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The influence of salinity on sodium lauryl sulfate toxicity in *Mytilus galloprovincialis*

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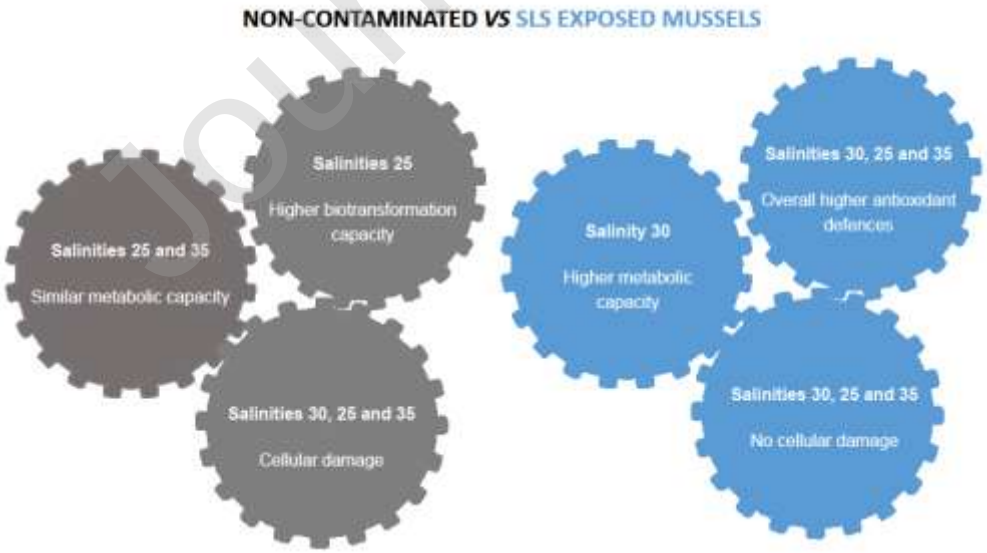
Graphical abstract



Highlights

- Higher Sodium Lauryl Sulfate (SLS) concentration in mussels' tissues at salinity 25
- At control salinity (30) contaminated mussels increased their metabolic capacity
- Lower metabolic and detoxification capacity in SLS mussels at salinities 25 and 35
- Higher antioxidant capacity in SLS exposed mussels (except for catalase)
- Higher cellular damage in non-contaminated mussels, especially at salinity 35

ABSTRACT

The influence of salinity on the effects of sodium lauryl sulfate (SLS) was evaluated using the Mediterranean mussel *Mytilus galloprovincialis*, exposed for 28 days to SLS (0.0 mg/L - control and 4.0 mg/L) under three salinity levels (Control-30, 25 and 35). The effects were monitored using biomarkers related to metabolism and energy reserves, defence mechanisms (antioxidant and biotransformation enzymes) and cellular damage. The results revealed that non-contaminated mussels tended to maintain their metabolic capacity regardless of salinity, without activation of antioxidant defence strategies. On the contrary, although contaminated mussels presented decreased metabolic capacity at salinities 25 and 35, they were able to activate their antioxidant mechanisms, preventing cellular damage. Overall, the present findings indicate that SLS, especially under stressful salinity levels, might potentially jeopardize population survival and reproduction success since reduced metabolism and alterations on mussels' antioxidant mechanisms will impair their biochemical and, consequently, physiological performance.

Keywords: Personal care products; bioaccumulation; metabolism; oxidative stress; bivalves.

1. INTRODUCTION

Global warming, leading to sea level rises, extreme weather events and acidification are among the main threats to estuaries and coastal lagoons (Elliott et al., 2019; IPCC, 2019). The average sea level leads to a consequent increase of seawater intrusion in estuaries, which can be exacerbated by periods of drought and changes in drainage due to human activities, which pose clear threats to biodiversity (Elliott et al., 2019; Hallett et al., 2018; Marques et al., 2017; Ross et al., 2015; Zahid et al., 2018). Recent studies have revealed that the frequency and intensity of extreme weather events, such as heavy rains are growing, which in transition environments can act as a disruptive agent (IPCC, 2019). These events lead to changes in the salinity of coastal areas, with implications for ecosystem processes and services, including alterations in the structure and functioning of marine and estuarine communities (Cardoso et al., 2008; Chollet and Bone, 2007; Grilo et al., 2011; Muresan et al., 2020; Smyth and Elliott, 2016; Velasco et al., 2019). At lower biological levels salinity changes can also influence the metabolic, physiological and biochemical patterns of various species (Carregosa et al., 2014a; Fazio et al., 2013; Freitas et al., 2020a, 2019a; Paital and Chainy, 2010 and 2012; Rodrigues et al., 2012; Velez et al., 2016, 2017). In addition, salinity changes may alter the sensitivity of species to pollutants and/or the behaviour of pollutants altering their toxicity (Rodrigues et al., 2014; De Marchi et al., 2020, 2018; Moreira et al., 2018; Freitas et al., 2020a).

As a consequence of population growth and industrialization, a vast variety of potential pollutants now reach coastal environments, that may pose risks to transitional systems and inhabiting species. The recent proliferation of human health care products and industrial activity have exacerbated the discharge of potentially toxic effluents into the aquatic environment, despite attempts at mitigation. Among the most common pollutants reaching the coastal systems are surfactants, including sodium lauryl sulfate (SLS), also known as sodium dodecyl sulfate (SDS). Surfactants are a diversified group of chemical compounds whose structure is characterized by the presence of hydrophobic and hydrophilic sites and are crucial for the solubilization of organic contaminants (Bhatt et al., 2020; Fink, 2020; Nakama, 2017). This property allows the surfactant to

interact with both polar and non-polar agents. These products are used in large volumes in cleaning products such as detergents and powdered soaps and in various cosmetics, such as bath salts, soaps, acne treatment creams, exfoliating products, hair paint, liquid soaps, conditioners, facial cleaning products and shampoos (Bondi et al., 2015; Holmberg and Laughlin, 1997; Lai, 2006; Singh et al., 2007). As an example, SLS has been identified as an ingredient of a wide variety of shampoo brands (AlQuadeib et al., 2018), and may represent 5% of the ingredients used in soaps (Chirani et al., 2021). Recently, as a consequence of COVID-19 pandemic, the world health organization (WHO) has recommended that individuals wash hands more frequently and at least for 20 s to ensure bacterial and virus removal from the skin, which has of course increased the use of detergents and soaps containing SLS (Jahromi et al., 2020; Chirani et al., 2021). The use of surfactants has been growing since the 1980s, from the 1.7 million tons used in 1984 and 9.3 million tons used in 1995 (Aboulhassan et al., 2006). Amid the COVID-19 crisis, the global market for SLS estimated at US$525.4 M in the year 2020, is projected to reach a revised size of US$ 666.4 M by 2027, growing at a compound annual growth rate (CAGR) of 3.5% over the period 2020-2027 (GLOBE NEWSWIRE). Though most surfactants are degradable (biodegradation of SLS ranges from 45% to 95% within 24 h), the sheer scale of their use contributes to increasing concentrations reaching coastal areas (Cserháti et al., 2002; Quiroga et al., 1989; Marcomini et al., 2000; Mustapha and Bawa-Allah, 2020; Olkowska et al., 2011, 2014; Rosety-Rodríguez et al. 2002). Accompanying the increasing production and use of surfactants, the information on SLS potential toxicity has been also increasing (Aguilar-Alberola and Mesquita-Joanes, 2012; Bhattacharya et al., 2021; Chaturvedi and Kumar, 2010; Freitas and Rocha, 2012; Lechuga et al., 2016; Messina et al., 2014; Mustapha et al., 2020; Nunes et al., 2005, 2008; Oliveira et al., 2020; Park et al. 2016; Rocha et al., 2007; Villegas-Navarro et al., 2001; Wang et al., 2015; Ying, 2006), although the impact of these agents on bivalves is underinvestigated (Freitas et al., 2020c).

In aquatic systems, the behavior and bioavailability of pollutants and, consequently, their potential for bioaccumulation and toxicity can be strongly altered by factors related to climate

change related factors. Previous studies already observed that salinity may influence metals bioavailability and toxicity (Zhang et al., 2014; Noyes and Lema, 2015). In addition, the response and sensitivity of organisms may be conditioned by factors related to climate change, altering their ability to respond to pollutants. Despite extensive investigation, the interaction between pollutants and climate change and their impact on marine organisms is not fully understood (Almeida et al., 2018; Costa et al., 2020a,b; De Marchi et al., 2020, 2019, 2018, 2017a; Freitas et al., 2020a, 2019a, 2017a,b; Morosetti et al., 2020; Munari et al., 2018).

Thus, this investigation aimed to determine the influence of salinity (Control, 30; 25 and 35) on the impacts caused by SLS (4.0 mg/L) in the mussel species *Mytilus galloprovincialis*. For this, biomarkers related with mussels' oxidative stress and metabolism were evaluated after a chronic exposure (28 days).

2. MATERIALS AND METHODS

2.1. Experimental conditions

Mytilus galloprovincialis specimens were collected in the Ria de Aveiro (Portugal). Organisms of similar size (condition index: 10.64 ± 1.92) were chosen to avoid interference with accumulation and biochemical responses.

After sampling, organisms were transported to the laboratory for acclimation during fifteen days before exposure, in aquaria containing 20 L of artificial seawater, prepared by the addition of artificial sea salt (Red Sea Salt®) to deionized water. The selection of the sea salt was based on its composition (containing recommended major elements such as Calcium, Magnesium, Potassium) trying to mimic as much as possible the natural seawater composition. During this period, organisms were maintained at 17.0 ± 1.0 °C; pH 8.0 ± 0.1, salinity 30 ± 1, under continuous aeration. Aquaria seawater was renewed every two days and, after the first week, animals were feed every two-three days with AlgaMac Protein Plus, Aquafauna Bio-Marine, Inc (150 000 cells/animal/day).

After this period, specimens were distributed in 7 L aquaria, with seven individuals per aquarium and three aquaria per treatment, with seawater conditions maintained as during the acclimation (salinity 30 ± 1, temperature 17 ± 1.0 °C, pH 8.0 ± 0.1).

During the experimental period of twenty-eight days mussels were exposed to control (non-contaminated mussels; CTL, 0.0 mg/L) and 4.0 mg/L of Sodium dodecyl sulfate (SLS), under 3 salinity levels (30, 25 and 35), making a total of 6 experimental treatments. Sodium lauryl sulfate (SLS salt) used in the experiment was obtained from Sigma-Aldrich (chemical purity ≥99%; molecular weight 288.4). The concentration selected already showed to occur in aquatic environments and to induce effects in non-target species (e.g., Bondi et al., 2015; Brunelli et al., 2008; Gibson et al., 2016; Marcomini et al., 2000; Rosety et al., 2001). Different seawater salinities

were achieved by the addition of different amounts of artificial sea salt (Red Sea Salt®) to deionized water.

During the experiment all the aquaria were monitored to maintain constant the conditions of the water medium and animals were fed as during the acclimation period. The exposure medium was renewed weekly, feces were removed and medium conditions were re-established. No mortality was recorded along the experimental period.

Weekly, after medium renewal and concentration reestablishment, water samples were collected from each aquarium to determine SLS concentration, aiming to determine real exposure concentrations.

At the end of the experimental period three individuals per aquarium (nine per treatment) were frozen with liquid nitrogen and preserved under -80 ºC until biochemical analyses and SLS quantification.

2.2. Sodium dodecyl sulfate quantification in water samples and mussels' tissues

Water

Concentrations of SLS in water samples were measured at 650 nm (see the supplementary material for details). A calibration curve (0.1-10 ppm) was established using SLS as the reference compound. The limit of detection (LOD) was 0.05 ppm (0.05 mg/L) (see Supplementary material for details).

Tissues

Tissues were extracted with 5 mL of water and centrifuged for 5 min. at 3000 rpm. The supernatant was collected after centrifugation and treated as reported for water samples (see the supplementary material for details). A calibration curve (0.01-10 ppb) was established using SLS as

the reference compound. The limit of detection (LOD) was 0.005 ppm (5 µg/g) (see Supplementary material for details).

For each treatment, Bioconcentration Factor (BCF) was determined by dividing the mean concentration of SLS present in mussels' tissues by the mean concentration of SLS determined in the exposure medium.

2.3. Biological responses

The whole soft tissues from each individual were homogenised separately with liquid nitrogen and distributed by aliquots of 0.5 g fresh weight (FW). Tissue samples from each individual were used to determine the following biomarkers: electron transport system activity, ETS; total protein content, PROT; glycogen content, GLY; lipids content, LIP; superoxide dismutase activity, SOD; catalase activity, CAT; glutathione peroxidase activity, GPx; glutathione reductase, GRed; glutathione S-transferases activity, GSTs; lipid peroxidation levels, LPO; protein carbonylation, PC. See the Supplementary Material for details.

2.4. Data analysis

Concentrations of SLS in water samples and mussels tissues as well as biochemical results (ETS, GLY, PROT, LIP, SOD, CAT, GPx, GRed, GSTs, LPO, PC) were submitted to hypothesis testing using permutational multivariate analysis of variance with the PERMANOVA+ add-on in PRIMER v6 (Anderson et al., 2008). Pairwise comparisons were performed and values lower than 0.05 were considered as significantly different. The null hypotheses tested were: i) for each SLS treatment (SLS30, SLS25, SLS30), 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 uppercase letters; ii) for each week, no significant differences were observed

among SLS treatments (SLS30, SLS25, SLS30) in terms of SLS concentration measured in water; significant differences among treatments are represented in Table 1 with different lowercase letters; iii) for SLS concentrations in mussels tissues, no significant differences exist among SLS treatments (SLS30, SLS25, SLS30); significant differences among treatments are represented in Table 1 with different lowercase letters; iv) for each biomarker, no significant differences exist among treatments; significant differences among treatments are presented in figures with different lowercase letters for non-contaminated mussels and uppercase letters for SLS exposed mussels.

The matrix containing the biochemical results was normalised, the Euclidean distance matrix was obtained and simplified through the calculation of the distance among centroids based on treatments, and then submitted to ordination analysis, performed by Principal Coordinates Ordination analysis (PCO). 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 samples and mussels' tissues

For each treatment, SLS concentrations in water samples collected weekly immediately after spiking showed, in general, no significant differences among weeks (Table 1). For each week, SLS concentrations found at salinity 35 treatment tended to be significantly lower than concentrations found at salinities 30 and 25 (Table 1).

Concentrations of SLS found in mussels' tissues were significantly higher at the lowest salinity (25) in comparison to control salinity (30) (Table 1).

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

3.2 Biochemical responses

3.2.1 Metabolic capacity and energy reserves

In terms of ETS activity non-contaminated mussels' showed no significant differences among salinities, while SLS exposed mussels' presented significantly higher values at control salinity (30) in comparison to salinities 25 and 35. Significant differences between non-contaminated and contaminated mussels' were only identified at control salinity, with higher values in SLS exposed organisms (Figure 1A).

Non-contaminated mussels' tended to present higher GLY content at stressful salinity conditions, with significantly higher values at the highest salinity in comparison to salinities 30 and 25. *M. galloprovincialis* exposed to SLS revealed significantly higher GLY content at salinities 25 and 35 in comparison to salinity control. Non-contaminated mussels' presented significantly higher GLY content than SLS exposed mussels' at salinities 25 and 35 (Figure 1B).

The PROT content in non-contaminated mussels showed no significant differences among salinities, while in SLS exposed mussels' significantly lower PROT concentration was observed at

the highest salinity. Significant differences between contaminated and non-contaminated mussels were identified only at salinity 35, with lower values in SLS exposed organisms (Figure 1C).

The LIP content was significantly higher at salinity 35 in non-contaminated mussels, while contaminated organisms showed significantly lower values at the lowest salinity. Significantly higher values were found in SLS exposed mussels comparing with non-contaminated mussels at salinity control and the lowest salinity (Figure 1D).

3.2.2 Oxidative stress

The activity of SOD was significantly higher in non-contaminated mussels maintained at salinities 25 and 35. SLS exposed mussels showed significantly lower SOD activity at salinity 25 in comparison to salinity 35. Significantly higher activity was found in SLS contaminated mussels at salinity control, while and opposite patterns was revealed at salinity 25 (Figure 2A).

The activity of CAT was significantly higher in non-contaminated mussels maintained at salinity 35 in comparison to control salinity. Mussels exposed to SLS presented significantly higher CAT activity at salinity 35 in comparison to salinities 30 and 25. Non-contaminated mussels showed significantly higher CAT values than SLS exposed mussels, regardless the salinity (Figure 2B).

The activity of GPx was significantly higher in non-contaminated mussels maintained at salinities 25 and 35. When in the presence of SLS mussels revealed no significant differences among salinities. Significantly higher values were found in SLS contaminated mussels at salinity control (Figure 2C).

The activity of GRed was significantly lower in non-contaminated exposed to salinity 25 and significantly higher at salinity 35. Mussels exposed to SLS and salinity 35 showed significantly lower GRed activity. Significantly higher GRed activity was observed in SLS exposed organisms at salinities 30 and 25 (Figure 2D).

In the absence of SLS mussels showed significantly higher GSTs activity at the lowest salinity, while contaminated mussels revealed significantly lower activity at salinities 25 and 35. Significant differences between non-contaminated and contaminated mussels were observed at salinity 25, with higher values recorded in non-exposed organisms (Figure 3).

Levels of LPO in non-contaminated mussels were significantly higher at salinities 25 and 35, with the highest values at the highest salinity. No significant differences were observed among salinities for SLS exposed mussels. Significantly higher LPO values were recorded in non-contaminated mussels maintained at salinities 25 and 35 (Figure 4A).

In the absence of SLS mussels showed significantly higher values at salinity 35, while in non-contaminated mussels no significant differences were observed among salinities. At each salinity, significantly higher PC values were observed in non-contaminated mussels (Figure 4B).

3.3 Data analysis

PCO analysis is presented in Figure 5. The first principal component axis (PCO1) represents 50.3% of the variability, separating non-contaminated (positive side) and individuals exposed to SLS (negative side). PCO2 axis explained 23.8% of the variability, separating control organisms (non-contaminated at salinity 30) and SLS exposed organisms at salinity 25 (negative side) from the remaining treatments (positive side). GLY, PC and CAT are the vectors that best explained PCO1 positive side ($p>0.85$), while ETS, LIP and GPx were the vectors that best explained PCO1 negative side ($p>0.75$).

4 DISCUSSION

Pollutants such as SLS, are already known to cause alterations in bivalves' oxidative status and metabolism (Freitas et al., 2020b; Messina et al., 2014). However, less is known concerning the impacts of pollutants when acting in combination with climate change related factors, especially under salinity shifts (De Marchi et al., 2018, 2020; Freitas et al., 2020a; Rodrigues et al., 2014; Velasco et al., 2019). Predicted extreme events, including heavy rain periods or marine heat waves, together with increasing sea level rise, will contribute to salinity shifts in coastal systems, especially estuaries and lagoons due to their limited hydrodynamic capacity and low depth (Frölicher et al., 2018; Remy et al., 2017; Grilo et al., 2011). Salinity is among the main environmental factors that limits the survival of species, biomass and distribution within estuarine systems. In the case of bivalves, being osmoconformers, salinity fluctuations can trigger internal adaptive processes which are energetically efficient but costly to the organism, in its efforts to maintain osmotic balance. Hence, such changes in the balance between metabolic performance, oxidative stress and energy production that characterize the body's acclimatization capacity can influence the ability of a species to maintain its population in a given environment (Carregosa et al., 2014a). Among other consequences, salinity changes in estuaries have been associated with changes on brackish and marine benthic and pelagic communities' spatial distribution, and a decrease in freshwater fauna diversity and abundance (Grilo et al., 2011). Furthermore, unpredictable frequency and intensity of rainfall events impact groundwater recharge patterns and associated contaminant leaching and waterborne transport (Jarsjö et al., 2020; Jonsson et al., 2017). In particular, climate change has been projected to induce 10 to 50% runoff increases for large coastal regions globally and, therefore, inputs of pollutants to ecosystems are expected to increase substantially in the future (Jonsson et al., 2017). In addition, physical disturbance associated with extreme weather events leads to faster release of metals accumulated in sediments compared to biological disturbances (e.g.,

bioturbation) (Coelho et al., 2014). Altogether, salinity shifts and increasing pollutants loads, will not only alter pollutants bioavailability and toxicity but also organisms' performance.

The present study clearly revealed the influence of salinity on SLS toxicity in *Mytilus galloprovincialis,* changing not only bioaccumulation patterns but also mussels' metabolic capacity and oxidative status. In fact, the results obtained demonstrated a clear distinction between non-contaminated and contaminated mussels, characterized by differences in organisms' metabolism, energy reserves content, antioxidant and biotransformation capacity and cellular damage (see Figure 5).

Regarding SLS concentrations in mussels' tissues, the present findings showed that SLS accumulation might be influenced by salinity, with contaminated mussels maintained under the lowest salinity (25) presenting the highest SLS concentration and bioaccumulation factor in comparison to mussels under salinities 30 and 35. Sammalkorpi et al. (2009) investigated the behavior of SLS micelles in the presence of NaCl and revealed that the excess of salt leads to larger micellar aggregates than in its absence. We can thus hypothesize that at the highest salinity larger aggregates were formed, which tend to sink at the bottom of the aquaria and, consequently, less amount was present in the medium for accumulation through mussels' filtration. In this context, it was demonstrated that in marine bivalves the uptake of metals increased with decreasing salinities (Ali and Taylor, 2010; Blackmore and Wang, 2003; Moreira et al., 2016; Wright, 1995), which was explained by the fact that low salinity and the inherent decrease in chloride concentrations, lead to decreased trace metal ion complexation, and thus leads to an increase of free metal ions. Such ions have been identified as the main forms that marine invertebrates can take up metals (Campbell, 1995), facilitating accumulation and related toxicity in bivalves. Although SLS is not a metal, the present results are in agreement with these findings that showed higher accumulation of metals and metalloids under low salinity conditions. For example, the mussels *M. galloprovincialis* maintained in low salinity seawater accumulated three times more arsenic (As) than those held at full strength seawater (Ünlu and Fowler, 1979). Also, higher As concentrations were measured in oysters

(*Crassostrea angulata*) under lower salinity (Moreira et al., 2016). The results here present further demonstrated that at salinity 30 mussels tended to present lower SLS concentration, which may reveal the capacity of organisms to limit pollutants accumulation under salinity control conditions.

Under stressful conditions, including the presence of pollutants and salinity levels alterations, bivalves may alter their metabolism, affecting their energy reserves, in an effort to limit accumulation of pollutants and/or activate strategies to eliminate pollutants and/or increase defenses to avoid injuries (among others, Coppola et al., 2019; Cruz et al., 2016; Freitas et al., 2017a; Freitas et al., 2019b; Morosetti et al., 2020; Oliveira et al., 2017). Therefore, energy reserves (such as glycogen, protein and lipids content) and the activity of the mitochondrial electron transport system (ETS) are important indicators of pollutants impacts (Smolders et al., 2004). Regarding mussel metabolic capacity, the results obtained showed that ETS activity in non-contaminated mussels was similar among different salinity levels, highlighting the ability of mussels to maintain their ETS activity at a salinity range between 25 and 35, which may reflect salinity fluctuations in estuaries. Similarly, Moreira et al. (2016) showed that *C. angulata* presented similar ETS at salinities 20, 30 and 40, while at salinity 10 the ETS activity strongly increased. Nevertheless, SLS exposed mussels evidenced a significant increase of ETS activity at control salinity (30) in comparison to organisms maintained at salinities 25 and 35 which showed similar ETS levels. These results may not explain higher SLS accumulation in mussels under salinity 25 since increased accumulation is normally accompanied by increased metabolic capacity, associated with higher filtration rate (Andrade et al., 2019a; Durieux et al., 2011). However, increased ETS activity at control salinity may indicate that mussels were under lower stress levels than the ones maintained at salinities 25 and 35, being able to enhance their metabolism, most probably to trigger defence mechanisms. These results could explain lower SLS accumulation at salinity 30 in comparison to salinities 25 and 35, being a result of higher mussels' detoxification capacity. The literature available shows that bivalves under stressful conditions can increase their antioxidant and biotransformation defences which result from organisms' increased metabolic capacity (De Marchi et al., 2017a, b) but can also follow a low-cost

energy strategy, by reducing their metabolism to avoid further injuries (Freitas et al., 2017a,b, 2019a,b; Pinto et al., 2019). As an example, Freitas et al. (2020b) showed an increase in mussels' metabolic capacity up to the concentration of 2.0 mg/L of SLS, while at the highest tested concentration (4.0 mg/L) mussels reduced their metabolism to control levels. These results may indicate that up to certain stress levels mussels increased their metabolism to activate and power defence mechanisms to combat the stress induced by SLS but at the highest concentration this capacity was no longer effective. The results here presented might indicate a similar response, with decreasing metabolism at the most stressful conditions, i.e., when mussels were exposed to the combined effects of SLS and salinities out of the control (30), evidencing an interactive effect between these two factors. Furthermore, the significantly higher ETS activity observed at control salinity in SLS exposed mussels in comparison to non-contaminated mussels could indicate that SLS had stronger impacts on mussels' metabolism than salinity since in non-contaminated mussels ETS activity was similar regardless the salinity level.

The deployment of energy reserves was already considered as a cellular protection mechanism when organisms are exposed to pollutants, most probably associated with increased metabolism and activation of defence mechanisms (Bielen et al., 2016; Coppola et al., 2019; Cruz et al., 2016). On the other hand, energy reserve accumulation may indicate a metabolic depression in an attempt to limit pollutants accumulation and toxicity. Results here presented revealed that in the absence of SLS mussels did not change their metabolic capacity regardless of the salinity, which resulted in the maintenance or increase (GLY and LIP at salinity 35) of their energy reserves. In SLS contaminated mussels' lower metabolic capacity at salinities 25 and 35 was accompanied by higher GLY content, indicating that the decreased metabolism was associated to the decrease in GLY expenditure. However, a different pattern was observed in terms of PROT content, with lower values at salinity 35 which may indicate lower enzymes production instead of PROT expenditure. Similarly, previous studies (Andrade et al., 2019b; Clements et al., 2019) demonstrated that bivalves exposed to pollutants revealed decreased GLY content in comparison to non-contaminated

organisms. Also, Freitas et al. (2020b) showed that mussels exposed to different SLS concentrations presented at the highest concentration of SLS (4.0 mg/L) a decrease in the PROT content, indicating that organisms were not capable to continue the production of PROT, including enzymes. In terms of LIP content, organisms exposed to SLS showed lower values at the lowest salinity, which may indicate that this combination represents the most stressful condition, demanding greater energy expenditure. Similarly, Andrade et al. (2019b) revealed that mussels under the most stressful condition (exposure to tides under temperature rise) mobilized LIP reserves, in an activity that the authors associated with the repair of damaged cellular structures.

Oxidative status may also be altered due to the presence of pollutants and salinity changes (Coppola et al., 2017; Freitas et al., 2020a,b; De Marchi et al., 2020; Messina et al., 2024). Regarding the impacts generated by salinity changes, in the absence of SLS, the present findings showed that the enzymes analysed (SOD, CAT, GPx, GRed) were mostly activated at stressful salinity levels, evidencing the capacity of mussels to increase their antioxidant defences to fight against the stress generated. Also Freitas et al. (2017b) showed enhanced antioxidant capacity in mussels exposed to the lowest tested salinity (14) but in this study organisms exposed to salinity 35 tended to present lower enzymes activity in comparison to organisms maintained at control salinity (30). These findings as well as the lowest GRed values observed in the present study at salinity 25 may indicate an overwhelmed condition at lower salinities, where enzymes are inhibited, a situation previously described in bivalves that are unable to overcome a stress condition (Morosetti et al., 2020; Franco et al., 2009). In what regards to SLS exposure, previous studies already revealed that this compound is responsible for higher production of reactive oxygen species (ROS) (Messina et al., 2014; Mizutani et al., 2016), leading to oxidative stress responses such as alteration in organisms' antioxidant mechanisms and cellular damage (among others, Freitas et al., 2020b; Messina et al., 2014; Nunes et al., 2008). The results obtained in the present study highlighted the capacity of mussels to increase their antioxidant defences when in the presence of SLS regardless the salinity tested, with higher enzymes activities (except for CAT) in comparison to non-

contaminated mussels. Furthermore, although lower PROT content was observed in SLS exposed mussels at salinity 35, mussels subjected to this treatment were able to significantly increase their antioxidant enzymes activities (except for GRed). Messina et al. (2014) studying the effects of SLS in *M. galloprovincialis* also demonstrated that enzymes SOD and CAT, involved in ROS scavenging, have higher activities in hepatopancreas and mantle of treated animals compared to untreated ones. Other authors also showed the increase of antioxidant defences in bivalves exposed to pollutants, namely pharmaceuticals and personal care products (PPCPs) (Almeida et al., 2018; Costa et al., 2020a,b; Freitas et al., 2020b). Lower CAT activity in the presence of SLS in comparison to non-contaminated mussels, regardless the salinity stress, could indicate an inhibitory effect of SLS towards this enzyme or could be related with the fact that GPx strongly increased it activity, an enzyme that performs the same function as CAT in ROS elimination. Previous studies exposing mussels to SLS also demonstrated greater activation of GPx along the increasing concentration gradient while in most of the concentrations CAT was inhibited (Freitas et al., 2020b).

Regarding biotransformation capacity, non-contaminated mussels showed higher GSTs activity at the lowest salinity which could result from higher ROS content (sourced from H_2O_2 dissociation). In particular, H_2O_2 can also activate GSTs activity, given that some GST isoforms may also catalyse the reduction of H_2O_2 to water (Regoli and Giuliani, 2014) which could explain higher GSTs activity in mussels maintained at salinity 25. Nevertheless, Moreira et al (2016) showed that *in Crassostrea angulata* varying salinity did not induce significant changes in GSTs activity. Also Zanette et al. (2011) did not find significant changes in GSTs activity in *C. gigas* exposed to salinities 9, 15, 25 and 35. In the presence of SLS mussels maintained at salinity 30 showed higher GSTs activity than non-contaminated mussels maintained under the same salinity, evidencing the detoxification of mussels in the presence of SLS and control salinity. Significantly lower GSTs activity in mussels under salinities 25 and 35 than at salinity control point out the

interactive effect of these two factors, which lead to enzyme inhibition and also contributed to higher SLS concentration at salinity 25.

The limited antioxidant defence capacity observed in non-contaminated mussels exposed to stressful salinity conditions led to higher LPO levels at salinities 25 and 35 and increased PC levels at salinity 35. Also Freitas et al. (2017) showed that mussels maintained at salinity 14 increased their cellular damages although antioxidant defences increased under this condition. Similarly, Moreira et al. (2014) demonstrated that oysters (*C. angulata*) presented higher LPO levels at salinities out of the control (10, 20, 40). On the other hand, no cellular damages were observed in SLS exposed mussels, regardless the salinity tested, indicating that the antioxidant defences were efficient in preventing damages of membrane lipids and proteins. The absence of LPO at salinities 25 and 35 may also result from lower ETS activity in mussels maintained under these conditions, since mitochondrial respiration is one of the major sources of ROS. Freitas et al. (2020b) previously demonstrated that only at the highest exposure concentration (4.0 mg/L of SLS) mussels showed increased LPO levels compared to non-contaminated mussels, but no PC was observed.

CONCLUSION

The combination of environmental stresses can result in a set of responses that vary from species to species, which may vary with changes in the environment (natural or anthropogenic). In aquatic systems, the behaviour and bioavailability of pollutants and, consequently, their bioaccumulation and toxicity potential can be strongly altered by factors related to climate change. In this context, very little is known about the interaction of climate changes with emerging pollutants. This study evaluated the influence of salinity on the toxicity induced by SLS in mussels. The results obtained demonstrated interactive effects between both factors, with greater accumulation at the lowest salinity and higher metabolic and defence mechanisms alterations in mussels exposed to SLS and salinities 25 and 35 compared to salinity control (30). Such biological

response, including decreased metabolism, lower energy reserves expenditure, activation of antioxidant defences prevented the occurrence of LPO and PC in contaminated mussels regardless the salinity level but might impair mussels' physiological performance or biochemical performance, limiting population survival when exposed to the combination of stressors.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure captions

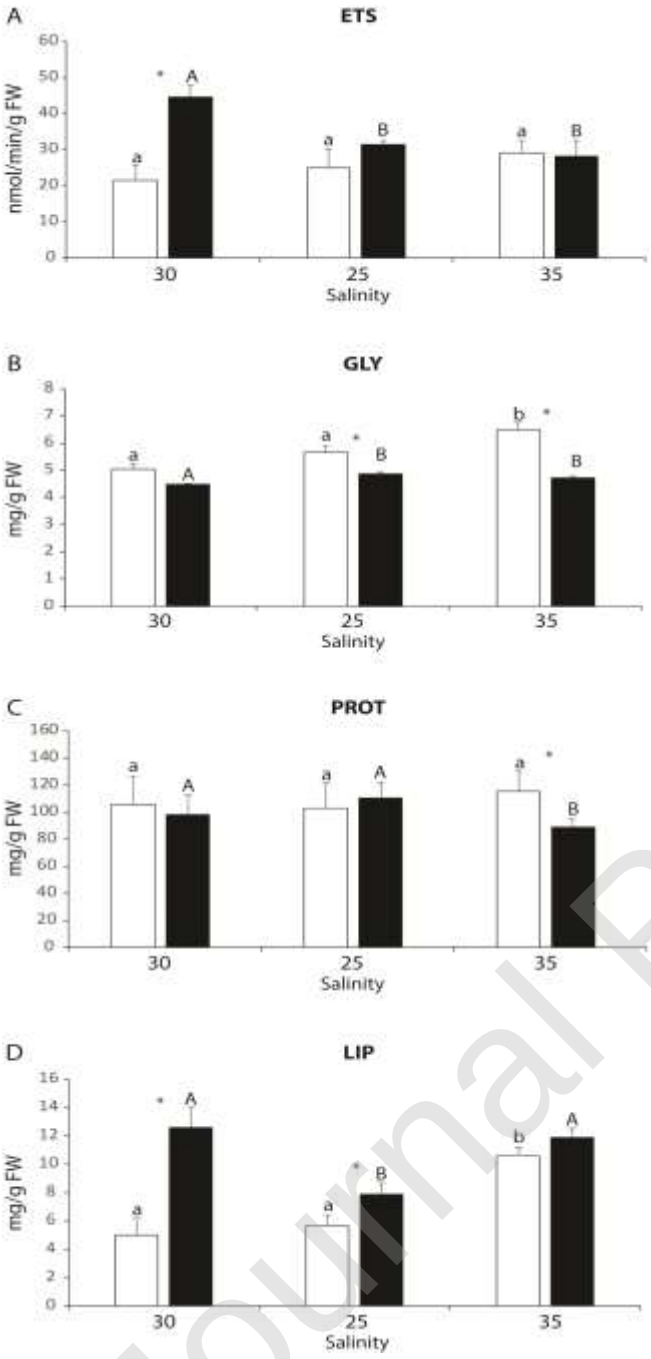

Figure 1. A: Electron transport system (ETS) activity, B: Glycogen (GLY), C: Protein (PROT) and D: Lipids (LIP) concentrations, in *Mytilus galloprovincialis* maintained for 28 days to control (0.0 mg/L) and 4.0 mg/L of Sodium dodecyl sulfate (SLS), under different salinity levels (30, 25 and 35). White bars represent non-contaminated mussels, while black bars represent SLS exposed mussels. Results are the mean+standard deviation. Different letters represent significant differences among tested salinities (lowercase letters for non-contaminated mussels, uppercase

letters for SLS exposed mussels). For each salinity, differences between non-contaminated and contaminated mussels are represented with an asterisk.

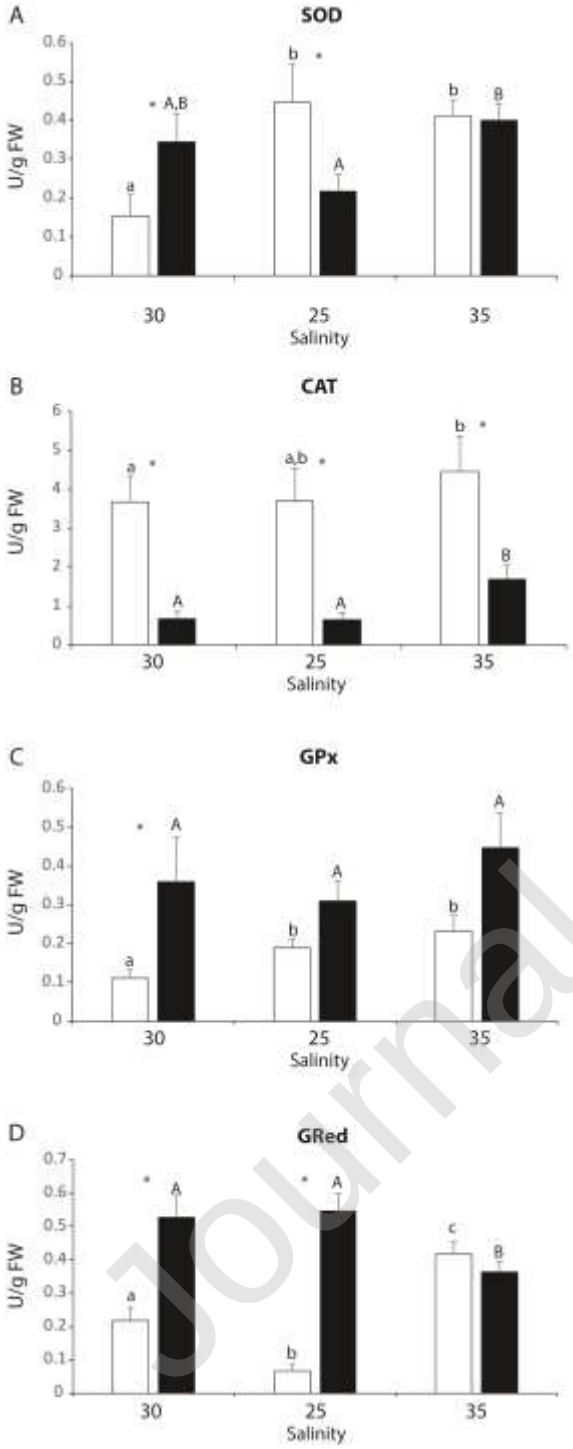

Figure 2. A: Superoxide dismutase (SOD), B: Catalase (CAT), C: Glutathione peroxidase (GPx) and Glutathione reductase (GRed) activities, in *Mytilus galloprovincialis* maintained for 28 days to control (0.0 mg/L) and 4.0 mg/L of Sodium dodecyl sulfate (SLS), under different salinity levels (30,

25 and 35). White bars represent non-contaminated mussels, while black bars represent SLS exposed mussels. Results are the mean+standard deviation. Different letters represent significant differences among tested salinities (lowercase letters for non-contaminated mussels, uppercase letters for SLS exposed mussels). For each salinity, differences between non-contaminated and contaminated mussels are represented with an asterisk.

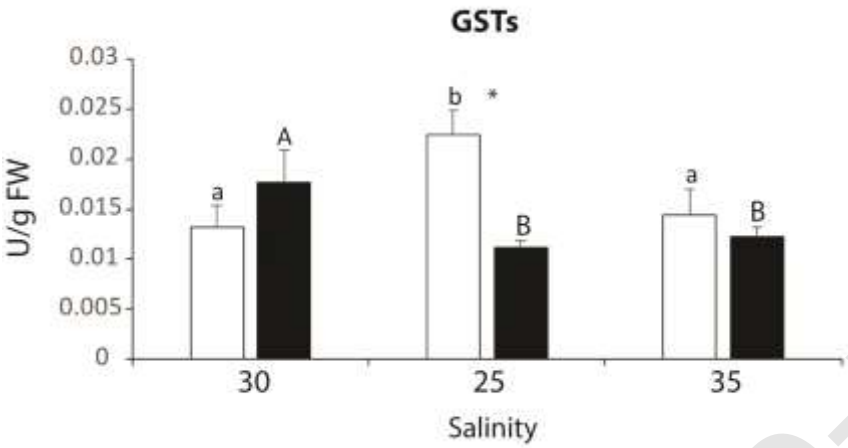

Figure 3. Glutathione S-transferases (GSTs) activity, in *Mytilus galloprovincialis* maintained for 28 days to control (0.0 mg/L) and 4.0 mg/L of Sodium dodecyl sulfate (SLS), under different salinity levels (30, 25 and 35). White bars represent non-contaminated mussels, while black bars represent SLS exposed mussels. Results are the mean+standard deviation. Different letters represent significant differences among tested salinities (lowercase letters for non-contaminated mussels, uppercase letters for SLS exposed mussels). For each salinity, differences between non-contaminated and contaminated mussels are represented with an asterisk.

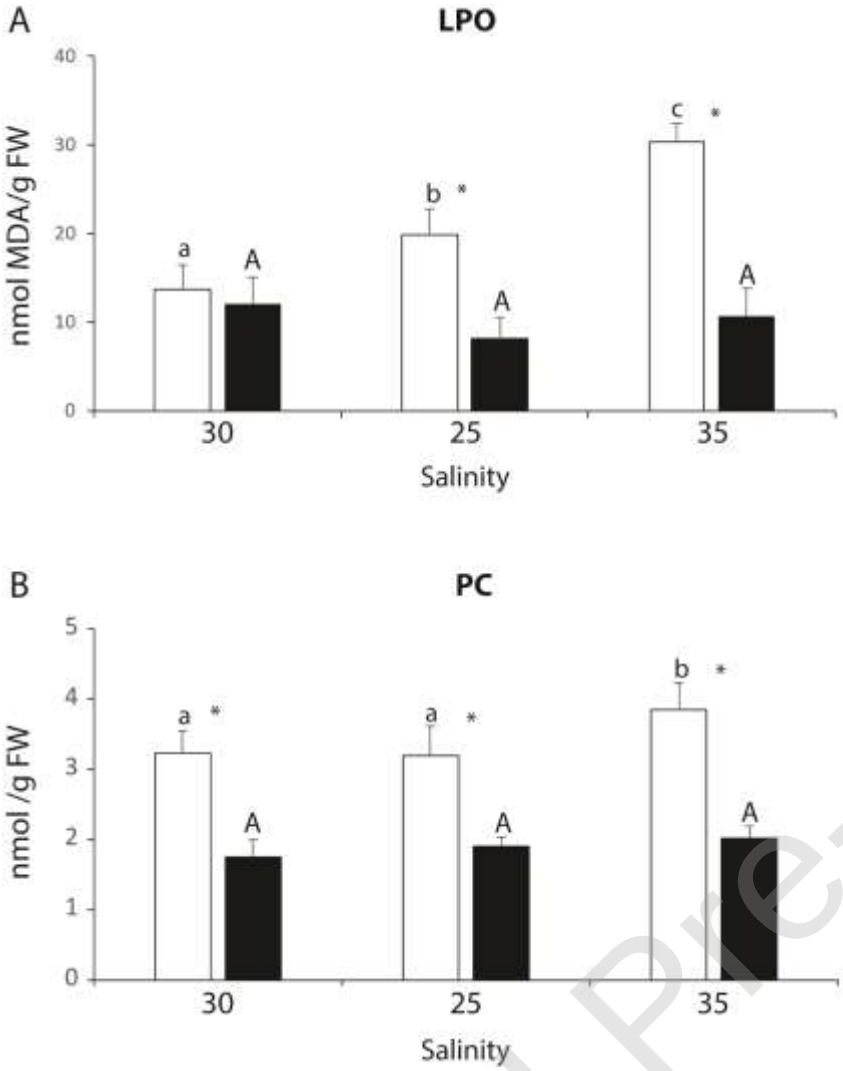

Figure 4. A: Lipid peroxidation (LPO) and B: protein carbonylation (PC) levels, in *Mytilus galloprovincialis* maintained for 28 days to control (0.0 mg/L) and 4.0 mg/L of Sodium dodecyl sulfate (SLS), under different salinity levels (30, 25 and 35). White bars represent non-contaminated mussels, while black bars represent SLS exposed mussels. Results are the mean+standard deviation. Different letters represent significant differences among tested salinities (lowercase letters for non-contaminated mussels, uppercase letters for SLS exposed mussels). For each salinity, differences between non-contaminated and contaminated mussels are represented with an asterisk.

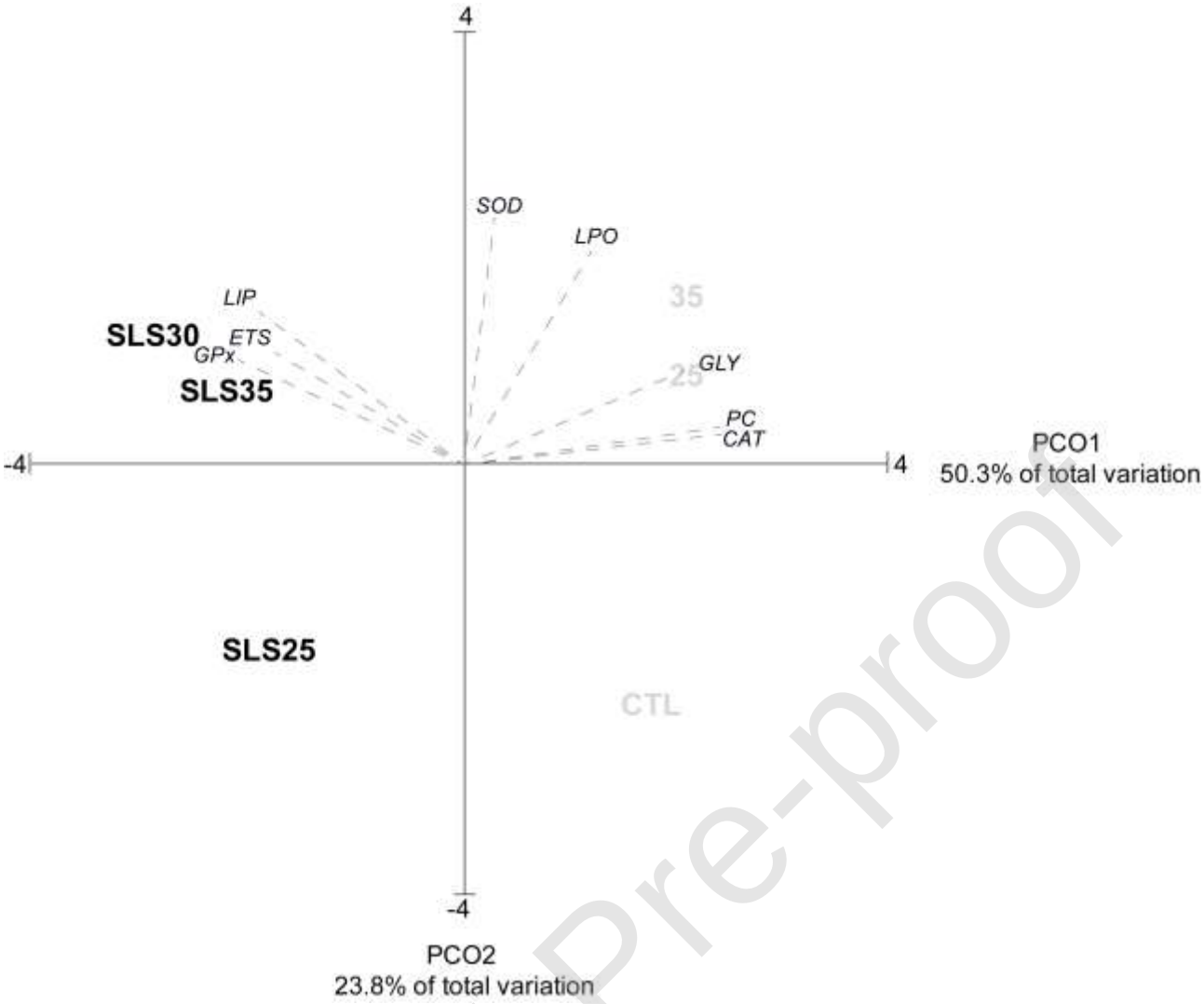

Figure 5: Centroids ordination diagram (PCO) based on SLS concentrations, physiological and biochemical parameters, measured in *Mytilus galloprovincialis* maintained for 28 days to control (0.0 mg/L) and 4.0 mg/L of Sodium dodecyl sulfate (SLS), under different salinity levels (30, 25 and 35). Pearson 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, LIP, SOD, CAT, GPx, LPO, PC. Grey letters represent non-contaminated mussels; black letters represent SLS exposed mussels.

Table 1- Sodium Lauryl Sulfate (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. Lowercase letters represent significant differences among salinities for each exposure week; uppercase letters represent significant differences among exposure weeks for each salinity.

Salinity	**Water (mg/L)**					**Mussels tissues (µg/g)**	**BCF**
	1st week	**2nd week**	**3rd week**	**4th week**		**4th week**	
Sal 30	3.8±0.1^{aA}	3.5±0.3^{a,bA,B}	3.2±0.3^{a,bB}	3.6±0.5^{a,bA,B}		0.2±0.01^{a}	0.05
Sal 25	3.9±0.2^{aA}	3.9±0.3^{aA}	3.7±0.2^{aA}	3.9±0.4^{aA}		0.4±0.1^{b}	0.11
Sal 35	3.8±0.3^{aA}	3.3±0.1^{bB}	3.2±0.1^{bB}	3.1±0.1^{bB}		0.3 ±0.1^{a,b}	0.08