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Research article

Sub-lethal effects induced in *Mytilus galloprovincialis* after short-term exposure to sodium lauryl sulfate: Comparison of the biological responses given by mussels under two temperature scenarios

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ABSTRACT

Surfactants are among the most common PPCPs that reach coastal systems, being often used in large quantities in cleaning products such as detergents and soap powders. Sodium lauryl sulfate (SLS) is listed in this group of emerging contaminants. Previous studies have already demonstrated the presence of SLS in aquatic environments and the negative effects on organisms living there. However, with ocean acidification and warming predictions, SLS-induced impacts may differ from those currently known. In this context, the present study aimed to reproduce environmental conditions by assessing the release of substances over a short period and to understand the influence of a rapid increase in temperature on the impacts caused. The marine bivalve *Mytilus galloprovincialis* was exposed to 2.0 mg/L SLS at 17 °C and 21 °C for 7 days. To assess the possible biochemical changes resulting from the exposure of mussels to SLS, a series of biomarkers related to oxidative stress/damage, detoxification, and metabolic capacity were measured. The SLS accumulation in soft tissues was low (about 0.7 ng/g) at both temperatures. The results evidenced increased metabolic activity, especially in mussels exposed to SLS at 17 °C. An increase in protein content was also observed upon exposure to SLS and increased temperature compared to controls at 17 °C. Although no effects on antioxidant enzymes were observed, protein damage was recorded, especially at 21 °C. These findings confirmed that SLS induces toxic effects and predicted climate change factors may increase the impact on *M. galloprovincialis*.

1. Introduction

Coastal areas are suffering from ongoing pressures related to population growth and industrialization with the associated release of contaminants into aquatic systems (Lotze et al., 2006). Humans bring most of the contaminants into coastal marine ecosystems through household and municipal wastes, sewage sludge, aquaculture activities, and accidental releases. So-called contaminants of emerging concern (CECs) are among the most detected in urban and sewage effluents and have been the subject of growing attention by the scientific community in recent years (Wilkinson et al., 2017). These CECs involve different substances, including pharmaceutical and personal care products (PPCPs) (da Silva and de Souza Abessa, 2019). Among the most common PPCPs reaching coastal systems are surfactants, including sodium lauryl sulfate (SLS), also known as sodium dodecyl sulfate (SDS). Surfactants are used in large quantities in cleaning products such as detergents and soap powders but also in various cosmetic products, including bath salts, soaps, acne treatment creams, exfoliating products, hair paints, conditioners, facial cleansers, and shampoos (Holmberg and Laughlin, 1997; Lai, 2005; Singh et al., 2007; Bondi et al., 2015).

Surfactants are released in large quantities into aquatic ecosystems as industrial and domestic wastes (Metcalfe et al., 2008). Zhang et al.

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(1999) reported surfactant concentrations in domestic wastewater ranging from 1 to 10 mg/L, while in industrial wastewater concentrations can be as high as 300 mg/L. In the United Kingdom, surfactant concentrations of up to 0.416 mg/L have been recorded in surface water (Fox et al., 2000), while concentrations of up to 1.09 mg/L have been documented in sewage effluent (Holt et al., 1989).

Even though the biodegradation of SLS ranges from 45 % to 95 % within 24 h, the constant introduction of SLS into the environment keeps high the levels of this pollutant in aquatic systems (Cserháti et al., 2002). The concentration of SLS present in consumer products varies by product and manufacturer, as reported by Bondi et al. (2015): it generally ranges from 0.01 % to 50 % in cosmetic products and from 1 % to 30 % in cleaning products. Despite the presumed toxic effects of SLS, a recent review (Asio et al., 2023) confirmed the need to further investigate environmental concentrations and possible effects of SLS, since most of the monitoring data available to date is related to the entire group of surfactants, with the associated threshold limits established as a group (drinking water limit 0.5 mg/L) applied in only 33 countries (WHO, 2021). In particular, SLS concentrations recorded several years ago in coastal areas (Bay of Cadiz, Spain) revealed values ranging from 0.48 mg/L to 1.80 mg/L (Quiroga et al., 1989). One of the most recently reported data shows a concentration of 0.10 mg/L of SLS in the Otamiri River (Nigeria) (Nwoye and Ifeoma, 2020).

The basis of SLS toxicity appears to be mainly related to the disruption of cellular ionic balance caused by alterations in membrane permeability (Grant and Acosta, 1996) and consequent induction of oxidative stress (Bromberg and Pick, 1985), which, in turn, can generate a range of physiological and biochemical stresses. The impact of SLS has already been demonstrated in aquatic organisms such as oligochaetes (Bhattacharya et al., 2021), fish (Mustapha and Bawa-Allah, 2020), nematodes (Oliveira et al., 2020) and mussels (Messina et al., 2014; Freitas et al., 2020). When considering anionic detergents, such as SLS, an important factor is their strong tendency to bind to the lipid component of the membrane, and high concentrations are responsible for alterations at the cellular level, particularly in fish (Brunelli et al., 2008).

Even though progress has been made regarding the impact of PPCPs on aquatic organisms, uncertainties remain regarding the consequences of combining these exposures with ongoing climate change (CC). Namely, CC-induced environmental alterations, such as temperature increases due to extreme weather events (Gazeau et al., 2013; Intergovernmental Panel on Climate Change - IPCC, 2022), are likely to significantly affect organisms (Freitas et al., 2016; Freitas et al., 2017), but may also influence the bioavailability and behavior of pollutants and consequently their bioaccumulation potential and impact on organisms. In this regard, little information is available on the combined effects of climate change and PPCPs in bivalves. To fill this gap of knowledge, the use of biomarkers indicative of temperature and PPCPs sub-lethal impacts in metabolic and oxidative stress in bivalves has already proven to be appropriate. As an example, Freitas et al. (2019) evaluated the influence of the temperature on the metabolic and oxidative status of *M. galloprovincialis* exposed to two pharmaceuticals (Diclofenac and Triclosan); Munari et al. (2018) further assessed the combined impact of pH and Diclofenac in the oxidative stress state of *M. galloprovincialis* and *R. philippinarum.* Another recent study by Freitas et al. (2021) concerning the influence of salinity on SLS-induced toxicity in mussels (*M. galloprovincialis*), demonstrates clear interactive effects between the two factors, including alterations in metabolism and defense mechanisms in mussels exposed to SLS and salinities 25 and 35 compared to the control salinity (30).

Considering that there is scarce information on the ecotoxicity of surfactants, especially SLS, and in particular when considering the influence of climate change-related factors, this study aimed to expose the model organism *Mytilus galloprovincialis* to SLS (2 mg/L) for one week (7 days) at two different temperatures: 17 °C representative of the actual mean temperature at the sampling site and 21 °C as the representative of predicted warming conditions. The objective was to reproduce environmental conditions, i.e., I) a release of substances over a short period with consequent concentration dilution over time due to currents and marine streams, and II) to understand the influence of a rapid temperature increase on the impacts caused. To our knowledge, there are no studies on the influence of temperature on SLS acute toxic effects on marine invertebrates, considering a short exposure period to simulate pulse contamination events. To assess the possible biochemical alterations resulting from mussel exposure to SLS, a battery of biomarkers related to mussels' oxidative status and metabolic capacity were measured after 7 days of exposure. Marine bivalves such as *M. galloprovincialis* are of global importance as bio-indicators of marine and estuarine pollution (Liu et al., 2010). Because they have a high geographical distribution, are easy to find, and are known to accumulate high levels of contaminants in their tissues, they provide a time-integrated indication of environmental contamination with an observable cellular response (Lau and Wong, 2003; Manduzio et al., 2004). The characteristics of mussels make them good bioindicators for environmental monitoring, the reason why this species was chosen as the model organism in the present study.

2. Materials and method

2.1. Experimental setup

Mytilus galloprovincialis specimens were collected in the Mira channel, at the Ria de Aveiro (northwestern Atlantic coast of Portugal) in May 2022. The Ria de Aveiro has a complex topography, with four main channels, several branches, islands, and intertidal mud and sand flats. This lagoon, and in particular the Mira channel, is considered a low-contaminated ecosystem (Calisto et al., 2011; Freitas et al., 2014), being part of the Natura 2000 network (EU Habitats Directive), has Special Protection Area (SPA) designation, with several areas classified as Sites of Community Importance (SCI), and is protected by the EU Birds Directive (79/109/EEC) (Lillebø et al., 2015). It encompasses a wide range of biotopes (e.g., wetlands, salt marshes, and mudflats) used as breeding areas for many valuable species, particularly bivalves, crustaceans, fish, and birds. Thus, considering the above mention environmental conditions, economic relevance as an important area for bivalves harvesting, and close location to the University of Aveiro, Ria de Aveiro was chosen as the sampling area.

After collection, mussels were transported to the laboratory (ECOMARE-University of Aveiro) and organisms of similar sizes were selected to avoid the effect of body weight on biological responses and SLS accumulation: a mean length of 5.8 ± 0.5 cm and a mean width of 3.6 ± 0.5 cm. The organisms were acclimated for 15 days before exposure in separate aquaria (20 L each) and the aquaria were set up by adding artificial sea salt (Tropic Marin® Sea Salt) to reverse osmosis water. During acclimation, the organisms were maintained at 17.0 ± 1.0 °C; pH 7.90 ± 0.10, and continuous aeration, in artificial seawater (salinity 30 ± 1) for one week. In the following week, mussels were divided into two groups: one maintained at 17 °C and the other one placed in a different climatic room and gradually acclimated to 21 °C. The seawater was renewed every two days after the third day in the laboratory, and the animals were fed with AlgaMac Protein Plus (150,000 cells/animal/day) three times a week, five days after arrival at the laboratory.

Subsequently, specimens were randomly distributed in 3 L aquaria, with 6 individuals per container and 3 containers per treatment: CTL17 (mussels maintained at 17 °C without SLS); SLS17 (mussels maintained at 17 °C with SLS); CTL21 (mussels maintained at 21 °C without SLS); SLS21 (mussels maintained at 21 °C with SLS). Mussels were exposed to these treatments for 7 days and the concentration used was 2.0 mg/L of SLS (obtained from Sigma-Aldrich; chemical purity ≥99 %; molecular weight 288.4). The choice of the concentration used was based on available literature showing that concentrations may reach these levels in coastal systems (Quiroga et al., 1989) but also considering previous

studies which have demonstrated that model organisms change their biochemical performance at this concentration, after chronic (Freitas et al., 2020; Freitas et al., 2021) and acute (Nunes et al., 2008) exposures. The warming scenario (+4 °C in relation to the control that resembles mean values at the sampling site) was selected to mimic projected values (IPCC, 2022). The short-term exposure was chosen to simulate a punctual discharge of SLS to the aquatic environment but also considering previous studies testing the toxicity of this compound using similar concentrations on other species acutely exposed (Nunes et al., 2008; Nunes et al., 2005) or the same mussel species chronically exposed (Freitas et al., 2020). The aquaria were kept at 17 °C and 21 °C in two different climatic rooms for the duration of the experiment and the animals were fed with Algamac protein plus (150,000 cells/animal/day) three times a week. All the aquaria were constantly aerated, the temperature and salinity were monitored daily and adjusted if necessary. At the end of the experiment, 100 % survival was observed, regardless of the treatment. After 7 days, individuals were collected from each aquarium and immediately frozen under liquid nitrogen and preserved at −80 °C.

Water samples were collected from each aquarium (10 mL) at the beginning of the experiment and used to determine SLS real exposure concentration. The stability of SLS during a one-week experimental period was already demonstrated by Freitas et al. (2021).

2.2. Sodium dodecyl sulfate quantification in water and tissue samples

The determination of SLS as Methylene Blue Active Substances (MBAS) was performed according to Latif and Brimblecombe (2004) and Arand et al. (1992). Water concentrations were determined, using the sample solution (15 mL), an alkaline buffer (2 mL) and 1 mL of neutral methylene blue solution followed by chloroform (6 mL) were added to a 50 mL vial. The vial was tightly closed and vigorously shaken for one min 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 of 0.1–10 ppm was established using SLS as the reference compound. LOD was 0.05 ppm (0.05 mg/L). Tissue samples (1.5 g) were extracted with 5 mL of water, vigorously shacked for one min in a vortex mixer and centrifuged for 5 min at 3,000 rpm. The supernatant was collected after centrifugation and treated as reported above for water samples. A calibration curve with a concentration range of 0.01–10 ppb was established using SLS. LOD for tissue samples was 0.005 ppm (0.005 ng/g).

2.3. Biochemical analysis

At the end of the experiment, as mentioned above, the organisms used for biochemical analysis were immediately sacrificed using liquid nitrogen to instantly freeze them. Then, the whole soft tissues of each animal were homogenized with liquid nitrogen and divided into aliquots of 0.5 g fresh weight (FW). After homogenization, different buffers were used for specific biomarkers (1:2 *w/v*) and tissue samples were sonicated for 90 s using a Tissuelyser (Qiagen) at 4 °C and centrifuged for 20 min at 10,000 *g* or 3,000 *g* (depending on the biomarker) and 4 °C. Tissue samples were used to determine biomarkers of: i) mussels' metabolic capacity (electron transport system activity, ETS (De Coen and Janssen, 1997) and total protein content, PROT (Robinson and Hogden, 1940)); ii) oxidative stress (activity of superoxide dismutase, SOD (Beauchamp and Fridovich, 1971), catalase, CAT (Johansson and Borg, 1988), glutathione reductase , GRed (Carlberg and Mannervik, 1985)); iii) oxidative damage and redox balance (lipid peroxidation levels, LPO (Ohkawa et al., 1979); protein carbonylation levels, PC (Mesquita et al., 2014); oxidized glutathione content, GSSG (Rahman, 2007)) and iv) detoxification capacity (activity of glutathione S-transferases, GSTs (Habig et al., 1974) and carboxylesterases, CbEs (Hosokawa and Satoh, 2001)). Supernatants were stored at −80 °C or used immediately. All biochemical parameters were performed in duplicate. All measurements were performed using a microplate reader. For details on biochemical parameters see Almeida et al. (2015), Andrade et al. (2019) and Coppola et al. (2017).

2.4. Data analysis

Concentrations of SLS in water samples and mussels' tissues as well as biochemical data (ETS, PROT, SOD, CAT, GRed, GSTs, Cbes_pNPA, Cbes_pNPB, LPO, PC, GSSG) were submitted to hypothesis testing using permutational multivariate analysis of variance with the PERMANOVA + add-on in PRIMER v6 (Anderson et al., 2008). *p*-values lower than 0.05 were considered significantly different and differences among treatments were identified with different lower-case letters, while the same letter represents no significant differences among treatments.

3. Results

3.1. SLS concentrations in water samples and mussels' tissues

SLS concentration in water samples collected immediately after the start of the experiment confirmed levels close to the nominal concentrations at both temperatures (Table 1). SLS concentrations measured in mussel soft tissues were <1 ng/ g, and no differences were observed between the two temperatures tested (17 °C and 22 °C) (Table 1).

3.2. Metabolic capacity and protein content

The ETS activity (Fig. 1A) increased significantly in SLS-exposed mussels at 17 °C compared to the control. At 21 °C mussels, regardless of the presence or absence of SLS, showed a higher ETS activity than non-contaminated ones at 17 °C but lower than the ones exposed to SLS at 17 °C, albeit not significant in any of the cases. The PROT content in mussels exposed to SLS (at 17 °C and 21 °C) was significantly higher than in the control (17 °C) and a similar response was observed in non-contaminated mussels maintained at 21 °C (Fig. 1B).

3.3. Oxidative stress/damage and detoxification

The activity of SOD revealed no significant differences among treatments, although mussels exposed to SLS at 21 °C revealed the lowest values (Fig. 2A). A similar pattern was found for CAT activity, with no significant differences among treatments (Fig. 2B). Regarding GRed activity, mussels exposed to 21 °C showed a clear decrease on enzyme activity, with significant differences between mussels maintained at 17 °C (with and without SLS) and contaminated ones at 21 °C (Fig. 2C).

The activity of the GSTs increased in contaminated mussels and 21 °C groups compared to the ones non-contaminated at 17 °C, with significant differences between 21 °C treatments and mussels at 17 °C in the absence of SLS (Fig. 3A). The activity of carboxylesterases (CbEs), with NPA and NPB substrates, showed a significant increase in contaminated mussels at 17 °C and mussels maintained at 21 °C in comparison to the

Table 1
SLS concentrations in water (mg/L) and in *M. galloprovincialis* soft tissues (ng/g) exposed to CTL and SLS at different temperatures (mean ± standard deviation). n.d. means below the limit of detection.

Treatment	Water		Soft tissue	
	17 °C	22 °C	17 °C	22 °C
CTL	n.d.	n.d.	n.d.	n.d.
SLS	1.88 ± 0.06	1.88 ± 0.03	0.79 ± 0.15	0.72 ± 0.15

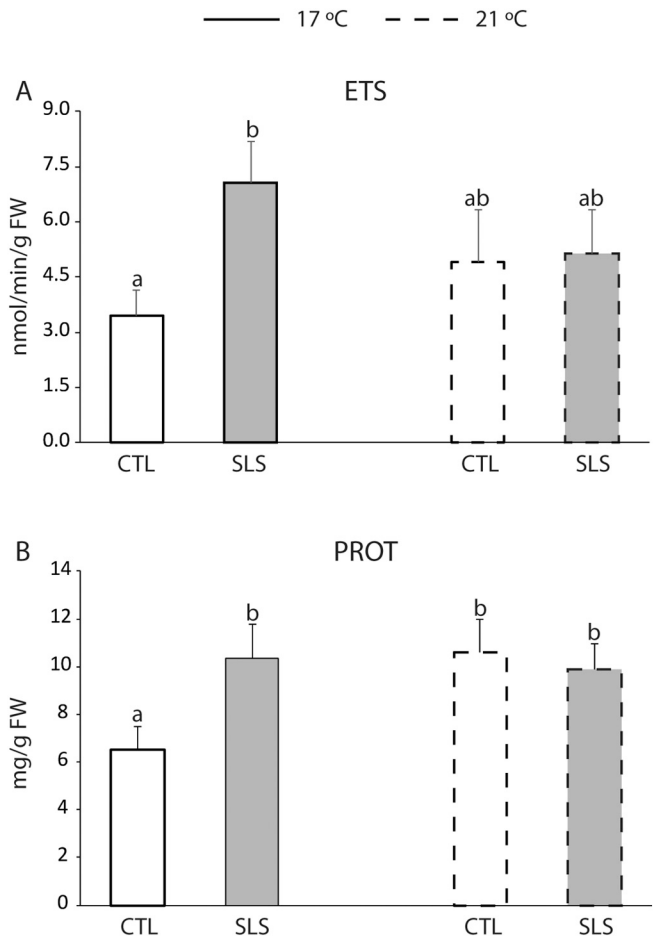

Fig. 1. A: Electron transport system (ETS) activity and B: Protein (PROT) concentration, in *Mytilus galloprovincialis* maintained at 17 °C and 21 °C to control (CTL, 0.0 mg/L) and 2.0 mg/L sodium lauryl sulfate (SLS) for 7 days. Results are mean values + standard deviation. Significant differences ($p < 0.05$) among treatments are represented by different letters, while the same letter represents no significant differences. White bars represent no contamination; grey bars represent SLS-exposed treatments.

ones maintained at 17 °C without SLS (Fig. 3B and C).

In terms of LPO, no significant differences were observed among treatments, although SLS-exposed mussels at 17 °C showed higher LPO levels (Fig. 4A). Exposure to SLS did not affect the PC levels in mussels at 17 °C. A significantly higher level of PC was observed in mussels maintained at 21 °C compared to the ones at 17 °C, regardless of the presence or absence of SLS (Fig. 4B). GSSG content was significantly higher in contaminated mussels (17 and 21 °C) and non-contaminated mussels at 21 °C in comparison to values recorded in mussels at 17 °C without SLS (Fig. 4C).

4. Discussion

Although marine organisms are exposed to periodic temperature fluctuations, several studies have provided evidence that temperature variations have a negative impact on their survival, reproduction, growth, population abundance, and biochemical performance (Pörtner et al., 2007; Matoo et al., 2013; Matozzo et al., 2013; Munari et al., 2011; Tomanek, 2012). Furthermore, exposure to xenobiotics can lead to alterations in the cellular homeostasis of organisms, possibly causing oxidative stress (Hoarau et al., 2004; Livingstone, 2003). On top of this, recent studies have proven that warming threatens aquatic ecosystems, by affecting the sensitivity of organisms to pollution and/or modifying the toxicity of contaminants (among others, Attig et al., 2014; Banni et al., 2014; Izagirre et al., 2014; Kamel et al., 2012; Nardi et al., 2017; Velez et al., 2017). Consequently, studies on the interactive effects of temperature with pollutants, including SLS, are significant for understanding the response patterns in aquatic organisms and the factors that may limit their survival. Regarding SLS, previous studies have already demonstrated the ability of this surfactant to stimulate intracellular ROS levels (Mizutani et al., 2016). However, pollutants such as SLS can also cause alterations in the metabolism of organisms (Freitas et al., 2020; Lopes et al., 2022) that may affect their ability to activate defense mechanisms, increasing oxidative stress-induced injuries.

In the present study, it was observed a very low accumulation of SLS

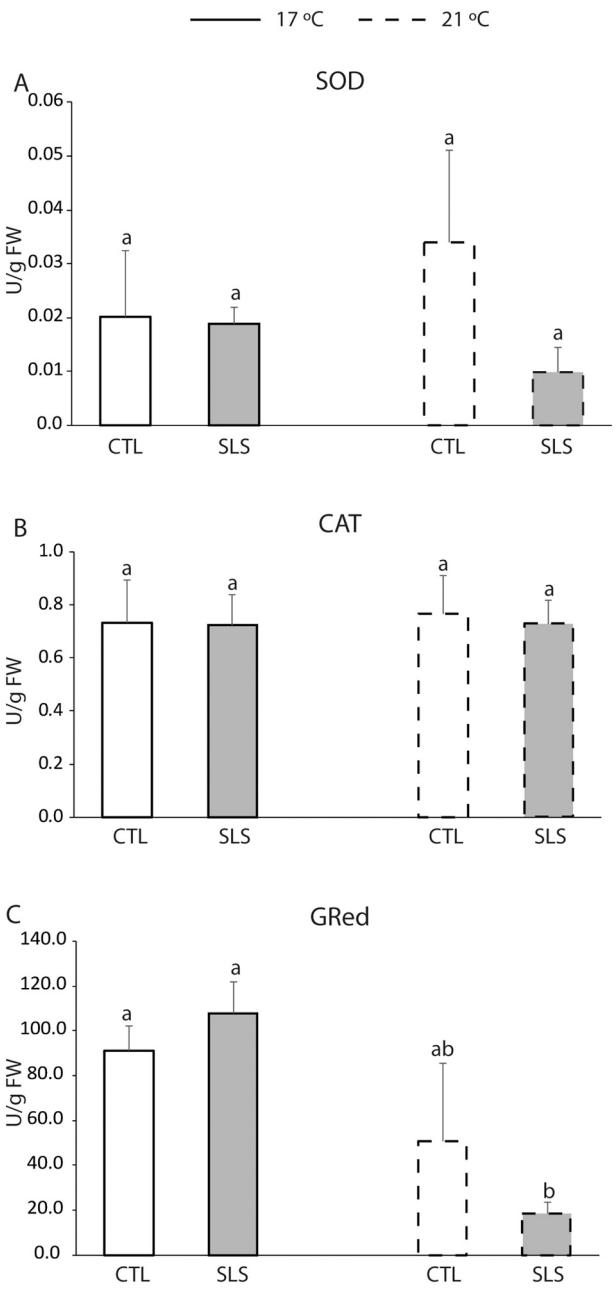

Fig. 2. A: Superoxide dismutase (SOD) activity, B: Catalase (CAT) activity and C: Glutathione reductase (GRed) activity in *Mytilus galloprovincialis* maintained at 17 °C and 21 °C to control (CTL, 0.0 mg/L) and 2.0 mg/L sodium lauryl sulfate (SLS) for 7 days. Results are mean values + standard deviation. Significant differences ($p < 0.05$) among treatments are represented by different letters, while the same letter represents no significant differences. White bars represent no contamination; grey bars represent SLS-exposed treatments.

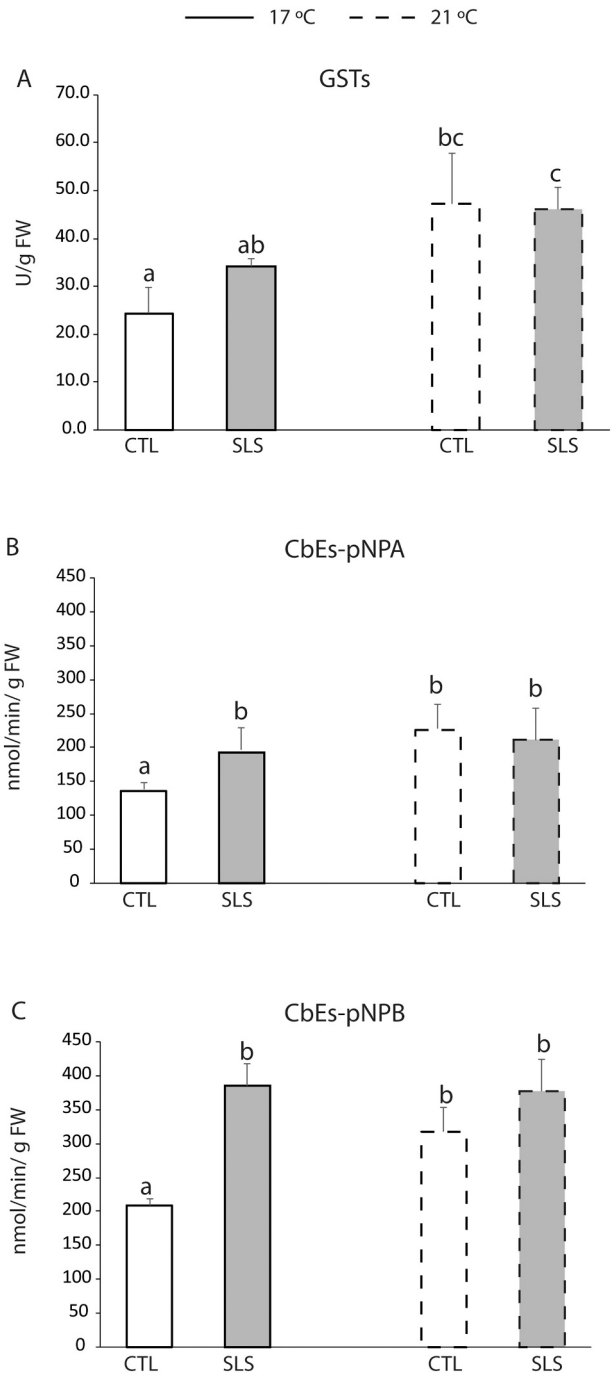

Fig. 3. A: Glutathione S-transferases (GSTs) activity, B: Carboxylesterase activity using *p*-nitrophenyl acetate substrate (CbEs_pNPA) and C: Carboxylesterase activity using *p*-nitrophenyl butyrate (CbEs_pNPB) substrate, in *Mytilus galloprovincialis* maintained at 17 °C and 21 °C to control (CTL, 0.0 mg/L) and 2.0 mg/L sodium lauryl sulfate (SLS) for 7 days. Results are mean values + standard deviation. Significant differences ($p < 0.05$) among treatments are represented by different letters, while the same letter represents no significant differences. White bars represent no contamination; grey bars represent SLS-exposed treatments.

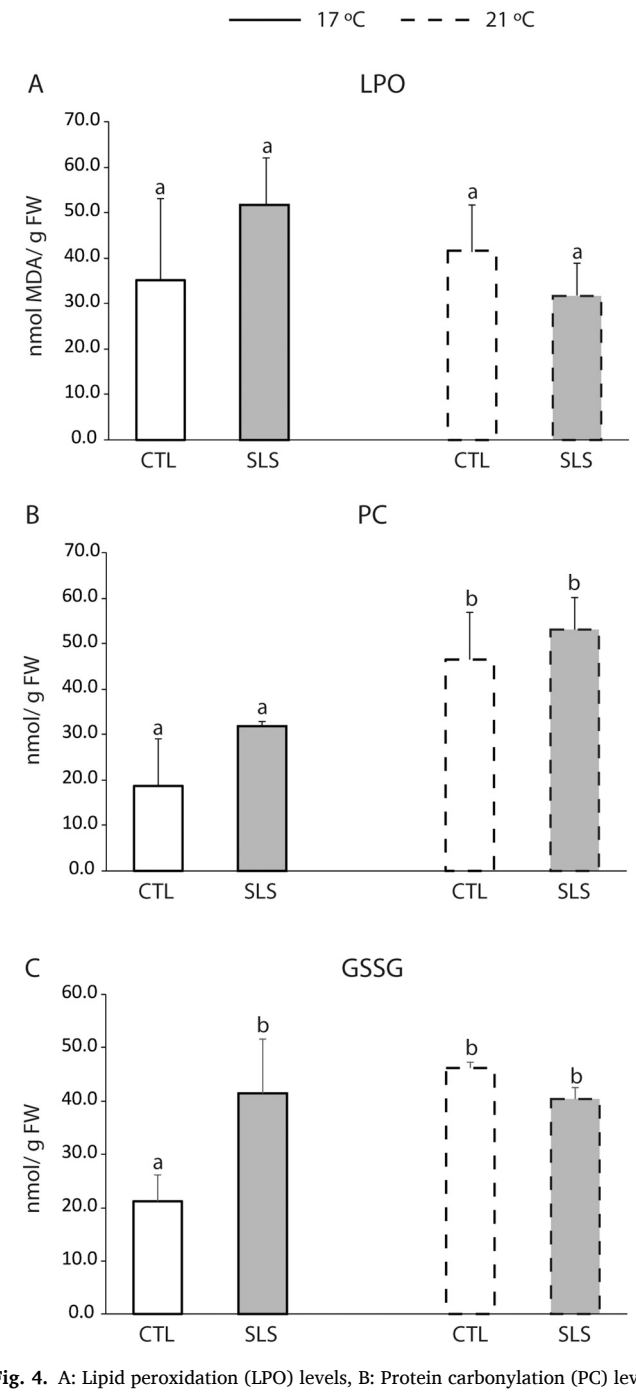

Fig. 4. A: Lipid peroxidation (LPO) levels, B: Protein carbonylation (PC) levels and C: oxidized glutathione (GSSG) content, in *Mytilus galloprovincialis* maintained at 17 °C and 21 °C to control (CTL, 0.0 mg/L) and 2.0 mg/L sodium lauryl sulfate (SLS) for 7 days. Results are mean values + standard deviation. Significant differences ($p < 0.05$) among treatments are represented by different letters, while the same letter represents no significant differences. White bars represent no contamination; grey bars represent SLS-exposed treatments.

by mussels within the 7 days of exposure. Differently from these results, Freitas et al. (2020), in a long-term (28-day) study assessed the toxic effects of SLS on *M. galloprovincialis* and observed a dose-dependent accumulation of this chemical, with higher values at the highest exposure concentration, identifying how, in a long-term, organisms can accumulate SLS, leading to subsequent alterations in organisms' metabolism and oxidative status. In particular, after 28 days at 2 mg/L (the same concentration used in our study), the SLS in tissues was 1.11 ± 0.38 μg/g, one order of magnitude higher than the levels measured in our study. Therefore, low concentrations of SLS in mussels' tissues observed in the present study may mostly result from the short exposure period but also from increased detoxification capacity observed, as phase I and II biotransformation enzymes (CbEs and GSTs, respectively) increased their activities in SLS-exposed mussels after a short exposure.

In line with our study, Capolupo et al. (2016), in a 7-day in vivo exposure of *M. galloprovincialis* to caffeine, found no drug accumulation, speculating a possible metabolism of the substance. Gomez et al. (2012) also reported how *M. galloprovincialis* absorbed and eliminated benzodiazepines (BZP) according to first-order kinetics while avoiding bioaccumulation in a 7-day *in vivo* assay. Since few studies exist on the effects of SLS, evaluating the effects of this substance over a short period is therefore critical to better understand the accumulation/detoxification of this chemical by filter-feeding organisms under realistic environmental conditions.

The measurement of the metabolic capacity has been used to assess the response of several marine invertebrate species to environmental disturbances (Bielen et al., 2016; Cammen et al., 1990; Freitas et al., 2016; Simčič et al., 2014) and ETS activity has been used as a reliable biomarker (De Coen and Janssen, 1997; Gagne et al., 2006). The results obtained in the present study showed that after 7 days, mussels exposed to stressful conditions (SLS and warming) tended to increase their metabolic capacity. Nevertheless, significant differences to control were only observed for SLS-exposed mussels at 17 °C. These results may indicate the capacity of mussels to activate their defense mechanisms in the presence of SLS at 17 °C while at increased temperature (both with and without SLS) mussels may have a different strategy limiting their metabolism (probably reducing their filtration rate). A similar response has already been observed with short-term (96-h) exposure to PPCPs. For example, Oliveira et al. (2017) reported an increase in ETS activity in *M. galloprovincialis* in relation to increased carbamazepine (CBZ) concentration. Bordalo et al. (2020) also reported a similar response, describing how ETS metabolic activity increased in mussels subjected to 96-h exposure to intermediate (0.0001 mg/L) concentrations of the UV filter benzophenone3 (BP3) but decreased at higher concentrations (0.001 mg/L), as the mussels are no longer able to increase metabolic activity at too high-stress levels. Regarding the effects of temperature on bivalves' metabolism, Coppola et al. (2017) already demonstrated the negative effect of warming on ETS activity, with limited metabolic capacity in *M. galloprovincialis* exposed for 28 days to Hg and warming (21 °C). This fact has already been confirmed by other authors, including Almeida et al. (2021a) who had described a metabolic depression in *R. philippinarum* exposed for 28 days to antiepileptic and antihistamine drugs (carbamazepine (CBZ) and cetirizine (CTZ) respectively) at 21 °C compared to those at 17 °C. Since the results here presented did not show an additional metabolic activation in contaminated mussels maintained at higher temperatures this may suggest that above certain levels of stress, mussels are not able to continue to active their metabolism. Such results might be in line with the published literature, reporting that bivalves decrease their ETS activity after a longer exposure period or when subjected to a combination of stressors.

Mussels exposed to SLS (17 °C and 21 °C) and warming conditions presented a higher protein content than CTL17 mussels, which may result from increased ETS activity and can indicate the ability of mussels to increase enzyme production under stress conditions, particularly enzymes involved in defense mechanisms against oxidative stress (e.g. antioxidant enzymes). According to Smolders et al. (2003), pollution triggers an increase in the synthesis of proteins related to defense processes in aquatic organisms. Other studies have shown how PPCPs can lead to higher PROT content in bivalves after acute exposures, as reported by Almeida et al. (2014) in *R. decussatus* after 96-h exposure to the highest concentrations (0.009 mg/L) of CBZ. Lopes et al. (2022) also reported that *M. galloprovincialis* subjected to a combination of increased temperature (21 °C) and 17 α-ethinylestradiol (EE2) had an increase in PROT content following a 28-day chronic exposure. Similarly, Cruz et al. (2016) showed that PROT content increased in *R. philippinarum* exposed to increased caffeine concentration for 28 days. Overall, these findings might point out that the PROT content can be maintained for longer periods under stressful conditions, which represents a tradeoff between enzyme production and elimination.

In addition to the altered metabolic capacity and energy reserve content, increased production of reactive oxygen species (ROS) may occur in response to exposure to stressful conditions. This can lead to responses of organisms to counteract oxidative stress and cellular damage, through the activation of their antioxidant defenses, such as antioxidant enzymes like SOD, CAT and GRed (Regoli and Giuliani, 2014). Nevertheless, above a certain limit, organisms may not be able to continue to enhance their antioxidant defenses and these may be even inhibited (Regoli et al., 2011). Especially after a short exposure period, before organisms may be able to adapt to stress conditions, antioxidant enzymes can be inhibited (Gonzalez-Rey and Bebianno, 2011) which was the response observed in the present study, in particular in contaminated mussels at 21 °C. Thus, we hypothesize that the lack of antioxidant defenses might be a consequence of high stress caused due to warming and especially warming associated with SLS exposure, which could be related to limited ETS activation observed at warming conditions and low organisms' adaptation capacity after short-term exposure.

Although no efficient antioxidant capacity was observed, detoxification was enhanced in mussels exposed to SLS, warming or the combination of both stressors. GSTs are a group of enzymes known for their ability to catalyze the conjugation of the reduced form of glutathione (GSH) with xenobiotic substrates as a detoxification mechanism (Regoli and Giuliani, 2014). Carboxylesterases (CbE) are also important biotransformation enzymes, involved in the phase I enzyme system, contributing to the excretion of contaminants (Ribalta et al., 2015). In the present study, mussels increased GSTs and CbEs activities when contaminated or maintained at 21 °C, with no additive effects when both stressors were acting together. Increased levels of GSTs have already been reported in several studies assessing the effects of short-term exposure to PPCPs (see for instance Franzellitti et al., 2014; Capolupo et al., 2016; Ehiguese et al., 2020). Pirone et al. (2019) reported increased activity of GSTs following exposure of *M. galloprovincialis* to PPCPs and metals at different temperatures (17 °C and 22 °C) for a period of 28 days. These authors demonstrated that GSTs activity was higher in mussels exposed at 22 °C, regardless of the type and presence of the contaminant. Assessing the effects of metals on CbEs, Moleiro et al. (2022) results showed higher CbEs activity in contaminated *M. galloprovincialis* mussels, especially in those exposed to higher temperatures (21 °C). Given the very low SLS bioaccumulation observed in the present study, we can hypothesize that GSTs and CbE enzymes acted as a detoxification strategy in mussels exposed to SLS, preventing its accumulation. As stated above, previous studies already demonstrated that GSTs catalyze the conjugation of GSH with a variety of toxic compounds, including xenobiotics such as SLS (Lopes et al., 2022). At the same time, considering their role as antioxidant enzymes (Regoli and Giuliani, 2014), GSTs can also protect macromolecules from attack by reactive electrophiles, such as ROS products. When under stressful conditions organisms tend to oxidize their glutathione, converting GSH into its oxidized form (GSSG), with the involvement of GSTs, which may occur when organisms were subjected to temperature rise (Verlecar et al., 2007). In terms of CbEs literature has described their role in xenobiotics detoxification (Ribalta et al., 2015) but no studies demonstrated their protective role in temperature increase. The results on the PROT content observed in mussels exposed to SLS, 21 °C and the combination of both stressors could thus support GSTs and CbEs results, with higher PROT content being associated with higher enzyme production, which resulted in increased enzyme activity.

Under stress conditions, organisms produce an excess of ROS that, if not eliminated by antioxidant defenses, can easily interact with membrane polyunsaturated fatty acids, causing lipid peroxidation (LPO) and protein carbonylation (PC) (Regoli and Giuliani, 2014). In the present study, although no LPO was observed, PC levels increased especially in mussels maintained at 21 °C (with and without SLS). The ability to avoid LPO could be related to increased detoxification, which in turn was not sufficient to avoid protein oxidation. Also, in agreement with this hypothesis, the study by Moleiro et al. (2022) on *M. galloprovincialis*, reported limited LPO and increased PC levels, especially with increasing

temperature (22 °C) and regardless of the presence of the contaminant, in this case Cobalt (Co). Other authors have also reported an increase in PC although no lipid damage occurred under heating conditions and/or in the presence of contaminants. Among them, Paital and Chainy (2013) described how LPO and PC were measured in different tissues (gills, hepatopancreas and muscle) of mud crabs (*Scylla serrata)* and at different temperatures (winter and summer). It was observed that while PC had higher values in summer than in winter, LPO remained at the same levels, with no changes related to temperature change. A study by Pirone et al. (2019) found a similar response, i.e., prevention of LPO and PC occurrence in mussels exposed for 28 days simultaneously to elevated temperatures (22 °C), lead and lead + Triclosan. The fact that no LPO occurred after 7 days of exposure, while the proteins were carbonylated, could indicate more rapid oxidation of proteins than lipids.

Glutathione reductase is an enzyme responsible for the conversion of GSSG to GSH and thus crucial for maintaining organisms' redox homeostasis. In the present study, GRed levels decreased significantly in mussels exposed to warming and SLS, which could explain the increased GSSG content in those treatments. This result is also in agreement with studies that have shown an accumulation of GSSG following 96-hour exposure to SLS in the fish *Gambusia holbrooki* (Nunes et al., 2008). Regarding the literature on long-term studies, Morosetti et al. (2020) observed similar responses in the same mussel species, with the inhibition of GRed after 28 days of exposure to cerium nanoparticles Ce NPs and Hg under warming conditions. These authors also associated the decrease in antioxidant capacity due to warming with a loss of metabolic performance, results that are in agreement with those observed in the present study.

5. Conclusions

This study provides relevant data on the adverse effects of SLS alone and combined with increasing temperature on the model species *Mytilus galloprovincialis*, representative of marine filter feeders. Results showed clear evidence of increased metabolic activity that can prevent SLS bioaccumulation and limit the onset of relevant oxidative stress and damage in organisms subjected to multiple stress conditions after a short-term exposure of 7 days. The study represents an important contribution to filling knowledge gaps regarding the impacts of short-term exposures to PPCPs, temperature rise and bioaccumulation of these substances since most studies have evaluated effects after chronic exposures. The inclusion of an additional external factor such as temperature may help to better understand the combined effects of climate change and PPCPs on aquatic organisms, addressing more realistic exposure scenarios.

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.

Data availability

Data will be made available on request.

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