on the maritime industry.

Abstract: The encapsulation of the biocide DCOIT in mesoporous silica nanocapsules (SiNC) has been applied to reduce the leaching rate and the associated environmental impacts of coatings containing this biocide. This research aimed to evaluate the effects of DCOIT in both free and nanostructured forms (DCOIT vs. SiNC-DCOIT, respectively) and the unloaded SiNC on different life stages of the bivalve Perna perna: (a) gametes (fertilization success), (b) embryos (larval development), and (c) juveniles mussels (byssus threads production and air survival after 72 h of aqueous exposure). The effects on fertilization success showed high toxicity of DCOIT (40 min-EC50 = 0.063 µg L⁻¹), followed by SiNC-DCOIT (8.6 µg L⁻¹) and SiNC (161 µg L⁻¹). The estimated 48 h-EC50 of SiNC, DCOIT and SiNC-DCOIT on larval development were 39.8, 12.4 and 6.8 µg L⁻¹, respectively. The estimated 72 h-EC50 for byssus thread production were 96.1 and 305.5 µg L⁻¹, for free DCOIT and SiNC-DCOIT, respectively. Air survival was significantly reduced only for mussels exposed to free DCOIT. Compared to its free form, SiNC-DCOIT presented a balanced alternative between efficacy and toxicity, inhibiting efficiently the development of the target stage (larvae that is prone to settle) and satisfactorily preventing the juvenile attachment.

Keywords: Perna perna; biofouling; nanotechnology; toxicity

1. Introduction

Long-term submerged structures are susceptible to aggregate fouling organisms, such as algae, barnacles, mussels, and other benthic organisms, known as biofouling [1]. In the shipping industry, this phenomenon causes extensive economic losses, resulting in a significant decrease in ships’ durability and operational efficiency, interfering with vessels’ navigability. The friction caused by the increase in the hull’s roughness exponentially increases maritime transport costs, as it demands...
greater engine power and fuel consumption [2]. Moreover, this increase in fuel consumption leads to an increase in greenhouse gas emissions. Another problem related with the biofouling regards the dispersion of invasive species associated with the hull’s vessels or its ballast water [3].

However, the traditional methods used to inhibit the establishment and growth of biofouling have arisen concerns since the 20th century due to the critical environmental impacts, particularly on non-target foulers/biota. The chemical formulation of antifouling (AF) paints contain biocides, which are chemical substances that neutralize, inhibit or exert control over undesirable fouling organisms and/or communities, preventing their settlement and further growth [4]. Despite the desired antifouling action, many of these compounds are toxic to non-target species [5,6], and may cause adverse effects to the non-fouling biota. The global ban on the organotin-based paints in 2008 increased the use of alternative AF booster biocides, such as 4,5-dichloro-2-octyl-2H-isothiazole-3-one (DCOIT). DCOIT is the biocidal ingredient of the AF products Sea Nine 211™ or Kathlon™ 910 SB, among other commercial products, which stands out as one of the most widely used AF agents in maritime topcoats [7]. This organic compound has been considered environmentally safe by USEPA [8], due to its reduced half-life (<1 day in seawater) [9,10]. However, recent studies have indicated that its half-life can be longer than four days in natural seawater [11], at least one week in artificial saltwater [12], and up to 13 days [13], depending on the environmental conditions, such as sunlight, dissolved oxygen or temperature [1]. Consequently, DCOIT has been found in water and sediment from several countries in Asia [14] and Europe [15]. Not surprisingly, studies have demonstrated this biocide’s high toxicity to non-target organisms [12,16]. Recently, DCOIT was classified as “very toxic to aquatic life, with long-lasting effects” by the European Chemicals Agency [17].

Because environmental regulations have become increasingly restrictive on national and international bases, new AF strategies need to attend some aspects such as environmental safety, AF efficacy and coating’s lifetime. In this sense, promising AF alternatives to regular state-of-the-art biocides have gained space, such as the encapsulation and immobilization of the biocides in low toxic nanocontainers [18–22]. Novel AF nanomaterials have been recently developed using silica mesoporous nanocapsules (SiNC) to encapsulate DCOIT [23]. SiNC have an empty core and shell with gradual mesoporosity which confers significant loading capacity and allows prolonged and stimuli-triggered release of the biocide, such as pH or chloride concentration [24], providing a safe environmental loading of the biocide together with specific targeting [23]. When used as coating additive, the biocide’s lifetime is increased and the percentage content of the main active ingredient in the coating is also greatly reduced. This feature has been triggering the core business of the company Smallmatek, Lda, which develops and produces functionalized engineered nanomaterials, including SiNC-DCOIT, to be added in protective coatings for maritime applications.

A recent study comparing the antifouling efficacy and toxicity of DCOIT in both free and encapsulated forms (DCOIT vs. SiNC-DCOIT) indicated that the novel AF nanostructured additive is much less toxic (up to 214-fold) towards non-target species from temperate regions [12]. However, there is no knowledge on the toxicity and efficacy of this AF nanomaterial on species from different climatic regions, a critical requirement on environmental risk assessment at global scale. Furthermore, the effects of SiNC-DCOIT on different life stages of a given species are also unknown so far. The brown mussel *Perna perna* occurs on rocky reefs, forming dense colonies at the low-tidal and intertidal levels in the tropical and subtropical zones. This bivalve species is regarded as a socio-economically relevant species with a very-broad natural distribution along Africa and the Arabic Peninsula, being an invasive species in the Gulf of Mexico and, more recently, in Portugal [25]. This bivalve species is economically important in Brazilian southern and southeastern coastal areas and represents a relevant food resource for coastal populations [26]. Therefore, due to its socio-economic importance, *P. perna* is a non-target fouling organism at adult life stages; however, it can be considered a target organism at the larval stage since it is prone to settle and become part of the undesirable fouling in human-made structures. Accordingly, this research aimed to evaluate and compare the effect of two DCOIT forms (free DCOIT vs. its nanostructured form SiNC-DCOIT) and the unloaded nanocarriers (SiNC) on different life
stages of the bivalve *P. perna*, namely, on gametes (fertilization success), fertilized eggs (embryo-larval development), and juveniles mussels (byssus threads production and air survival capacity).

2. Materials and Methods

2.1. Chemical Compounds

DCOIT (CAS nr. 64359-81-5) was purchased from Sigma-Aldrich (São Paulo, SP, Brazil). Nanomaterials (SiNC; SiNC-DCOIT) were supplied by Smallmatek, Lda. (Aveiro, Portugal). Tested nanomaterials were fully characterized by Figueiredo et al. [12]. Briefly, SiNC has a diameter of 129 nm and SiNC-DCOIT has a diameter of 152 nm and a biocidal content of 18.3% [12]. Stock solutions/dispersions were prepared in natural seawater (salinity 33 ± 2), filtered through a 0.22 µm microporous membrane filter and dispersed in an ultrasonic bath (40 kHz) for 30 min. Diluted dispersions were sonicated for 15 min., immediately before the exposure test.

2.2. Animals

Adult and juvenile *Perna perna* mussels were acquired from a mariculture farm located on the north coast of São Paulo (Brazil). In the laboratory, the animals were placed on 60 L plastic boxes filled with seawater (salinity 33) and maintained under constant aeration, temperature (25 ± 2 °C), and photoperiod of 12 h:12 h (light:dark) for 72 h prior to the experiments [27].

2.3. Fertilization Assay

The fertilization assay was carried out according to the protocol proposed by the United States Environmental Protection Agency [28], adapted for *P. perna* by Zaroni et al. [27]. The exposure concentrations used in the fertilization assay were: 1, 3.33, 10, 33 and 100 µg L⁻¹ of free DCOIT; 24.7, 74.1, 222.2, 666.7 and 2000 µg L⁻¹ for SiNC-DCOIT (expressed as DCOIT content); and 250, 500, 1000, 2000 and 4000 µg L⁻¹ for SiNC. These concentration ranges were chosen based on previous toxicity data acquired for temperate marine species [12].

The mussel’s gametes (eggs and sperm) were obtained by the thermal induction [27]. The experiment was carried out in glass test tubes containing 10 mL of each test solution. Four replicates were prepared for each treatment and experimental control (filtered seawater). In each replicate, 150 µL of sperm solution were transferred to each replicate and incubated (25 ± 2 °C) for 40 min. Next, approximately 2000 eggs were added to the sperm suspensions. Sixty minutes after the eggs are added, the test was terminated by adding 500 µL of formaldehyde (10%) buffered with borax to each replicate. The percent fertilization was determined by microscopic examination of an aliquot from each replicate of the treatments placed on a Sedgwick-Rafter chamber. The first 100 eggs observed were counted and the average percentage of fertilization was determined, considering the 4 replicates used.

2.4. Embryo-Larval Development Assay

The short-term chronic exposure assay using *P. perna* embryos followed the standard method ABNT NBR 16,456 [29]. In this experiment, six exposure concentrations were tested for each chemical: 1, 3, 3.3, 10, 33 and 100 µg L⁻¹ of free DCOIT, 0.064, 0.32, 1.6, 8, 40 and 200 µg (of DCOIT) L⁻¹ in SiNC-DCOIT, and 6.5, 32, 162, 808 and 4040 µg L⁻¹ of SiNC. These concentrations were established based on the findings of a preliminary experiment.

Gametes obtention and fertilization were conducted according to the procedure described in the fertilization assay. Approximately 400–500 fertilized eggs were added to the glass test-tubes containing 10 mL of test solutions. Four replicates per concentration were prepared. The experiment was incubated (25 ± 2 °C) for 48 h with a photoperiod of 12 h:12 h (light:dark). Development confirmation was verified in the experimental control (above 70% of normal larvae, according to Zaroni et al. [27]). The test was then terminated by adding 500 µL of 10% buffered formaldehyde to each replicate. The first
100 organisms observed were counted and identified: and the normal veliger larvae were determined as those presenting symmetrical and closed valves, visible internal content, and a “D shape”. On the other hand, abnormal larvae included those undeveloped, those exhibiting delays and/or morphological abnormalities and the absence of development of eggs.

2.5. Short-Term Exposure Assay with Juveniles

The tested concentrations were 10, 100 and 1000 µg L\(^{-1}\) for SiNC and SiNC-DCOIT (as mg of DCOIT L\(^{-1}\)) and 0.81, 8.1 and 81 µg L\(^{-1}\) for DCOIT (free form), based on the sublethal toxicity of these compounds for adult mussels of the species *Mytilus galloprovincialis* [12]. Negative control (filtered seawater) was also prepared.

Juvenile mussels with approximately 2.5 cm long were selected. Their byssus threads were cut with surgical scissors aiming at to examine their growth during exposure and counting at the end of the experiment. A total of 300 animals were randomly divided into 500 mL glass flasks with 400 mL of test solution (\(n = 6, 5\) organisms per replicate). The animals were exposed for 72 h with constant gentle aeration, at 25 ± 2 °C and photoperiod of 12:12 h (light:dark). During the experimental period, mussels were not fed and the test solutions were not renewed. In order to maintain the quality of the experiment, survival of the organisms were evaluated daily and dead organisms were removed; physical-chemical parameters were measured in the beginning and in the end of the exposure.

2.5.1. Byssus Threads Formation

The number of byssus threads produced by the organisms (\(n = 6\) per treatment) was counted after the exposure period. This procedure consisted of viewing both upside and underside of the mussel through the transparent glass flasks that were clearly visible, allowing the identification and counting of all the byssus threads.

2.5.2. Survival-in-Air Test

Mussels (\(n = 3\)) of each treatment were exposed to air, on empty containers (6-wells cell culture plates), after the aqueous exposure period of 72 h aiming at assessing the fitness to survive in prolonged extreme conditions. Containers were kept at constant room temperature (25 ± 1 °C) and photoperiod (12:12 h). Organisms’ survival was checked daily by an inspection of the valve closure. Mussels were considered dead when their valves did not close after mechanical stimulation.

2.6. Statistical Analysis

Data normality and homoscedasticity obtained in each experiment (fertilization, embryotoxicity, byssus threads produced, and survival in air tests) were tested using the Shapiro Wilk and the Levene tests (\(p < 0.05\)), respectively. For each experiment, statistical differences between the negative control and each treatment were analyzed using one-way ANOVA, followed by Dunnett’s multiple comparison tests whenever significant differences were observed (\(p < 0.05\)). Then, the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) were derived for fertilization and embryotoxicity tests.

The median effective concentrations (EC\(_{50}\)) that reduce 50% of fertilization success, embryo-larval development and byssus threads production were determined using dose-response curves through nonlinear regression analysis using a 4-parameter log-logistic model, performed with the statistical software GraphPad Prism v.6 (GraphPad Software, La Jolla, CA, USA). The number of byssus threads for each chemical concentration were normalized to the total threads production of the control (considered as 100%). For each chemical, the nonlinear regression equation that best fits the data was selected considering the R\(^2\) value, absolute sum of squares, and the 95% confidence intervals.
3. Results and Discussion

3.1. Fertilization Assay

All tested treatments exhibited significant effects compared to the negative control (Figure 1; Table 1). The 40 min-EC$_{50}$ value of DCOIT (free form) was estimated at 0.063 µg L$^{-1}$ (Table 1), roughly 137-fold more toxic than the nanostructured form SiNC-DCOIT (8.6 µg DCOIT L$^{-1}$) and 4 orders of magnitude more toxic than the unloaded SiNC (161 µg L$^{-1}$). The high difference between toxicity of SiNC-DCOIT in comparison with DCOIT may be related to the biocidal controlled release property of SiNC-DCOIT, which occurs gradually in time and by predefined stimuli [12,13].

Figure 1. Fertilization rates observed for P. perna gametes exposed to (a) SiNC, (b) DCOIT, and (c) SiNC-DCOIT. Asterisks (*) indicate significant differences relative to the control ($p < 0.05$). Data are presented as average ± standard deviation (SD).

Table 1. NOEC, LOEC, EC$_{50}$ values and respective confidence intervals (95%) of DCOIT, SiNC-DCOIT, and SiNC on gametes fertilization, embryo-larval development, byssus threads production, and survival-in-air of P. perna. Units are given in µg L$^{-1}$. SiNC-DCOIT values correspond to the concentration of encapsulated DCOIT (µg DCOIT L$^{-1}$). “nd”: not determined.

| Parameter          | NOEC  | LOEC  | EC$_{50}$ | 95% CI     |
|--------------------|-------|-------|-----------|------------|
| SiNC               |       |       |           |            |
| Fertilization      | <250.0| 250.0 | 161.3     | 139.3–186.8|
| Embryotoxicity     | <6.5  | 6.5   | 39.8      | 10.5–150.8 |
| Byssus threads     | 1000  | >1000 | 1323      | 218.2–8019 |
| Air survival capacity | 1000 | >1000 | nd        | –          |
| DCOIT              |       |       |           |            |
| Fertilization      | <1.0  | 1.0   | 0.063     | 0.016–0.255|
| Embryotoxicity     | 1.0   | 3.3   | 12.4      | 9.9–15.4   |
| Byssus threads     | 81.0  | >81.0 | 96.1      | 6.3–470    |
| Air survival capacity | <0.810 | 0.810 | nd        | –          |
| SiNC-DCOIT         |       |       |           |            |
| Fertilization      | <24.7 | 24.7  | 8.6       | 4.9–15.0   |
| Embryotoxicity     | 0.064 | 0.320 | 6.8       | 2.7–16.9   |
| Byssus threads     | 100.0 | 1000  | 305.5     | 124.2–751.5|
| Air survival capacity | <10.0 | 10.0  | nd        | –          |

These results stress the high toxicity of the conventional form of DCOIT by inhibiting the fertilization of P. perna gametes even at very low and environmentally relevant exposure concentrations. As a comparison, the EC$_{50}$ reported for the sea urchin Paracentrotus lividus fertilization assay was 198 µg L$^{-1}$ [30]. However, it is worth pointing out that the experiment was slightly different since sperm was pre-exposed for 45 min, and the subsequent fertilization was carried out in artificial seawater without DCOIT. In the present study, the fertilization was carried out in the same test-solutions that sperm was exposed for 40 min.

Previous studies demonstrate that DCOIT was more toxic (i.e., efficient) towards temperate target species, namely the bacterium Vibrio fischeri (IC$_{50}$ = 299 µg L$^{-1}$ of free DCOIT vs. IC$_{50}$ = 459 µg DCOIT L$^{-1}$ for SiNC-DCOIT) and the diatom Phaeodactylum tricornutum, involved in the biofilm formation...
(IC$_{50}$ = 4 µg L$^{-1}$ and 7 µg L$^{-1}$ for free DCOIT and SiNC-DCOIT, respectively [12]). Nevertheless, in such study, the sibling nanomaterial containing also silver (SiNC-DCOIT-Ag), demonstrated the opposite being much more efficient than the dissolved forms of DCOIT or Ag towards the tested target species [12].

3.2. Embryo-Larval Development Assay

The effects of SiNC, DCOIT, and SiNC-DCOIT on the larval development rate of $P.\ perna$ are shown in Figure 2 and Table 1. Significant effects were observed as low as at the lowest tested exposure concentration of unloaded SiNC (6.5 µg L$^{-1}$). Additionally, at 4040 µg SiNC L$^{-1}$ no developed veliger larvae were found (Figure 2a). The 48 h-EC$_{50}$ value was set at 39.8 µg SiNC L$^{-1}$, the highest value amongst the three tested compounds. Despite being the less toxic among the tested chemicals, the embryotoxicity caused by SiNC was much higher than expected since silica was expected to be, in principle, inert. According to Figueiredo et al. [12] this effect can be attributed to the cationic surfactant cetyltrimethylammonium bromide (CTAB) used in the synthesis of the mesoporous silica nanocapsules [23]. CTAB is a quaternary ammonium compound (QACs), a class of toxic surfactants towards aquatic species [12,31]. Besides, residues of this organic component can be detected even after several washes [12] explaining the recently reported effects of SiNC on the fertilization and embryo-larval development of the sea-urchin $Paracentrotus\ lividus$ [32] and the inhibition settlement of the bryozoan $Bugula\ neritina$ larvae [33]. This raw nanomaterial is not considered environmentally dangerous when compared with the AF biocides, but our results indicate the importance of knowing its potential toxicity against a wide set of organisms in order to improve the manufacturing process of the engineered nanomaterial, as recently proposed by Kaczerewska et al. [34].

Regarding the tested biocide, the development of mussel embryos was significantly affected at 3.3 µg L$^{-1}$ of DCOIT (LOEC). Above 33 µg L$^{-1}$ (Figure 2b) no tested organisms reached a well-developed veliger larvae stage. The embryotoxicity of DCOIT to $P.\ perna$ is in agreement with previous findings for other bivalve species from temperate regions, namely, the mussel $Mytilus\ edulis$, for which Bellas et al. [35] and the USEPA Office of Pesticides Programs [36] reported 48 h-EC$_{50}$ values of 10.7 and 2.7 µg L$^{-1}$, respectively. Moreover, Shade et al. [10] estimated the DCOIT 48 h-EC$_{50}$ at 6.9 µg L$^{-1}$ for embryos of the oyster $Crassostrea\ virginica$.

In the SiNC-DCOIT treatment, the embryonic development rates differed significantly from the control at 0.32 µg L$^{-1}$ (Figure 2c). Moreover, the estimated 48 h-EC$_{50}$ of SiNC-DCOIT was set on 6.77 µg DCOIT L$^{-1}$ (Table 1), nearly 2-fold more toxic to the embryos of $P.\ perna$ than the free form of DCOIT (12.36 µg L$^{-1}$). The present findings are the first evidence that SiNC-DCOIT can exhibit a better antifouling efficacy comparing with the non-nanostructured form of DCOIT in the pre-settlement stage of a fouler organism. This can be justified by the very low and slow release of DCOIT from the nanocapsules through diffusion, during the exposure period (undetectable by HPLC with a high
The parameters estimated for the studied chemicals on byssus threads production are presented in Table 1. The number of byssus threads secreted by mussels exposed to the different treatments showed no significant differences comparing to organisms from the experimental control, except for SiNC-DCOIT (at 1000 µg DCOIT L⁻¹), in which the number was significantly lower. However, increasing concentrations of DCOIT and SiNC-DCOIT reduce byssus production (Figure 3b,c, respectively). The 72 h-EC₅₀ value of DCOIT was estimated at 96.1 µg L⁻¹, being 3-fold more toxic than the SiNC-DCOIT (305.5 µg DCOIT L⁻¹) and one order of magnitude more toxic than the unloaded SiNC (1323 µg L⁻¹). These data confirm the high antifouling efficacy of free DCOIT, which inhibit the attachment of P. perna. In temperate and subtropical regions, mussels have been successfully used as a model system to test the antifouling activity of both free-forms and nanostructurated biocides by determining their continued attachment by byssus threads [33].

3.3. Short-Term Exposure Assay with Juvenile Mussel Stages

Regardless to evaluation of the capacity of organisms to survive to aerial exposure after the chemical exposure in water, mussels from the control group survived for approximately 53 h (Table 1). Mussels previously exposed to SiNC treatments survived in air, in average, 40 ± 17 h at 10 µg L⁻¹, 40 ± 21 h at 100 µg L⁻¹ and 36 ± 23 h at 1000 µg L⁻¹. The air survival capacity of mussels exposed to unloaded SiNC did not differ significantly concerning the experimental control. SiNC-DCOIT impaired the mussels physiological capacity to survive to a prolonged aerial exposure, set on 33 ± 12 h at 10 µg L⁻¹ and 31 ± 11 h at 100 µg DCOIT L⁻¹ treatments (not possible to measure on the highest SiNC-DCOIT tested concentration (1000 µg DCOIT L⁻¹) due to the high lethality during the aqueous exposure). Juvenile P. perna exposed to dissolved DCOIT presented the lowest survival-in-air rate. In average, the air survival was 31 ± 17, 29 ± 14, and 24 ± 13 h for animals previously exposed to 0.81, 8.1, and 81 µg DCOIT L⁻¹, respectively, being significantly different from the control. It was possible to conclude that the average survival was reduced by less than 50% for mussels exposed to
81 µg DCOIT L⁻¹ (Figure 4). Since P. perna mussels live mainly at the intertidal level and are periodically exposed to air during tidal cycles, less ability to survive may indicate limited physiological capacity.

![Graph showing air survival capacity](image)

**Figure 4.** Results of the juvenile mussel survival-in-air after exposition to DCOIT, SiNC-DCOIT and SiNC. Concentration is expressed as DCOIT content (µg DCOIT L⁻¹) in the case of SiNC-DCOIT. Asterisks (*) indicate significant differences relative to the control (p < 0.05). Data are presented as average ± standard deviation (SD).

Overall findings indicate that the biocide’s encapsulation protected the mussels from significant effects compared with the DCOIT exposure. Similarly, the acute toxicity of DCOIT, in its free form, in the mussel *Mytilus galloprovincialis*, from the temperate region was much higher than the nanostructured form SiNC-DCOIT (72 h-EC₅₀ = 1270 µg DCOIT L⁻¹ and 38,500 µg DCOIT L⁻¹, respectively; [12]). According to the authors, these mussels close the valves as a defense mechanism to prevent the animals’ exposure to contaminants. Thereby, the survival of mussels during the 72 h exposure to free DCOIT solutions can also be related to this mechanism to avoid exposure. In this sense, these individuals possibly remained without oxygenation of gills for a longer time, characterizing physiological effects that reduced their survival capability in a dry environment.

### 3.4. Environmental Relevance

No information regarding the presence or levels of DCOIT throughout tropical and subtropical zones has been reported in literature. However, DCOIT has been detected in coastal waters of the temperate climate zone in the northern hemisphere, such as Greece [13], Japan [37] or Sweden [38], reaching a maximum of 3.7 µg L⁻¹ reported in a Spanish marina [39]. Since this level is above the LOEC value determined for the DCOIT exposure in the fertilization bioassay and above both LOEC values determined in the embryotoxicity test with DCOIT and SiNC-DCOIT, adverse effects on the reproduction of bivalves may occur. In the other hand, the statistical predicted no effect concentration (PNEC) based on L/E/IC 50 values of DCOIT was recently set on 0.2 µg L⁻¹ [40], one order of magnitude higher comparatively to the EC₅₀ of DCOIT on *P. perna* fertilization. Thus, environmental concentrations of DCOIT may critically impair natural populations of this species, indicating potential ecological risks worldwide. Fonseca et al. also shown that the viability of haemocytes of mussels of *P. perna* are affected by DCOIT exposure as early as 24 h of exposure [41]. It is important to emphasize that the lowest exposure concentration of DCOIT (with environmental relevance) of the present study also caused a reduction on the air survival capacity of *P. perna* mussels by 45%. Since the natural populations of *P. perna* mussels inhabits intertidal rocky shores, a continuous exposure, particularly close to marinas or harbors, may cause incapacity of mussels to properly cope with the presence of DCOIT. Thus, a hypothetical decline of the established populations caused by the continuous exposure...
to DCOIT can have a negative socioeconomic impact in mid and low-income countries. In Brazil, for instance, *P. perna* mussels has both ecological and economical relevance [42], and many natural populations and mussel farms are located close to marinas, being under potential influence of AF compounds, including DCOIT.

In this sense, deleterious levels of DCOIT can foster technological developments to control the biocidal leaching from maritime coatings with environmental and economic benefits for the maritime industry. Recently, Figueiredo et al. [40] demonstrated that the encapsulation of DCOIT was able to promote a 25-fold decrease on the marine hazard of DCOIT in temperate ecosystems shown by the increase of the PNEC values from 0.2 to 5 µg L\(^{-1}\) on the conventional DCOIT and SiNC-DCOIT, respectively [40]. In the same direction, the present study demonstrated for the first time the promising antifouling efficacy of the novel nanostructured biocide in early life stages of the mussel *P. perna* together with the reduced toxicity on non-target stages of this neotropical species compared with the conventional DCOIT. These results are explained by the slow and controlled release of biocide in time, as recently demonstrated by Figueiredo et al. [12] in artificial saltwater. Since this is the first ecotoxicological study ever conducted in a tropical species, future research should focus on the holistic assessment of the fate, behavior, toxicity and hazard on the tropical environment of this novel nanoadditive for maritime coatings to avoid the same mistakes of the past when conventional biocides entered in the market without an appropriate assessment of their effects on the environment.

4. Conclusions

This is the first study assessing the ecotoxicological effects of SiNC-DCOIT, a novel AF nanomaterial, in a tropical marine species. The present findings reinforce the importance of balancing toxicity towards non-target species and efficacy against fouler species when developing a novel AF biocide. Using the mussel *P. perna*, as a model to holistically assess DCOIT in a dissolved and nanostructured forms, it was possible (a) to confirm that DCOIT is very efficient inhibiting the attachment of juvenile *P. perna*, nevertheless extremely toxic towards gametes and very early stages of the tested mussels; (b) to demonstrate that SiNC-DCOIT can be even more efficient than DCOIT, by preventing the formation of the *P. perna* veliger larvae, which can be the critical to control the undesired settlement of such fouler species on coated immersed surfaces; (c) to corroborate the lower toxicity of SiNC-DCOIT (comparing with DCOIT), now on fertilized gametes and juveniles of *P. perna*, thus ensuring the ecological success of the natural populations of this species.

Therefore, SiNC-DCOIT can be regarded as promising AF additive for maritime coatings that poses lower environmental risk while can successfully tackle the adhesion of larvae of *P. perna* thanks to the controlled biocidal release of this engineered nanomaterial. Future integrative studies would unveil the holistic effects on tropical marine biota and environment.

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