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Integrated Assessment of CO_2-Induced Acidification Lethal and Sub-Lethal Effects on Tropical Mussels *Perna perna*

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Abstract: Leakages of CO_2 capture and storage systems from the seabed are able to cause significant adverse biological effects in marine species. Adult mussels were exposed to different CO_2 enrichment scenarios (pH from 8.3 to 6.0) for 96 h, and endpoints (lysosomal membrane deterioration, lipid peroxidation and primary damages in DNA) were assessed. Mortality and reduced health status can occur after short exposure of the tropical mussel *Perna perna* to pH levels lower than 7.5. Results pointed out cytogenotoxic effects in the hemolymph and gills after 48 and 96 h of exposure, respectively. These findings should be considered when environmental monitoring approaches are performed in tropical marine areas employing CCS strategies.

Keywords: CO_2 enrichment; mollusks; ocean acidification; biomarkers; carbon capture storage

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1. Introduction

Rising atmospheric carbon dioxide (CO_2) concentration is causing global warming and ocean acidification [1–3], which increasingly are recognized as important drivers of change in biological systems [4–7]. In aquatic environments, the CO_2 undergoes a series of chemical transformations, and elements recombine giving rise to new compounds [8]. First, the carbonic gas mixes with the water, producing aqueous CO_2 and carbonic acid (H_2CO_3). This compound, classified as a weak acid, quickly dissociates, producing bicarbonate (HCO_3^-) and protons (H^+), which react with other molecules present in the aquatic environment. Bicarbonate ions can also undergo chemical reactions, transforming into CO_3^{2-} and releasing hydrogen (H^+) in the water column, lowering the pH [9,10].

Since the industrial era, oceanic uptake of CO_2 has resulted in acidification of the ocean, and the pH of ocean surface water has decreased by 0.1, corresponding to a 26% increase in acidity [11]. In order to reduce atmospheric CO_2 levels, many mitigation strategies have been developed and proposed. One such strategy is large-scale carbon dioxide capture and storage strategies (CCSs) in geological formations. According to the International Energy Agency [12], it could contribute to a reduction of 19% in CO_2 emissions by 2050. This technology consists of trapping CO_2 from industrial and energy-related sources, transporting it to a storage site, injecting and storing it for a long time instead of releasing this gas into the atmosphere [13].

Transportation of CO_2 in high-pressure pipelines from source to storage location constitutes an important link in the CCS chain, especially when transporting large quantities of CO_2 over long distances [14]. The possibility of leakage from the sub-seabed reservoirs and transportation pipelines into the waters and atmosphere causes great concern, with

the timeframe for safe CO_2 storage being relatively unknown [15]. Considering that oceans have the largest capacity for CO_2 storage, sub-seabed geological formations, such as depleted oil and gas reservoirs and saline aquifers, have been designated as potential storage locations for CO_2 sequestration. The complexity in foreseeing the location and magnitude of possible seepages makes difficult the evaluation of potential effects on aquatics ecosystems. Two main potential sources of CO_2 escape are transport facilities and storage areas [16]. The effects of CO_2 leakage will depend on the amount and/or rate of leakage, the transport, the dispersion processes and the chemical buffering capacity of the sedimentary or water system, contributing to the imbalance of seawater's chemistry. There are also natural sources of CO_2 enrichment, such as natural CO_2 vents [17,18], bacterial organic matter degradation, diagenesis process [19] and submarine eruptions such as in the Canary Islands (Spain), where pH values between 5.13 and 8.04 have been measured [20,21].

In this context, many studies have been performed in order to assess the impacts of changes in the marine carbonate system as well as pH reduction via CO_2 enrichment to organisms [22–26]. Several authors mimicked a CO_2 leakage in the lab, assessing different responses related to decreases in pH values in diversified organisms, for instance: growth rate of bacteria (*Roseobacter* sp. CECT 7117 and *Pseudomonas litoralis* CECT 7670) [27] and bacterial communities [28]; mortality rate applied to amphipods (*Ampelisca brevicornis* and *Hyale youngi*) [29]; impact on the early life stages of marine mussel *Perna perna* [30,31]; and effects on growth, cell viability and oxidative stress using three microalgae species (*Tetraselmis chuii*, *Phaeodactylum tricornutum* and *Nannochloropsis gaditana*) [32]. Histopathological effects and lysosomal membrane stability were also assessed in mollusks such as clams (*Ruditapes philippinarum*) and mussels (*Mytilus edulis*) [33,34] apart from a battery of biomarkers using the clam *Scrobicularia plana* [35].

Ocean acidification impacts other aspects of a marine organism's physiology, including acid–base balance, energy metabolism, redox balance and behavior [36,37]. Acidification of ocean surface water is a currently developing scenario that warrants a broadening of research foci in the study of acid–base physiology, ensuring a strong basis for the physiological interactions of ocean acidification with pollutants that may affect the same molecular and physiological pathways [38].

The cellular mechanisms of CO_2-induced changes in the physiology of mollusks are not yet fully understood but are likely to involve multiple pathways of metabolism, biomineralization and acid–base balance [39,40]. Metabolic effects of elevated pCO_2 vary between different species [41,42] and depend on the CO_2 concentration in seawater.

For decades, bivalve filter feeders have been used for environmental assessments [43–45]. Due to their sedentary habits, low metabolic transformation rates and their ability to bioconcentrate pollutants, bivalves have been used as bioindicators suitable for monitoring studies in coastal areas such as useful bioindicators of persistent pollutants [46]. Studies using clams [47–50], mussels [51–54] and oysters [55–57] have been performed worldwide.

It has been reported that the early life stages of marine invertebrates are more susceptible to environmental toxicants than are the adult forms [58]. However, the mechanism of action-oriented toxicity assays has been performed with adult organisms, and the results are sensitive to environmentally relevant concentration [59,60]. The use of biological endpoints (biomarkers) has been advocated as an important tool for assessing the bioavailability of contaminants and the general health of individual organisms [61]. These responses are taken into consideration for monitoring different sources of anthropogenic contamination in coastal areas using bivalves as bioindicator species [44,62].

The aim of this study is to assess adverse effects of CO_2-induced acidification on individuals of mussel *Perna perna* using a battery of sub-cellular effect biomarkers (lipid peroxidation, DNA primary damage and lysosomal membrane stability). Additionally, the mortality of the organisms was used to establish the toxicity related to the increase of proton concentration associated with the enrichment of CO_2. For this purpose, organisms

were exposed over four days to different acidification scenarios with different pH values, and the influence of acidification associated with CO_2 enrichment was analyzed.

2. Materials and Methods

2.1. Laboratory Tests

To simulate the acidification process based on CO_2 enrichment event such as CCS, a CO_2 injection system was adapted from the experimental set up described by [63,64], with further detailed experiments in [65]. Briefly, the different acidification scenarios reached pH values of 8.0, 7.5, 7.0, 6.5, and 6.0, controlled using the injection system, and non-CO_2 injected treatment as control (pH 8.3). The experiment was developed using clean natural seawater from Enseada Beach in Guarujá, SP/Brazil [66]. Adult mussels *Perna perna* were acquired from a farming zone located at the Cocanha beach–Caraguatatuba municipality in São Paulo's coastal zone. The organisms were transported to the laboratory, where they were kept for 24 h prior to the assays under temperature (22 °C) and salinity (35 ppt) controlled in a 300 L aquarium with seawater.

Ten individuals of mussel *Perna perna* were introduced per 20 L chamber (two replicates per treatment). The laboratory conditions were controlled: seawater temperature at 20 ± 2 °C, salinity 35 ppt, 8.0 mg L^{-1} dissolved oxygen, and photoperiod 12:12. Water quality was maintained through renewing every 48 h.

After 48 and 96 h of exposure, survival was recorded, and ten organisms from each pH treatment (five organisms from each tank) had the hemolymph withdrawn before dissection gills were frozen (−80 °C). The hemolymph was collected to assess the lysosomal membrane stability through neutral red retention time assay (NRRT), while gills were used to assess DNA damage (strand breaks) and lipid peroxidation (LPO).

2.2. Chemical Characterization

The pH values and total alkalinity (TA) were measured using a potentiometric titration system (Metrohm 794 Basic Titrino, Switzerland, ref.6.0210.100) (pH scale calibration with 0.1 M HCl). Dissolved inorganic carbon (DIC) was determined via the experimental values and the seawater carbonate system speciation: HCO_3^-, CO_3^{2-}, CO_2, calcite saturation (Ω_{Cal}), aragonite saturation index (Ω_{Arag}) and partial pressure of carbon dioxide (pCO_2), using the CO2SYS v2.1 program as described in [65].

2.3. Neutral Red Retention Time Assay

The NRRT assay was carried out following the method described by [67]. This non-destructive method employed hemolymph withdrawn from the posterior adductor muscle of living mussels. The hemolymph was mixed to physiological saline solution (pH 7.3 containing HEPES 4.77 g L^{-1}, NaCl 25.48 g L^{-1}, $MgSO_4$ 13.06 g L^{-1}, KCl 0.75 g L^{-1}, $CaCl_2$ 1.47 g L^{-1}), spread on slides and transferred to a lightproof chamber for 15 min to allow cell attachment. Excess liquid was removed and 40 µL of the neutral red (NR) dye was added to the cell monolayer. A cover slip was added. After a 15 min incubation period, slides were examined every 15 min via optical microscopy (400×) for both structural abnormalities and NR dye loss from the lysosomes to the cytosol. The test was terminated when at least 50% of the examined cells exhibited these characteristics, and an NRRT mean value was calculated for each group. The same analyst carried out the assessment for all slides during the study.

2.4. Lipid Peroxidation and DNA Strand Breaks

For the LPO and DNA damage assays, mussel gills were homogenized with a buffer solution containing Tris-HCl (50 Mm), EDTA (1 mM), dithiothreitol (DTT) (1 mM), sucrose (50 mM), KCl (150 mM) and phenylmethylsulfonyl fluoride (PMSF) (100 mM).

LPO assay was carried out as demonstrated by [68]. LPO was determined in gill homogenates using thiobarbituric acid. Thiobarbituric acid reactants (TBARS) were determined via fluorescence at 530 nm for excitation and 630 nm for emission using a fluorescence

microplate reader. Because the reagent could react with other aldehydes, the results were expressed as μg of TBARS mg^{-1} total protein.

The mitochondrial DNA damage was determined as outlined by [69], based on the K-SDS precipitation of DNA–protein crosslink. Fluorescence was used to quantify DNA strand breaks. DNA quantitation was achieved using Hoescht dye at a concentration of 100 nM in 200 mM Tris–HCl, pH 8.5, containing 300 mM NaCl and 4 mM sodium cholate. Salmon sperm DNA standards were used for calibration, and fluorescence readings were taken at 360 nm excitation and 460 nm emission. The results were expressed as μg of DNA·mg^{-1} total protein.

The Bradford methodology [70] was used to determine the total protein content (the calibration curve with bovine serum) and normalization.

2.5. Data Treatment

A normal distribution and equal variance among groups of ANOVA residues from the experiment were confirmed by means of the Shapiro–Wilk test and Levene's F-test, respectively. Two-way factorial ANOVA (pH values, exposure times and their combination) followed by the Dunnett's test identified significant differences between control group and treatments using the SPSS 15.0 statistical software program. In addition, the effect size estimated for ANOVAs (NRRT, DNA damage and LPO) were calculated to measure how much variance in the response variables (biomarkers) are accounted for by the explanatory variables (pH values and exposure times). Therefore, the "omega squared" (effect size, ES) method was used because it is considered a less-biased alternative, especially when sample sizes are small. Ref. [71] suggests the following interpretation of calculated effect sizes: 0–0.01 = very small; 0.01–0.06 = small; 0.06–0.14 = medium and >0.14 = large. The calculations were performed in the R Environment 4.0.4 [72] (using the software package "effectsize") [73].

Thereafter, the original data set including all biological responses (means) was analyzed through an integrative approach via factor analysis, employing principal component analysis as the extraction procedure. The data set was rearranged in a correlation matrix and two factors (or new variables) were extracted considering eigenvalues higher than 1.0 (Kaiser's criteria). For the factor analysis, the variables were auto scaled (Varimax normalized) to be treated with equal importance. The criterion for consideration of a variable as being associated with a particular factor was defined as it having a loading of 0.40 or higher. Besides the analysis of the variables aggregated by PCA, a representation of estimated factor scores from each studied area to the centroid of all cases for the original data was performed in order to confirm the factor descriptions and to characterize the studied stations. All analyses were performed using the PCA option of the multivariate exploratory technique procedure, followed by the basic set-up for factor analysis procedure from the STATISTICA software tool (Hamburg, Germany, Stat Soft, Inc., 2001; version 6.0).

3. Results and Discussion

Data had sufficient homogeneity of variance for ANOVA. There was no significant difference between mussels held in tanks at any treatment.

3.1. Chemical Analysis

The averaged values for the carbonate system speciation are presented in Table 1. This data refers to the carbon parameters measured and calculated at the end of the experiments. The salinity parameter used was 35, and the temperature was 23.5 ± 0.5 °C. As expected, the total inorganic carbon (TIC) was higher as pH was reduced, and the saturation index for calcite (Ω_{Cal}) and aragonite (Ω_{Arag}) decreased as pH decreased and pCO_2 increased.

Table 1. Carbonate system speciation in assays exposed to the different pH treatments for both bioassays of fertilization and embryo-larval development.

pH Treatment	TA (µmol/kg)	TCO_2 (µmol/kg)	TIC/DIC (µmol/kg)	HCO_3^- (µmol/kg)	CO_3^{2-} (µmol/kg)	CO_2 (µmol/kg)	pCO_2 (µatm)	Ω_{Cal}	Ω_{Arag}
8.3	1644	1404	1404	1249	147	8	262	3.53	2.31
8.0	1662	1511	1511	1397	99	14	472	2.38	1.56
7.5	1731	1718	1718	1629	39	50	1672	0.93	0.61
7.0	1682	1828	1828	1650	12	165	5451	0.29	0.19
6.5	2483	3249	3249	2469	6	774	25,996	0.14	0.09
6.0	3172	5996	5996	3167	3	2827	97,130	0.06	0.04

Mussel shells are made of up to 83% aragonite [74]. Aragonite is more soluble than calcite, therefore shell dissolution may be a considerable problem faced by mussels in acidified seawater [75]. The Ω_{Arag} remains favorable for calcification when >1 [73]. The present study shows a decrease in the Ω value related to an increase in protons H^+, being a critical value for calcification when pH $\leq$ 7.5 (Table 1). According to [76], $CaCO_3$ is of high ecological relevance, acting on the calcification kinetics and interfering in bivalve shell formation. Its reduction and dissolution depend on $\Omega_{Arag}/\Omega_{Cal}$ availability, which are changed via H^+ variance.

3.2. Biological Responses

The percentage of mussel mortality for each treatment assay is shown in Table 2. In general, the mortality increases as pH values decrease. For pH values up to 7.5, no mortality was registered, while for pH values below 7.0, mortality was recorded at a minimum of 20% after 96 h of exposure. These results concur with study [77], in which the clam *Scrobicularia plana* showed a mortality of 20% at the pH value of 7.1 at 96 h of exposure.

Table 2. *Perna perna* mortality percentage registered after 48-and 96 h exposure for the different pH levels (8.0, 7.5, 7.0, 6.5, and 6.0) and control (8.3 with no CO_2 added).

pH Value	Mortality (%)	
	48 h	96 h
8.3	0	0
8.0	0	0
7.5	0	0
7.0	0	20
6.5	10	20
6.0	0	30

Lysosomes are central to many key biological processes, including reproduction, digestion and immune response; thus, any deleterious changes to their function can have serious consequences for those processes. The general health status of the mussels was therefore considered in terms of changes to the lysosomal system of the blood cells [75].

The NRRT assay is based on the principle that only lysosomes in healthy cells take up and retain the vital dye neutral red. Lysosomal membrane damage caused by the impact of xenobiotics can decrease the NRRT times through inducing the leaking of lysosomal components. Lysosomal membrane damage caused by the impact of xenobiotics [78] or by CO_2-induced seawater acidification [75] can decrease the NRRT through inducing the leaking of lysosomal components [79].

The results of the biomarker of exposure NRRT assay for *Perna perna* involving acidified seawater are shown in Figure 1. The "exposure times" presented very small estimates ($F = 0.197$; $df = 1$; $p = 0.65$; ES = 0.001), while the isolated "pH values" ($F = 10.328$; $df = 5$; $p < 0.001$; ES = 0.37) and the combination of both explanatory variables ($F = 3.518$; $df = 5$; $p = 0.007$; ES = 0.15) also evidenced a large effect on NRRT responses. The range in NRRT

values observed for lysosomes from control mussels (n = 10; no CO_2 added) at time zero, 48 h and 96 h of assay were 81 ± 26 min, 93 ± 23 min and 77 ± 24 min, respectively. In the first 48 h, the RT significantly ($p < 0.05$) decreased at pH 7.0 and 6.5. After 96 h, the organisms exposed to different pH values showed a significant decrease ($p < 0.01$) in the dye retention time in lysosomes only at pH 6.0. There was no significant difference between RT of the dye in the control group for the highest pH values (8.0 and 7.5).

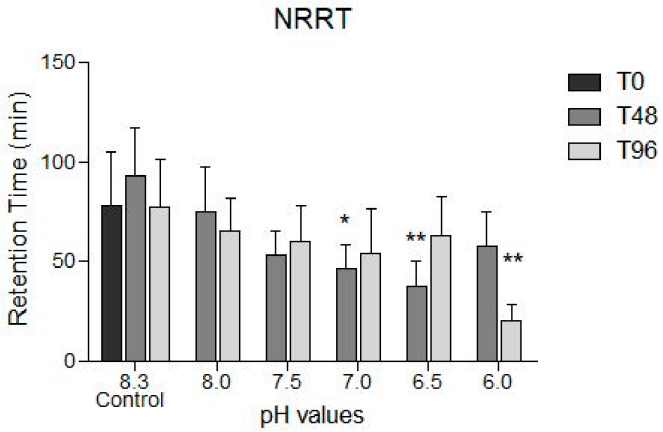

Figure 1. Mean (and standard error) of LMS assessed through NRRT assay in the hemocytes of *P. perna* exposed to different pH levels (Dunn's test: * $p < 0.05$; ** $p < 0.01$).

The results presented in this study for the control sample (81 ± 26 min) are reliable and fit with those obtained in previous studies reporting an NRRT ranging from 60 to 90 min in a study on healthy or non-exposed *P. perna* [80] and 60 min in a study using hemolymph of *Perna viridis* [81]. Furthermore, [82] reported NRRTs of 90 min using *Mytilus galloprovincialis* hemolymph, and 106 min in the [83] study using clam *Ruditapes philippinarum* hemolymph.

According to the criteria established by [84], in the cytochemical method, animals are considered to be stressed but compensating if NRRT were between 50 and 120 min. However, studies have demonstrated that lysosomal membrane stability differs in tropical zones compared to temperate zones, as higher temperatures in the tropics lead the neutral red dye to be retained in the lysosomes for a shorter period of time [85], agreeing with the results of this work and previous studies on NRRT of *P. perna* populations from the Brazilian coast [43,65,86,87]. Therefore, the present study may contribute to the development of a health index for tropical mussels.

Lysosomal integrity is directly correlated with physiological scope for growth and is also mechanistically linked with the processes of protein turnover [88]. Ref. [89] showed that lysosomal stability in adult oysters is also correlated with larval viability. Therefore, the integrity or stability of this membrane is considered an indicator of the cell "well-being", acting as an important cellular and nonspecific biomarker of stress [46] once its activity is directly related to ion transportation [59].

The significant reduction in lysosomal health observed for pH below 7.0 (48 h) may be related to the increase in Ca^{2+} concentrations in the hemolymph [75], which can cause lysosomal destabilization [90]. According to [22], decreasing pH promotes the dissolution of shells and, consequently, an increasing concentration of Ca^{2+} in the hemolymph, altering the cellular metabolism and reducing immune function. According to [91], extracellular calcium enters in the cells via pinocytosis, and once in the cytosol, Ca^{2+} can activate calcium-dependent phospholipase A2, destabilizing the lysosome membrane.

The compensation of acid–base imbalance is also of great importance, since it is related to ion transportation through the cell membrane. The CO_2 produced in the cells during routine metabolism is typically hydrated to form bicarbonate and H^+. The H^+ ions are buffered, while the bicarbonate is transported out of the cell in exchange for Cl^- via ion transport proteins. Refs. [92,93] indicated that mussels responded to maintain acid–

base balance through carbonic anhydrase activity, so that it was significantly higher in mussels exposed to low pH in comparison with control individuals. As observed in the present study, the increasing proton concentration in the environment, once more, may be associated with a decrease in the lysosome's capacity to retain the dye as a healthy cell.

DNA damage results evidenced that "exposure time" (F = 0.298; df = 1; p = 0.58; ES = 0.001) was not significant, presenting a very small influence on DNA-SB. The "pH values" variable, on the other hand, demonstrated a large significance (F = 20.766; df = 5; $p < 0.001$; ES = 0.47) and the combination of these explanatory variables showed a significantly medium effect (F = 3.727; df = 5; p = 0.003; ES = 0.11). Lipid peroxidation results showed that "exposure time" (F = 34.953; df = 1; $p < 0.001$; ES = 0.24), the "pH values" (F = 4.940; df = 5; $p < 0.001$; ES = 0.15) and the combination of these explanatory variables (F = 5.603; df = 5; $p < 0.001$; ES = 0.17) had a large statistical effect on LPO responses. The DNA strand break values and the LPO results are shown in Figure 2. In the present study, the DNA strand breaks exhibited significantly higher values ($p < 0.05$) than T0 in the gills of *Perna perna* mussels exposed to a pH value of 6.0 in both exposure times. Only LPO was significantly different ($p < 0.01$) at pH 6.0 after 96 h exposure.

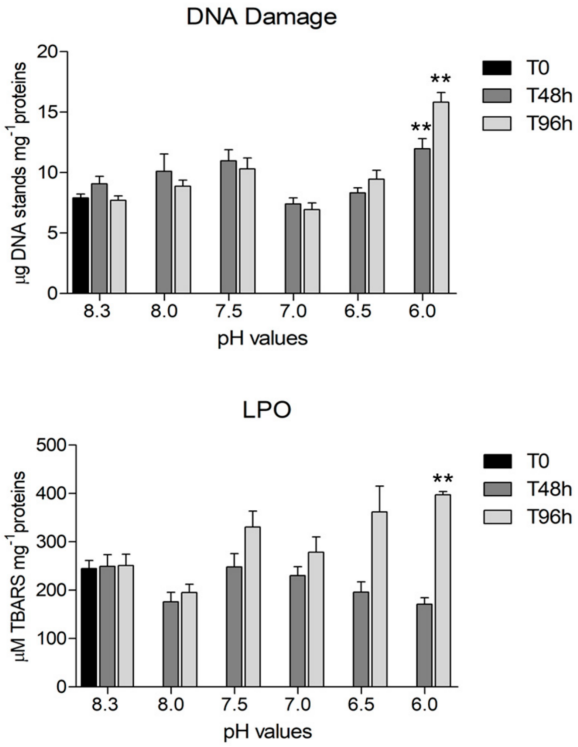

Figure 2. Mean (and standard error) of DNA damage (stand break) and LPO assessed in *P. perna* exposed to different pH levels for 96 h (** $p < 0.01$).

Oxidative stress occurs due to an imbalance between the production of reactive oxygen species (ROS) and their elimination via the antioxidant system defense, leading to protein degradation and enzyme inactivation [94]. If it is continuously generated, ROS can damage important biomolecules such as DNA, proteins and LPO [95]. Results obtained from this study may be related to the increase in the ROS production in acidified scenarios, agreeing with the study [96], where bivalve *Crassostrea gigas* showed a significant increase in the production of ROS in hemocytes, as well as DNA damage (comet assay) in different scenarios of ocean acidification (pH 7.6 and 7.8).

Ref. [97] reported that low pH caused a decrease in electron supply from NADH to the electron transport chain in the oyster *Crassostrea gigas*, and their response mechanism changed the cellular balance between resource supply and oxidative stress. As a protective system against the consequences of oxidative stress, lysosome autophagy consists

of breaking down longer-lived proteins and organelles and recycling the products into protein-synthesis and energy production pathways [98].

3.3. Principal Component Analysis (PCA)

A multivariate analysis approach was applied to all data to help discriminate the main variables responsible for the variance of biological effects detected in mussels (Table 3, Figure 3). The application of PCA to the original 13 variables at the pH bioassay indicates that they can be grouped in two new factors. These new variables explain more than 96% of the total variance of the original data set. In the present study, we selected a group of variables associated with a component loading ≥ 0.40, corresponding to an associated explained variance of more than 30%.

Table 3. Sorted rotated factor loadings of 13 variables for the two principal factors.

Variables	Components	
	Factor 1	**Factor 2**
NRRT 48 h		−0.92
NRRT 96 h	−0.76	−0.52
DNA 48 h	0.69	
DNA 96 h	0.91	0.24
LPO 48 h	−0.76	
LPO 96 h	0.51	0.72
Mortality 48 h		
Mortality 96 h	0.55	0.68
TA	0.88	0.40
DIC	0.89	0.41
Ω_{Cal}	−0.26	−0.94
Ω_{Arag}	−0.26	−0.94
pCO_2	0.91	
Variance (%)	5.63	4.51
Cumulative (%)	0.43	0.34

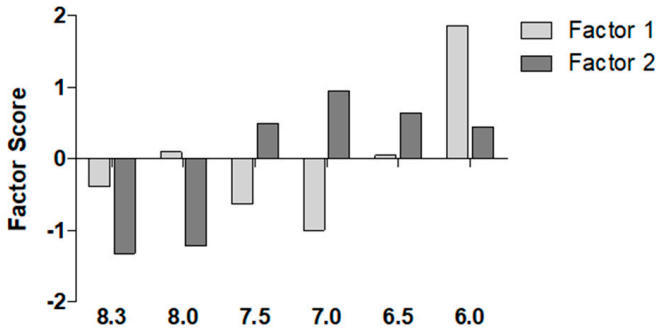

Figure 3. Estimated factor scores in relation to the pH treatments used in the study.

The F1 (61%) includes the lethal response and DNA damage of the mussel. According to [89], the positive LPO 96 h value may demonstrate a relationship between an increase of antioxidant enzymes and LPO levels as defense mechanisms due to possibly being overwhelmed by ROS produced in organism tissues. It was more representative for pH 6.0 with higher positive values (Figure 3).

The F2 (21%) showed the relationship between NRRT, DNA damage, LPO, mortality and pH reduction. The effects of this factor could be related with acidification by CO_2 enrichment because of the significant variance in lysosome membrane stability (NRRT) and the increase in DNA damage, the oxidative stress and the mortality.

The presented results corroborate the toxic effects observed from a decrease in pH value $\leq$ 6.5, while from pH values $\leq$ 7.5 a stress showed to be produced through activating enzymatic systems maintained at the lowest pH values. In summary, the stress produced via increasing proton concentration can be noticed in the enzymatic systems of the mussels (NRRT and LPO) in pH values lower than 7.5, being accentuated when the value of the concentration of protons is higher, which produces toxic effects related to pH 6.5 and 6.0.

A similar integration analysis was carried out for the same pH range, but with other toxicity species and endpoints (amphipods mortality, sea-urchin embryo-larval development and the benthic community) in the Bay of Santos [29]. Similar results were obtained; they determined that pH 6.0 was extremely toxic for marine life.

The responses of the mussels might be affected by the presence of other toxic compounds in the environment. The Santos Bay is affected by sewage discharges that carry illicit drugs, such as crack cocaine. This, in combination with acidification conditions (also below pH 7.5), provokes alterations in *Perna perna* reproduction [31].

4. Conclusions

The results obtained in this study suggest that mortality and reduced health status can occur when the tropical mussel *Perna perna* is exposed to pH levels lower than 7.5 for a short term (48 and 96 h). Lysosomal membrane stability of hemocytes showed the first signs of effects, followed by DNA damages and LPO in gills under acidified conditions. These findings should be considered when environmental monitoring approaches are performed in tropical marine areas receiving petroliferous or other activities employing CCS technologies.

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