Toxic impacts induced by Sodium lauryl sulfate in Mytilus galloprovincialis

Rosa Freitas[a],* rosafreitas@ua.pt, Serena Silvestro[a,b], Francesca Coppola[a], Silvana Costa[a], Valentina Meucci[c], Federica Battaglia[c], Luigi Intorre[c], Amadeu M.V.M. Soares[a], Carlo Pretti[c,d], Caterina Faggio[b]

a: Departamento de Biologia & CESAM, Universidade de Aveiro, 3810-193 Aveiro, Portugal

b: Department of Chemical, Biological, Pharmaceutical and Environmental Sciences University of Messina, Italy

c: Dipartimento di Scienze Veterinarie, Università di Pisa, Italy

d: Consorzio per il Centro Interuniversitario di Biologia Marina ed Ecologia Applicata "G. Bacci" (CIBM), Livorno, Italy

*Corresponding author.

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ABSTRACT

Pharmaceuticals and personal care products (PPCPs) are continuously dispersed into the environment, as a result of human and veterinary use, reaching aquatic coastal systems and inhabiting organisms. However, information regarding to toxic effects of these compounds towardsmarine invertebrates is still scarce, especially in what regards to metabolic capacity and oxidative status alterations induced in bivalves after chronic exposure. In the present study, the toxic impacts of Sodium lauryl sulfate (SLS), an anionic surfactant widely used as an emulsifying cleaning agent in household and cosmetics, were evaluated in the mussel Mytilus galloprovincialis, after exposure for 28 days to different concentrations (0.0; 0.5; 1.0; 2.0 and 4.0 mg/L). For this, effects on mussels respitation rate, metabolic capacity and oxidative status were evaluated. The obtained results indicate a significant decrease on mussel's respiration rate after exposure to different SLS concentrations, an alteration that was accompanied by a decrease of bioconcentration factor along the increasing exposure gradient, especially at the highest exposure concentration. Nonetheless, the amount of SLS accumulated in organisms originated alterations in mussel's metabolic performance, with higher metabolic capacity up to 2.0 mg/L followed by a decrease at the highest tested concentration (4.0 mg/L). Mussels exposed to SLS revealed limited antioxidant defense mecanhisms but cellular damage was only observed at the highest exposure concentration (4.0 mg/L). In fact, up to 2.0 mg/L of SLS limited toxic impacts were observed, namely in terms of oxidative stress and redox balance. However, since mussel's respiration rate was greatly affect by the presence of SLS, the present study may highlight the potential threat of SLS towards marine bivalves, limiting their filtration capacity and, thus, affecting their global physiological development (including growth and reproduction) and ultimely their biochemical performance (afecting their defense capacity towards stressful conditions).

Keywords: Personal care products, metabolism, respiration, oxidative stress, bivalves

1. INTRODUCTION

Currently, a vast diversity of substances reaches the aquatic environment, including newly developed chemicals, identified as contaminants of emerging concern (CECs) (Ebele et al., 2017; Lorenzo et al., 2018; Richardson and Ternes, 2014, 2018; Richardson and Kimura, 2016), posing at risk a wide diversity of marine biological resources with ecological and economical relevance. For decades' classical pollutants (as trace metals) have been monitored worldwide, but regarding CECs studies information on their environmental concentrations has recently been a topic of concern. Nevertheless, although for some of the CECs information has increased on the last decade, still scarce data is available on the concentration levels of these substances for the majority of the coastal systems worldwide, and less is known on their impacts towards inhabiting organims.

Among the most worldwide dispersed CECs are Personal care products (PCPs), with increasing information on their occurrence and, as a consequence, with a high number of these substances already included in the Watch List adopted by European Union (EU). Although impacts towards marine species were already identified, still scarce information exists on their concentrations and toxic impacts towards non-target organisms, and in particular in what regards to marine invertebrate species (see for review: Brausch and Rand, 2011; Montesdeoca-Esponda et al., 2018). PCPs represent a group of substances that includes surfactants, foaming agents, wetting agents, detergents, emulsifier solubilizers and antimicrobials. As a consequence of their properties, including the capacity to lower the surface tension of a liquid, allowing easier spreading, PCPs are used in a wide variety of detergents and cosmetics (Daughton and Ternes, 1999; Loraine and Pettigrove, 2006; Olkowska et al., 2015; Ramos et al., 2015; Sharma et al., 2009; Teo et al., 2015). Many of these compounds are not susceptible to biodegradation, being eventually discharged into receiving waters in their pareting forms. Also, metabolic conjugates can also be converted back into their free parenting forms. In addition, many of these PCPs and their metabolites have bioaccumulation potential and/or are bioactive substances that can put living organisms at risk (Peck, 2006; Mackay and Barnthouse, 2010; Biel-Maeso et al., 2019).

Among the most widely used PCPs is Sodium lauryl sulfate (SLS), also identified as sodium dodecyl sulfate (SDS), an anionic surfactant used as an emulsifying cleaning agent in household detergents such as dishwashing soaps, but also in cosmetics, including toothpastes, shampoos, shaving foams, hand soap, facial cleanser, body wash, and shaving creams (Chaturvedi and Kumar, 2010). In the pharmaceutical industry, SLS is used as an agent to improve the absorption of chemicals through the skin, gastrointestinal mucosa and other mucous membranes (Hauthal, 1992).
In the industry SLS can be used infire fighting products, detergents and soaps, flocculant, de-inking agent (among others, Chaturvedi and Kumar, 2010). The concentration of SLS found in these products may vary depending on the product and the manufacturer, and although biodegradation of SLS ranges from 45% to 95% within 24 h (Fatma et al., 2015). Nevertheless, the continuous introduction of SLS into the environment through domestic and industrial waste discharges result into high concentrations of this pollutant (Cserhati et al., 2002). Anionic detergents, as SLS, have a strong tendency to bind to the lipid component of the membrane, with high concentrations being responsible for alterations at the cellular level (Brunelli et al., 2008). Although impacts of SLS were already demonstrated in aquatic organisms, still scarce information is available regarding molecular

mechanisms of surfactants toxicity, and in particular in what regards to SLS (Freitas and Rocha, 2012; Messina et al., 2014; Nunes et al., 2005, 2008; Rocha et al., 2007). However, recent studies pointed out that toxic effects of SLS may be related to the disruption of the osmotic balance and induction of oxidative stress, as demonstrated by Messina et al. (2014) and Nunes et al. (2008). It has been shown that oxidative stress is induced by a wide variety of pollutants, with published studies revealing the impacts of a wide diversity of pharmaceuticals and PCPs (PPCPs), including surfactants, on marine species oxidative stress performnce (among others, Almeida et al., 2015; Freitas et al., 2019; Messina et al., 2014; Nunes et al., 2005; Nunes et al., 2008). Among the biomarkers most widely measured as a consequence of PPCPs exposure are: i) lipid peroxidation (LPO), which reveal injuries at a cellular level caused by the overproduction of reactive species (ROS); ii) activity of antioxidant enzymes, corresponding to organisms defense mechanisms to eliminate the excess of ROS; and iii) content of reduced glutathione (GSH) that considered to be one of the most important scavengers of ROS, and its ratio with oxidised glutathione (GSSG) may be used as a marker of oxidative stress. Besides oxidative stress related biomarkers, measurements of organisms' metabolic capacity and energy reserves have been used to assess the impacts of different stressful conditions in marine organisms, namely bivalves. Commonly used as a proxy of the organism metabolism, the electron transport system (ETS) activity revealed to be an efficient marker to identify the impacts resulting from pollutants. When under stressful conditions, such as in the presence of pollutants, bivalves are able to change their metabolic activity to cope with the stress induced (Coppola et al., 2017, 2018; Fanslow et al., 2001; Freitas et al., 2017). In what regards to energy reserves, different studies demonstrated that organisms have the capacity to control the use of glycogen or lipids according to the stress level that they are subjected to (Anacleto et al., 2014; Coppola et al., 2017; 2018; Duquesne et al., 2004; Freitas et al., 2017; Timmins-Schiffman et al., 2014; Velez et al., 2018).

Therefore, the present study aimed to assess the effects induced in the mussel species Mytilus galloprovincialis after a chronic exposure (28 days) to SLS (0.00-4.00 mg/L). For this, biochemical markers related with organisms metabolism and oxidative status were evaluated.

2. MATERIALS AND METHODS

2.1. Experiment set up

Mytilus galloprovincialis specimens were collected in the Ria de Aveiro (northwest Atlantic coast of Portugal), in February 2018. To avoid the effect of body weight on biological responses and SLS accumulation, organisms with similar weight (0.45±0.11 g dry weight, DW; condition index 10.64±1.92) were selected.

After arriving to the laboratory organisms were acclimated for fifteen days prior to exposure, in separate aquaria (20 L each). Aquaria were set up by the addition of artificial sea salt (Tropic Marin® Sea Salt) to reverse osmosis water. During acclimation, organisms were maintained at 17.0 ± 1.0 °C; pH 7.90 ± 0.10 and continuous aeration, in artificial seawater (salinity 30 ± 1). Seawater was renewed every two days and animals were feed every two-three days with AlgaMac Protein Plus (150 000 cells/animal).

After this period, specimens were randomly distributed in 7 L aquaria, with 7 individuals per container and 3 containers per condition, maintaining species separation, salinity and temperature conditions as during the acclimation period (salinity 30 ± 1, temperature 17 ± 1.0 °C). For the experimental assay organisms were exposed for 28 days to four test conditions plus control: 0.5; 1.0; 2.0 and 4.0 mg/L of Sodium dodecyl sulfate (SLS), control (CTL, 0.0 mg/L). Lauryl Sulfate (SLS sodium

salt) used in the experiment was obtained from Sigma-Aldrich (chemical purity ≥ 99%; molecular weight 288.4).

The concentrations selected were necessary to test: i) a wide range of contamination; ii) determine the threshold concentrations that may cause toxic impacts to M. galloprovincialis mussels; iii) based on bibliography that showed effects in non-target species; iv) concentrations in the environment (among others, Bondi et al., 2015; Brunelli et al., 2008; Gibson et al., 2016; Rosety et al., 2001). During the exposure period containers were continuously aerated, temperature and salinity were daily checked and adjusted when necessary. Mortality was daily checked. During the entire exposure period (28 days) animals were fed with Algamac protein plus (150 000 cells/animal) three times per week and exposure medium (seawater at salinity 30) was renewed weekly, after which SLS concentration was reestablished.

Immediately after water renewal and concentrations reestablishement, water samples were collected every week from each aquarium (10 mL) and used for the SLS quantification. At the end of the experimental period 100% survival was observed in all conditions. After exposure (28 days), 2 individuals per aquarium (6 per condition) were used to measure respiration rate (RR), while 3 individuals per aquarium (9 individuals per condition) were used for biochemical analyses and SLS quantification.

2.2. Sodium dodecyl sulfate quantification in water and mussel's tissues

Water

The sample solution (15 mL), an alkaline buffer (2 mL) and 1 mL of neutral methylene blue solution (0.35 g/) followed by chloroform (6 mL) were added to a 50 mL vial. The vial was tightly closed and vigorously shaken for one minute in a vortex mixer. It was then left to stand until the phases had separated, after which the chloroform layer was transferred to a second vial containing distilled water (22 mL) and 1 mL of acid methylene blue solution. The second vial was shaken and the separated chloroform was collected. The absorbance of the chloroform phase was measured spectrophotometrically at 650 nm. A calibration curve with a concentration range 0.1-10 ppm was established using SLS as the reference compound. The lower limit of detection (LOD) for anionic surfactant analysis was 0.05 mg/L.

Tissues

Tissue samples (1.5 g) were extracted with 5 ml of water, vigorously shacked for one minute in a vortex mixer and centrifuged for 5 min. at 3000 rpm. The supernatant was collected after centrifugation and treated as reported above for water samples. A calibration curve with a concentration range 0.01-10 ppb was established using SLS. The lower limit of detection (LOD) for anionic surfactant analysis was 5 µg/g. This was estimated using the mean and standard deviation ofthe blank value.

For each tested condition, Bioconcentration factor (BCF) was calculated by dividing the mean concentration of SLS found in organism's tissues by the mean concentration of SLS measured in the water medium immediately after each spiking.

2.3. Biological responses: Physiological parameters

The respiration arete (RR) was measured at the end of the experimental period (28 days). Measurements were performed by simple static respirometry, using two organisms of the same aquarium per respirometric chamber. RR was recorded as a function of declining O2 concentration

(mg/L) over time every 15 min during 2h, with a multi-channel fiber optic oxygen meter (Multi-channel oxygen meter, PreSens GmbH). Organisms were posteriorly dried and weighed. RR was expressed in mg O2 consumed per h per g dry weight (DW) (see for more details Andrade et al., 2019).

2.4. Biological responses: Biochemical parameters

After exposure to SLS for 28 days organisms used for biochemical analyses were immediately frozen after the experimental period and the whole tissue was pulverized individually with liquid nitrogen and divided into aliquots of 0.5 g fresh weight (FW). For each biochemical determination, 0.5 g FW of soft tissue per organism was used. For each condition indicators of mussels metabolic capacity (electron transport system activity, ETS), energy reserves (total protein content, PROT; glycogen content, GLY), and oxidative stress biomarkers (including, superoxide dismutase activity, SOD; catalase, CAT; glutathione peroxidase activity, GPx; glutathione S-transferases activity, GSTs; lipid peroxidation levels, LPO; protein carbonylation, PC; reduced glutathione content, GSH; oxidized glutathione content, GSSG) were determined. Tissue samples from each organism were homogenized individualy, for 15 s at 4 ºC and centrifuged for 20 min at 10000 g (or 3000 g for ETS) and 4 ºC. Supernatants were stored at -80 ºC or immediately used. All biochemical parameters were performed in duplicate. All measurements were done using a microplate reader. For details on biochemical parameters see Almeida et al. (2015); Andrade et al. (2019) and Coppola et al. (2017).

2.5. Data Analysis

Concentrations of SLS in water samples and mussels tissues as wel as biochemical results (ETS, GLY, PROT, SOD, CAT, GPx, GSTs, LPO, PC, GSH/GSSG, RR) were submitted to hypothesis testing using permutational multivariate analysis of variance with the PERMANOVA+ add-on in PRIMER v6 (Anderson et al., 2008). A one-way hierarchical design, with SLS exposure concentration as the main fixed factor, was followed in this analysis. The pseudo-F values in the PERMANOVA main tests were evaluated in terms of significance. When the main test revealed statistical significant differences pairwise comparisons were performed. The t-statistics in the pairwise comparisons were evaluated in terms of significance. Values lower than 0.05 were considered as significantly different. The null hypotheses tested were: i) for each SLS test concentration, no significant differences were observed among weeks in terms of SLS concentration measured in water; significant differences among weeks are represented in Table 1 with different letters; ii) for SLS concentrations in mussels tissues, no significant differences exist among tested SLS concentrations; significant differences among SLS test concentrations are represented in Table 1 with different letters; iii) for each biomarker, no significant differences exist among SLS test concentrations; significant differences among SLS test concentration, are presented in figures with different letters.

The matrix containing biochemical results as well as SLS concentrations in mussels' tissue for each condition was normalised and the Euclidean distance similarity matrix calculated. This similarity matrix was simplified through the calculation of the distance among centroids based on SLS concentrations, which was then submitted to ordination analysis, performed by Principal Coordinates Ordination analysis (PCO). In the PCO graph, the variables that best explained the samples spatial distribution ($r>0.75$) were represented as superimposed vectors.

3. RESULTS

3.1 Sodium dodecyl sulfate quantification in water and mussel's tissues

For each tested concentration, SLS values obtained in water samples colected every week immediately after spiking revealed no significant differences among weeks. Concentrations measured each week showed significant differences among SLS tested concentrations, with nominal concentrations close to the measured ones, validating the spiking process (Table 1).

Concentrations of SLS measured in mussels tissues increased with the increasing SLS exposure concentration, with significantly lower values at the lowest exposure concentration in comparison to the remaining ones (Table 1).

The lowest BCF value was observed at the highest exposure concentration (Table 1).

3.2 Physiological responses

The respiration rate evaluated in M. galloprovincialis is presented in Figure 1. Contaminated mussels significantly reduced their filtration rate, with no significant differences among mussels exposed to different SLS concentrations.

3.3 Biochemical responses

3.3.1 Metabolic capacity and energy reserves

Results on ETS activity (Figure 2A) revealed an increasing trend with increasing exposure concentration up to 2.0 mg/L of SLS, with significantly higher values in mussels exposed to 2.0 mg/L. At the highest concentration (4.0 mg/L) a significant decrease in the activity of the ETS was observed, with no significant differences to the control.

M. galloprovincialis exposed to SLS showed no significant differences in GLY content among treatments (Figure 2B), although compared to control mussels exposed to 1.0 and 2.0 mg/L tended to accumulate more GLY while mussels exposed to 4.0 mg/L tended to present lower GLY content in comparison to contol.

The PROT content in mussels exposed to the highest concentrations (2.0 and 4.0 mg/L) was significantly lower than the concentration observed in control organisms (Figure 2C).

3.3.2 Oxidative stress

The results obtained revealed that contaminated mussels significantly reduced SOD activity in comparison to control organisms, with no significant differences among mussels exposed to different SLS concentrations (Figure 3A).

CAT activity was significantly lower in organisms exposed to 1.0 and 2.0 mg/L in comparison to control and organisms exposed to the highest SLS concentrations (Figure 3B).

On the other hand, the activity of GPx significantly increased in mussels exposed to SLS compared to uncontaminated organisms (Figure 3C).

No significant differences were observed in terms of GSTs activity among treatments (Figure 3D).

In terms of LPO significantly higher values were obtained in mussels exposed to the highest SLS concentration in comparison to the remaining conditions (Figure 4A).

No significant differences were observed in terms of PC levels among treatments (Figure 4B) as well as in terms of GSH/GSSG (Figure 4C).

3.4 Multivariate analysis

Results from the PCO analysis are presented in Figure 5. The first principal component axis (PCO1) represents 41.9% of the variability, with a clear distinction between individuals exposed to SLS (negative side) and individuals under control condition (positive side). PCO2 axis explained 29.4% of the variability, separating organisms exposed to the highest SLS concentration (negative side) from the remaining conditions (positive side). SOD and RR were the variables that best explained PCO1 positive side, with the highest values at control organisms. LPO, CAT, GPx and SLS concentration were the variables presenting higher correlation with PCO2 negative side, being close related with organisms exposed to the highest SLS concentration. GLY and ETS were the variables that best correlate with PCO2 positive side, being associated with animals exposed to 0.5, 1.0 and 2.0 mg/L of SLS.

4. DISCUSSION

The exposure of an organism to xenobiotics can lead to alterations in cell homeostasis, probably causing oxidative stress (Hoarau et al., 2004; Livingstone, 2003). In the case of SLS, previous studies already demonstrated the capacity of this compound to stimulated intracellular ROS levels Mizutani et al., 2016), which will result into oxidative stress. Nevertheless, pollutants may also cause alterations on organism's metabolism (among others, Coppola et al., 2019; Cruz et al., 2016; Freitas et al., 2016; Oliveira et al., 2017) which may impact their capacity to activate defence mechanisms, enhancing oxidative stress injuries.

The present study demonstrated that in the presence of SLS mussels significantly decrease their respiration rate, which was accompanied by a decrease in BCF values at the two highest exposure concentrations. Such findings highlight the capacity of bivalves to develop strategies to avoid accumulation of pollutants, limiting their toxic effects. However, still accumulation of SLS was observed along the exposure gradient, with higher values at the highest exposure concentration, resulting into alterations in organism's metabolism and oxidative status.

The balance between energy reserves and the activity of the mitochondrial electron transport system (ETS) is an important tool to understand if lower energy availability can result in negative effects to organisms physiological and biochemical performance (Smolders et al., 2004). ETS has bee used as a measure of metabolic capacity in various marine invertebrate species in response to environmental disorders (Bielen et al., 2016; Cammen et al., 1990; Freitas et al., 2016; Simčič et al., 2014), and can be used as a marker to predict changes due to xenobiotics (De Coen and Janssen, 1997; Gagne et al., 2006). The results obtained in the present study have clearly shown that after 28 days of exposure to SLS mussles significantly increased their metabolic capacity up to 2.0 mg/L of SLS, while at the highest test concentration (4.0 mg/L) mussels decreased their metabolism to values close to control. Such findings may indicate that by increasing their metabolism mussels were trying fuel up and activate their defense mechanisms to fight against the stress induced by SLS but at the highest concentration this capacity was no longer effective. Other studies revealed similar patterns, with organisms presenting an increasing metabolic capacity along the increasing exposure

concentration gradient (De Marchi et al., 2017a, b) while at highly stressful conditions organisms metabolic capacity decreased (Freitas et al., 2017, 2019; Pinto et al., 2019), indicating that organisms may have limit capacity to continuously increase their metabolic capacity if stress conditions exced their tolerance limits.

Energy metabolism plays a fundamental role in the survival of organisms and in vital functions, as well as in adaptation and tolerance to stress (Sokolova et al., 2012). It has already been shown that energy expenditure tends to increase when organisms are exposed to pollutants, considering this phenomenon as a cellular protection mechanism (Bielen et al., 2016). However, recent studies have shown that some marine invertebrates exposed to certain pollutants may show a decrease in GLY and PROT concentrations due to high metabolic rates (among others, Coppola et al., 2019; Cruz et al., 2016). The present study showed that overall M. galloprovincialis tended to maintain their GLY and PROT content regardless the SLS concentration, a result that may indicate that although leading to metabolism increase the stress experienced by the organisms was not suficient to lead to energy reserves expenditure. Nevertheless, at the highest concentration of SLS (4.0 mg/L) PROT content decreased, probably indicating that organisms were at high stress and were not able to maintain the production of PROT, including enzymes, a consequence of lower metabolic activity (shown as lower ETS activity). Such results are further highlighted by the PCO analysis, that clearly demonstrated the close relationship between higher ETS and GLY values with intermediate SLS concentrations (1.0 and 2.0 mg/L).

Besides metabolism related parameters, biomarkers of oxidative stress were evaluated in the present study, including the ones representing organisms antioxidant defense capacity. The activity of the enzyme superoxide dismutase (SOD) showed a significant decrease compared to the control, which can probably indicate that the antioxidant system fails to adapt efficiently to the changes induced by exposure to SLS. SOD is a protein that works as a "scavenger" of free radicals, catalyzing the dismutation reaction of the superoxide radical (O2 -) into hydrogen peroxide (H2O2), the latter subsequently converted into water (H2O) and oxygen (O2) by the enzymes catalase (CAT) and glutathione peroxidase (GPx). Acompaining the decrease of SOD activity in contaminated mussels CAT was also reduced at all exposure concentrations except the highest one, which could indicate that SLS also has an inibitory capacity against this enzyme. Nevertheless, organisms were able to increase the activity of GPx when exposed to SLS, showing that this enzyme was involved in the elimination of ROS and conversion of GSH into GSSG. Our findings may indicate that the hydrogen peroxide produced is possibly being converted not by CAT (whose activity is inhibited) but by GPx contributing to the defense against oxidative stress.

Furthermore, because the stress induced by SLS was not extremely high the ratio GSH/GSSG was similar among tested conditions. GSH/GSSG ratio represents the major homeostatic regulator of redox equilibrium inside the cell and can be useful as a biomarker to detect protective or injurious cellular reactions by measuring the rate and level of ratio alterations (Mocan et al., 2010). Therefore, the present findings may indicate that the concentrations of SLS tesed were not enough to induce great laterations on mussels defense mechanisms and redox balance. Such results are corroborated by the activity of GSTs, that was inaltered among tested conditions. GSTs is a group of detoxifying enzymes that catalyzes the conjugation of various toxic molecules with glutathione making them less reactive and more easily eliminated from the body (Sturve et al., 2008). Also studies conducted by Almeida et al. (2017) and Oliveira et al. (2017) revealed inibithion or limited capacity of antioxidant defense of clams (Ruditapes philippinarum) and mussels (M. galloprovincialis) when exposed to environmentally relevant concentrations of the drugs cetirizine and carbamazepine. Such results may highlight that up to a certain concentration level, organisms are not able to activate their

defense mechanisms in the presence of PPCPs, being however capable of maintain their redox balance.

When under stressful conditions ROS are produced in excess by organisms, which in conditions of excessive stress can not be completely eliminated by defense mechanisms such as antioxidant enzymes. In this case, ROS can interact promptly with polyunsaturated fatty acids of the membrane, causing lipid peroxidation (LPO) and protein carbonilation (PC) (Regoli and Giuliani, 2014). In the present study, the levels of LPO and PC were maintained similar to control values up to 2.0 mg/L of SLS, which can probably derive from low stress levels exerted by SLS up to this concentration and the capacity, even limited, of antioxidant defense mechanism to eliminate the excess of ROS produced. Nevertheless, as demonstrated by the PCO analyses, significantly higher LPO levels were strongly correlated with the highesr SLS exposure concentration (4.0 mg/L of SLS), which may have resulted from the overproduction of ROS that were not promptly eliminated by antioxidant enzymatic responses. However, at this SLS concentration level no PC was observed, once again corroborating the hypothesis that the toxic impacts induced by SLS were limited.

CONCLUSION

As a result of increasing application of PPCPs and the consequent release in aquatic ecosystems, the present study provides relevant data on the potential risk of SLS in the aquatic environment and inhabiting organisms, namely in Mytilus galloprovincialis. Nevertheless, the study of the toxicity of these SLS may be lacking in ecological importance since in the environment different conditions can act in combination modifying the behavior and toxicity of the different PPCPs. Therefore, future studies should address more realistic exposure scenariosfor a more relevant risk assessment.

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Conflict of interest

The Authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affi liations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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Figure 1: Respiration Rate (RR), in Mytilus galloprovincialis maintained for 28 days to control (CTL, 0.0 mg/L), 0.5; 1.0; 2.0 and 4.0 mg/L of Sodium dodecyl sulfate (SLS). Results are the means + standard errors. Different letters represent significant differences among tested conditions.

Figure 2. A: Electron transport system (ETS) activity, B: Glycogen (GLY); and C: Protein (PROT), in Mytilus galloprovincialis maintained for 28 days to control (CTL, 0.0 mg/L), 0.5; 1.0; 2.0 and 4.0 mg/L of Sodium dodecyl sulfate (SLS). Results are the means + standard errors. Different letters represent significant differences among tested conditions.

Figure 3. A: Superoxide dismutase (SOD); B: Catalase (CAT); C: Glutathione peroxidase (GPx); and Glutathione-S-transferases (GSTs) activities, in Mytilus galloprovincialis maintained for 28 days to control (CTL, 0.0 mg/L), 0.5; 1.0; 2.0 and 4.0 mg/L of Sodium dodecyl sulfate (SLS). Results are the means + standard errors. Different letters represent significant differences among tested conditions.

Figure 4. A: Lipid peroxidation (LPO) and B: protein carbonylation (PC) levels; and C: reduced/oxidised glutathione (GSH/GSSG) ratio in Mytilus galloprovincialis maintained for 28 days to control (CTL, 0.0 mg/L), 0.5; 1.0; 2.0 and 4.0 mg/L of Sodium dodecyl sulfate (SLS). Results are the means + standard errors. Different letters represent significant differences among tested conditions.

Figure 5: Centroids ordination diagram (PCO) based on SLS concentrations, physiological and biochemical parameters, measured in in Mytilus galloprovincialis maintained for 28 days to control (CTL, 0.0 mg/L), 0.5; 1.0; 2.0 and 4.0 mg/L of Sodium dodecyl sulfate (SLS). Pearson correlation

vectors (r > 0.75) of physiological and biochemical descriptors were provided as supplementary variables being superimposed on the top of the PCO graph ETS, GLY, SOD, CAT, GPx, LPO, RR, SLS.

Table 1- Sodium Lauryl Sulphate (SLS) concentrations in water (mg/L), collected immediately after spiking at the 1st, 2nd, 3rd and 4th weeks of exposure, and in mussel's tissues (µg/g dry weight) at the end of the experimental period (28 days). LOD for water samples 0.05 mg/L; LOD for tissue samples 5 µg/g. Lower case letters represent significant differences among tested concentrations for each exposure week; upper case letters represent significant differences among exposure weeks for each tested concentration. BCF: Bioconcentration factor: mean concentration of SLS measured in mussel's tissues / mean concentration of SLS measured in the water every week after spiking.

S/A concentrations (mg/L)	**Water (mg/L)**				**Mussels tissues (µg/g)**	**BCF**
	1st week	2nd week	3rd week	4th week	4th week	
CTL	<LOD	<LOD	<LOD	<LOD	<LOD	
0.5	$0.57 \pm 0.21^{a,A}$	$0.62 \pm 0.22^{a,A}$	$0.42 \pm 0.04^{a,A}$	$0.52 \pm 0.13^{a,A}$	0.43 ± 0.19^{a}	0.80
1.0	$0.86 \pm 0.04^{b,A}$	$0.97 \pm 0.11^{b,A}$	$0.99 \pm 0.09^{b,A}$	$1.05 \pm 0.08^{b,A}$	1.16 ± 0.41^{b}	1.19
2.0	$1.72 \pm 0.24^{c,A}$	$1.91 \pm 0.06^{c,A}$	$1.80 \pm 0.12^{c,A}$	$1.88 \pm 0.01^{c,A}$	1.11 ± 0.38^{b}	0.61
4.0	$3.73 \pm 0.18^{d,A}$	$3.79 \pm 0.07^{d,A}$	$4.13 \pm 0.12^{d,A}$	$3.74 \pm 0.19^{d,A}$	3.92 ± 1.09^{d}	0.49

Graphical abstract:

Highlights:

- Mussels bioaccumulated SLS, with lower BCF values at the highest exposure concentration
- Respiration rate strongly decreased in contaminated mussels
- Metabolic capacity was increased at intermediate concentrations
- Defence mechanisms presented limited activity, although redox balance was maintained
- Cellular damage was observed at the highest exposure concentration