Scilight

Earth: Environmental Sustainability
https://www.sciltp.com/journals/eesus

Article

Responses of Health- and Growth-Related Biomarkers in Corals Exposed to Iron

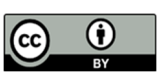
Juliana da Silva Fonseca [1], Letícia May Fukushima [1], João Vitor Langorte Bueno [2], Thales Jean Vidal [3], Kely Paula Salvi [4], Carlos Henrique Figueiredo Lacerda [4], Patrícia Gomes Costa [1], Miguel Mies [4,5] and Adalto Bianchini [4,6,*]

[1] Postgraduate Program in Physiological Sciences, Institute of Biological Sciences, Federal University of Rio Grande—FURG, Av. Itália, km 8, Rio Grande 96203-900, RS, Brazil
[2] Postgraduate Program in Biological Oceanography, Institute of Oceanography, Federal University of Rio Grande—FURG, Av. Itália, km 8, Rio Grande 96203-900, RS, Brazil
[3] Postgraduate Program in Oceanography, Department of Oceanography, Federal University of Pernambuco—UFPE, Av. Prof. Moraes Rego 1235, Cidade Universitária, Recife 50670-901, PE, Brazil
[4] Coral Vivo Institute, Rua dos Coqueiros, Parque Yaya, Santa Cruz Cabrália 45807-000, BA, Brazil
[5] Oceanographic Institute, University of São Paulo, Praça do Oceanográfico, 191, São Paulo 05508-120, SP, Brazil
[6] Institute of Biological Sciences, Federal University of Rio Grande, Av. Itália, km 8, Rio Grande 96203-900, RS, Brazil
* Correspondence: adaltobianchini@furg.br; Tel.: +55-53999681818

How To Cite: da Silva Fonseca, J.; Fukushima, L.M.; Bueno, J.V.L.; et al. Responses of Health- and Growth-Related Biomarkers in Corals Exposed to Iron. *Earth: Environmental Sustainability* **2025**, *1*(2), 366–382. https://doi.org/10.53941/eesus.2025.100028

Received: 12 October 2025
Revised: 9 December 2025
Accepted: 15 December 2025
Published: 24 December 2025

Abstract: Coral reefs biodiversity and productivity are currently in decline due to the impacts of human activities, especially those associated with chemical pollutants, including metals. In this context, iron (Fe) contamination of coastal waters associated with land runoff and disasters associated with mining activities has drawn attention around the globe, especially in the Southern Atlantic coast. Fe is an essential metal involved in photosynthesis, respiration, and oxidative metabolism, which can thus influence parameters associated with photosynthesis and the activity of ATPases. Therefore, we evaluated the acute and chronic effects of Fe on the maximum quantum yield of photosystem II and carbonic anhydrase and Ca^{2+}-ATPase activities in three corals species: *Mussismilia harttii*, *Siderastrea* sp., and *Millepora alcicornis*. Corals were maintained in control condition (no Fe addition in seawater) and acutely (4 days—laboratory conditions) or chronically (up to 28 days—mesocosm conditions) exposed to different increments of Fe (0.1, 0.3, and 0.9 mg L^{-1}) in seawater. The tested concentrations were selected based on the range of total and dissolved Fe concentrations observed in seawater in reef environments of the South Atlantic Ocean after the collapse of the Fundão mine dam occurred in Mariana (state of Minas Gerais, southeastern Brazil) in 2015. In the acute and chronic experiments, three and four replicates were performed for each experimental condition, respectively. In the acute exposure, all biological parameters were measured after 4 days of exposure. In the chronic exposure, the maximum quantum yield of photosystem II was measured at 5, 10, 17, and 24 days of exposure while enzyme activities were analyzed at 14 and 28 days of exposure. Results indicated that the maximum quantum yield of photosystem II was decreased by 20.5% ($p < 0.05$) in *Mi. alcicornis* exposed for 17 days to 0.1 mg L^{-1} Fe, when compared to the control condition at the same experimental time. Along the experimental time, it was decreased ($p < 0.05$) by 19.8% and 20.9% in *Mu. harttii* exposed for 24 days to 0.3 and 0.9 mg L^{-1} Fe, respectively. In *Mu. harttii*, carbonic anhydrase activity was reduced by 31.7% after acute exposure of corals to 0.3 mg L^{-1} Fe and increased by 102.4% when they were exposed to 0.9 mg L^{-1} Fe. Also, carbonic



Copyright: © 2025 by the authors. This is an open access article under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).
Publisher's Note: Scilight stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

anhydrase activity was reduced ($p < 0.05$) by 62.1% and 54.5% in *Mi. alcicornis* exposed for 14 days to 0.3 and 0.9 mg L^{-1} Fe, respectively. After 28 days of Fe exposure, no significant change is CA activity was observed in the three species of corals. Furthermore, Ca^{2+}-ATPase activity of the three coral species was not altered by the Fe increments in seawater, regardless of the exposure time. Overall, our findings indicates that exposure to increments of Fe in seawater influenced the health- (maximum quantum yield of photosystem II) and growth-related (carbonic anhydrase activity) biomarkers evaluated. The observed effects were specific to the three coral species tested and highlight the need to test the impacts of the seawater contamination with Fe over longer exposure periods than those tested in the present study.

Keywords: calcification; coral reefs; environmental contamination; metal; Fundão dam collapse; *Fv/Fm*

1. Introduction

Coral reefs are ecosystems showing high productivity and biodiversity [1,2], occupying less than 1% of the ocean surface. However, because of their elevated structural complexity, they harbor approximately 32% of marine biodiversity [3]. This elevated diversity is responsible for providing essential ecosystem services, including fisheries, tourism, and coastal protection [4,5]. The Brazilian Province hosts the vast majority of the shallow-water coral reefs in the South Atlantic, displaying low species diversity but a high level of endemism [6,7]. The massive-shaped species belonging to the *Mussismilia*, *Siderastrea*, and *Montastraea* genera are among the main reef builders in this region [8,9]. Calcifying hydrozoans (i.e., hydrocorals) belonging to the *Millepora* genus are the main structural complexity providers due to their branching morphology [10,11].

The calcification process carried out by some corals is an interesting process and relies on fine control for the formation of $CaCO_3$, with the involvement of some enzymes, such as carbonic anhydrase and Ca^{2+}-ATPase [12–17]. Calcification is also supported by the presence of dinoflagellate symbionts (Symbiodiniaceae family) [18], which are housed intracellularly by corals and aid in light-enhanced calcification [19,20]. This relationship is fundamental to the growth of carbonate structures and allows coral reefs to develop in environments with low nutrient supply. In general, the host coral provides CO_2, inorganic nutrients produced by its metabolism, and greater protection against predation in the plankton. In contrast, symbionts offer photosynthetically-derived carbon that meet up to 100% of the host energetic requirements [20–22]. The health of the photosynthetic apparatus can be accessed based on a chlorophyll a fluorescence technique allowing easy assessment of the Symbiodiniaceae photosynthetic efficiency [23–25].

Local and global stressors are negatively affecting coastal coral reefs [26–29]. Anthropogenic stressors have promoted a progressive degradation of coral reef environments worldwide [30–32]. Organic and inorganic pollutants, including metals, has increasingly threatened the coral reef ecosystems worldwide [28,33,34]. Metals present in seawater are often originated from coastal runoff, sewage discharge, and mining activities [35–37]. In this context, environmental accidents involving the collapse of mining dams in Brazil have put the South Atlantic coral reefs at risk [38]. In November 2015, a great amount of iron ore tailings were released into the Doce River basin (Southeastern Brazil) as a consequence of the rupture of mining dam. Several months later, these tailings impacted the Abrolhos Reef Complex [38–41], the most important coral complex of the Southern Atlantic Ocean [8,42,43]. Metal contamination associated with the tailings plume has caused physiological impacts in corals from the Abrolhos Reef Complex [44]. Notably, an increased concentration of Fe in coral growth bands was observed in early 2016 [45]. Furthermore, along with the metal accumulation observed in corals, the evidence of elevated metal levels in surrounding water and sediments was reported [45,46].

At this point, it is worth noting that Fe plays a dual role in corals, depending on its concentration in surrounding water. At low concentrations, it serves as a crucial micronutrient for photosynthesis and metabolism, while becoming a potent toxicant when in excess [47,48]. Considering the background described above on the high levels of Fe contamination in reef environments and the Fe duality in corals and their symbionts, this metal was selected as the target contaminant to be tested in the present study.

Exposure to high concentrations of metals can cause cellular damage, bleaching, and hamper the calcification process in corals [49–54]. High concentrations of metals in coastal ecosystems is also responsible for the increased sensitivity of coral reefs to global stressors [23,55–58]. Tissue bioaccumulation of Fe can generate free radicals and lead to a condition of oxidative stress [59–62]. Excessive concentrations of this metal is capable of generating free radicals due its role in oxidation and reduction cycles, mainly in the presence of H_2O_2, generating even more

unstable and toxic reactive species. It is well known that ROS can cause several biological injuries, including enzyme activity inhibition [60,63]. Additionally, it is reported that metals, including essential metals like Fe, can directly bind to important functional groups of enzymes (thiol and histidyl groups), as well as replace the enzyme cofactors, such as Zn^{2+} in the case of CA [64–66].

Therefore, parameters involved in the calcification process (carbonic anhydrase and Ca^{2+}-ATPase activity), as well as those related to the photosynthesis process (maximum quantum yield of photosystem II) carried out by the symbiotic dinoflagellates, are key biomarkers of metal exposure [23–25,67] with a significant ecological relevance, as they are early-warning indicators of coral reef health and development [68–71]. Therefore, we assessed the impacts of the exposure to increments of Fe in seawater on the maximum quantum efficiency of photosystem II and enzyme (carbonic anhydrase and Ca^{2+}-ATPase) activity, as proxies of health and growth, in three species of corals, the hydrocoral *Mi. alcicornis* and the scleractinian corals *Siderastrea* sp. and *Mu. harttii*. We hypothesize that increasing Fe concentrations impair coral photosynthetic efficiency and calcification-related enzyme activity in a species-specific manner.

2. Materials and Methods

2.1. General Aspects

The hydrocoral *M. alcicornis* and scleractinian corals *Mu. harttii* and *Siderastrea* sp. used in the experiments were obtained from the Recife de Fora (−16.402901, −38.982132; Northeastern Brazil) by scuba diving at a depth of 3–4 m. Genotypic diversity was ensured and maximized by collecting coral colonies at distances from each other [72]. After collection, corals were immediately brought to the laboratory and the marine mesocosm system (Coral Vivo Project (Porto Seguro, BA, Northeastern Brazil).

The impacts of exposing corals to increments of Fe in seawater were assessed through two experiments: (i) short-term exposure under controlled laboratory conditions; and (ii) long-term exposure in a mesocosm system. In both experiments, we tested four different increments of Fe concentration in seawater (nominal concentrations): 0 (control), 0.1, 0.3, and 0.9 mg L^{-1}. The range of tested concentrations brackets the typical concentrations of Fe in natural seawater [73] and those observed following severe pollution events [46,74]. Few months after the Fundão dam collapse, total Fe concentrations were close to or higher than 1 mg L^{-1} in the Doce River mouth [74,75]. Regarding dissolved Fe concentrations, they still exceeded 0.8 mg L^{-1} in the continental shelf 4 years after the disaster [76]. In the Abrolhos reef, the mean values of total Fe concentration in surface and bottom water from four sampling sites ranged from 0.024 to 1.149 mg L^{-1} over the six-year period of monitoring performed by the Programa de Monitoramento da Biodiversidade Aquática [77].

2.2. Acute Exposure to Fe

This experiment was performed with twelve 70-L glass aquaria, all connected to a 1200-L recirculating system. Therefore, a closed and independent setup was used for the acute experiment. In each aquarium, seawater temperature was kept constant (28 °C), with a 300-W heater (Aquael Platinum Heater). Seawater pH and salinity was monitored using portables pH meter (Gehaka, Brazil) and refractometer (Soma, Brazil), respectively. Lighting was kept constant (~120 µmol photons m^{-2} s^{-1}) using Maxspect MJ-LI 65 (65 W) lamps. The light-dark regime was 12:12. In each aquarium, the circulation and filtration of seawater was carried out using a pump and a mechanical filter. Synthetic reconstituted seawater adjusted to a salinity of 35 ppt was employed.

For this experiment, thirty-six fragments of each coral species were used. They were preconditioned to the control condition for 1 day, and further subjected to the 4-day experimental period. For each experimental condition, three aquaria (experimental units) were used. Each replicate was performed with three fragments of each coral species (n = 3 fragments per aquarium × 3 replicates = 9 biological replicates per treatment for each coral species). At the beginning (time 0) and after 4 days of experiment, samples belonging to the different treatments were collected. The collected samples were immediately frozen and kept at −80 °C for further analysis.

2.3. Chronic Exposure to Fe

The chronic exposure experiment was performed using a semi-open flow-through experimental system at the Coral Vivo research facilities. This marine mesocosm system consists of 16 tanks (130 L each) receiving seawater from a nearby reef (Mucugê reef). Thus, this experimental system is able to simulates the natural environmental conditions found at Recife de Fora reef, both in biodiversity and water parameters [78,79].

In each tank of the mesocosm system, a simulated reef environment was set up, comprising the corals *Mussismilia harttii*, *Siderastrea* sp., and *Millepora alcicornis*, along with other reef organisms (zoanthids,

rhodoliths, turf algae, macroalgae, ophiuroids, and dusky damselfish). Each treatment (experimental condition) consisted of four replicates. This setup was part of a broader experimental framework designed to evaluate the influence of Fe increment in seawater on various groups of reef organisms and their ecological interactions [79].

Forty-eight fragments of each coral species (n = 3 fragments per tank × 4 replicates = 12 biological replicates per treatment) were used in this experiment. Corals were subjected to a 7-days preconditioning period and then exposed to the designated levels of Fe increment in the mesocosm seawater. An iron chloride ($FeCl_3$) solution, prepared with seawater and 24 h before use, was continuously pumped from a 1000-L plastic reservoir into the seawater flowing into the experimental tanks. The iron chloride solution was pumped at different ratios to reach the desired Fe concentration to be tested [79]. Coral fragments (n = 4; one fragment per experimental unit for each coral species) were sampled at different exposure times: before (1 day) and after (14 and 28 days) the beginning of the experiment. Tested coral fragments were frozen and stored (−80 °C) for further analysis.

2.4. Monitoring of the Experimental Medium Conditions

Two times a day (06:00 and 18:00), we used a mercury thermometer and the portable refractometer to monitor the temperature and salinity of the experimental media of the acute experiment, respectively. In the mesocosm system, seawater conditions were measured at 6 h, 12 h, 18 h, and 24 h. Salinity, temperature, O_2 concentration, and pH were monitored using the portable refractometer, mercury thermometer, a hand-held oximeter (model MO900, Instrutherm, São Paulo, Brazil), and the portable pH meter, respectively. The volume of seawater flowing into each experimental unit (8–10 L min^{-1}) was measured using a TM050 flowmeter (GPI, Houston, TX, USA). A shade cloth was used to kept constant (~200 µmol photons m^{-2} s^{-1}) the solar radiation reaching the experimental units.

In both experiments, Fe concentrations in the experimental media were measured using a hand-held photometer (AKSO, São Leopoldo, Brazil). Measurements were performed at 06h and 18h. Whenever needed, adjustments in the Fe concentrations of the test aquaria (acute experiment) were performed manually. In the mesocosm (chronic) experiment, peristaltic pumps were used to deliver the appropriate volume of the Fe standard solution and thus maintain the desired Fe concentrations in the experimental units. In both experiments, water from the experimental media were collected, acidified (1% final concentration) with ultrapure HNO_3 (Merck, Darmstadt, Germany), and stored (~2.5 °C) until analysis. Non-filtered water seawater samples were used for measurement of the total Fe concentration. In turn, filtered (0.45 µm-mesh filter) seawater samples were employed for quantification of the dissolved Fe concentration. Fe concentrations (total and dissolved) were determined by mass spectrometry (ICP-MS, Analytik Jena, Germany) using a calibration curve (r^2 = 1.00). A commercial (Merck, Germany) standard solution (1.0 g L^{-1} Fe) was employed to prepare the calibration curve. The limit of detection (LOD) corresponded to 0.033 µg L^{-1} and the relative standard deviation (%RSD) of measurements was 2.1%. The procedures used in the Fe analysis were as previously described [79].

2.5. Health-Related Biomarker

The maximum quantum yield of photosystem II was used as a proxy of coral health. It was measured using an underwater pulse-amplitude modulated (PAM) fluorometer (diving-PAM, Heinz Walz, Effeltrich, Germany). The initial fluorescence signal (*Fo*) was obtained using a weak pulsed light (<1 µmol photons m^{-2} s^{-1}). In turn, the maximal fluorescence level (*Fm*) was determined using a short saturating pulse of actinic light. The variable fluorescence (*Fv*) was calculated from Fm–Fo, and the maximum quantum efficiency of photosystem II was obtained from the ratio *Fv/Fm*. The diving-PAM system was configured with the following parameters: measuring light intensity = 6; saturation pulse intensity = 8; saturation pulse width = 0.8, gain = 1; and damping = 1. Measurements were performed in triplicate and the average among them was used to minimize the chance of inaccurate values due to slight variation in probe position.

In the laboratory (acute) experiment, measurements were performed immediately after coral preconditioning (T0) and 4 days after exposure to the experimental conditions. In the mesocosm (chronic) experiment, measurements were performed at 0, 5, 10, 17, and 24 days after exposure to the experimental conditions. Procedures employed for measuring *Fv/Fm* values were as previously described [23]. For measurements of the minimum and maximum fluorescence levels used to calculate the *Fv/Fm* values, the coral fragments were subjected to a dark acclimation period (1 h). This was necessary to ensure that the photosystem II was momentarily inactive, ensuring that all remaining chlorophyll a fluorescence from the photosynthetic process had dissipated, and that the reaction centers were capable of performing their maximum photochemistry [80].

2.6. Growth-Related Biomarkers

Enzyme (CA and Ca^{2+}-ATPase) activities were used as proxies of coral growth. The procedures employed for sample preparation were as previously described [54,81]. Small fragments were cut (~0.2 g) and homogenized on ice with a buffer solution (1:1; *w*/*v*). For this, coral fragments were sonicated at 70 kHz (Sonaer Ultrasonics, Melville, NY, USA). For CA, the buffer solution contained 0.01 M tris(hydroxymethyl)aminomethane (pH 8.5), 0.075 M sucrose, 0.001 M phenylmethanesulfonylfluoride, and 0.001 M dithiothreitol. For Ca^{2+}-ATPase, the buffer solution consisted of 0.1 M tris hydrochloride (pH 7.6), 0.5 M sucrose, 0.001 M phenylmethanesulfonylfluoride, and 0.001 M dithiothreitol. In both cases, sample homogenized were subjected to centrifugation (20 min at 10,000× *g*) at 4 °C. Enzyme activity was measured in the homogenized supernatant and normalized by its content of total proteins. Protein content in the homogenized supernatant was measured with an assay kit (Sigma-Aldrich, USA) prepared according to the method described by Bradford [82].

Measurement of CA activity was conducted according to procedures already described [83]. The homogenate supernatant (enzyme source) was pipetted into a buffer solution containing 0.01 M tris(hydroxymethyl)aminomethane (pH 8.5), 0.075 M sucrose, 0.225 M mannitol, and 0.01 M phosphate. The enzyme substrate was prepared by saturating ultrapure water with CO_2. Every 5 s over 30 s, the reaction medium pH was determined. Blank reactions (reaction buffer solution and enzyme substrate) were also performed and used to calculate the pH variation only associated with the enzyme activity. The resulting pH values were plotted against time and CA activity (units of CA mg^{-1} protein) was determined based on the linear regression slope value.

Ca^{2+}-ATPase activity was performed following procedures already described [63]. The enzyme activity was determined used a reaction medium containing 0.02 mM tris hydrochloride (pH 7.6), 0.189 M NaCl, 0.005 M $MgCl_2$, 0.005 M $CaCl_2$, 0.001 M ouabain, and 0.003 M ATP. The concentration of inorganic phosphate (Pi) resulting from the enzymatic reaction was quantified with a phosphate assay kit (Sigma-Aldrich, St. Louis, MO, USA). The Pi and protein contents in the reaction medium and the reaction time were used to calculate the enzyme activity (mmol Pi mg^{-1} protein min^{-1}).

2.7. Statistical Evaluation

Data on the maximum quantum yield of the photosystem II and enzyme activities are presented as mean values (±standard error). Analysis of variance (ANOVA) assumptions were verified and no data transformation was required. Data normality was checked using the normal probability plot for raw residuals. Homogeneity of variances was checked using the Cochran C test. For each coral species, the Fe concentration effect on the maximum quantum yield was tested using one-way (acute experiment) or two-way (chronic experiment) ANOVA followed by the Fisher's post-hoc test for multiple comparisons. The Fe concentration effect on the CA and Ca^{2+}-ATPase activity was also verified through one-way ANOVA followed by the Fisher's post-hoc test for multiple comparisons. This analysis was performed for every exposure time. The significance level was at $p < 0.05$. The software Statistica 7.0 (StatSoft, Tulsa, OK, USA) and SigmaPlot 11.0 (Systat Software, Chicago, IL, USA) were used to perform the statistical analyses and build the graphs, respectively.

3. Results

3.1. Physical and Chemical Conditions

In the laboratory (acute) experiment, mean values (±SE) of seawater temperature, salinity, and pH corresponded to 27.2 ± 0.01 °C, 31.6 ± 0.51 ppt, and 8.23 ± 0.14, respectively. In the mesocosm (chronic) experiment, mean values (± SE) of seawater temperature, salinity, pH, and O_2 concentration corresponded 28.6 ± 0.12 °C, 35.2 ± 0.08 ppt, 8.33 ± 0.01, and 5.94 ± 0.12 mg L^{-1}.

Regarding Fe concentration, blank seawater samples collected in the Recife de Fora and Mucugê reefs were analyzed. Mean values of total and dissolved Fe concentrations were 0.019 ± 0.003 and 0.017 mg L^{-1}, respectively. For both experiments, procedural blanks were performed and results indicated no significant contamination of seawater associated with the infrastructure used in the laboratory and mesocosm. In both experiments, seawater contamination with Fe was prevented using only Fe-free equipment and materials, including the experimental tanks and pumps. Furthermore, all mesocosm structure (pumps, pipes, containers, and experimental tanks) were made of plastic material. In the laboratory (acute) experiment, mean values (±SE) of total Fe concentration in seawater samples from the procedural blank before and after the experimental period (4 days) corresponded to 0.020 ± 0.002 and 0.023 ± 0.001 mg L^{-1}, respectively. Regarding dissolved Fe concentration, they were 0.018 ± 0.003 and 0.017 ± 0.004 mg L^{-1}, respectively. In the mesocosm (chronic) experiment, mean values (±SE) of total Fe concentration in seawater samples from the procedural blank before and after passing the mesocosm system

corresponded to 0.021 ± 0.001 and 0.022 ± 0.002 mg L^{-1}, respectively. Regarding dissolved Fe concentration, they were 0.020 ± 0.002 and 0.019 ± 0.001 mg L^{-1}, respectively.

In the laboratory (acute) experiment, mean values (± SE) of total Fe concentration in the experimental media were 0.023 ± 0.002, 0.101 ± 0.003, 0.314 ± 0.007, and 0.904 ± 0.006 mg L^{-1} for the nominal increments of Fe in seawater of 0 (control), 0.1, 0.3, and 0.9 mg L^{-1}, respectively. In turn, the dissolved Fe concentration corresponded to 0.017 ± 0.001 mg L^{-1}, 0.085 ± 0.004 mg L^{-1}, 0.268 ± 0.013 mg L^{-1}, and 0.857 ± 0.012 mg L^{-1}, respectively. Regarding speciation, the measured concentration of dissolved Fe corresponded to 75.2%, 84.2%, 85.2%, and 94.7% of the measured total concentration of Fe in seawater, respectively.

In the mesocosm (chronic) experiment, mean values (±SE) of total Fe concentration in the experimental media were 0.022 ± 0.002, 0.118 ± 0.004, 0.323 ± 0.005, and 0.911 ± 0.010 mg L^{-1} for the nominal increments of Fe in seawater of 0 (control), 0.1, 0.3, and 0.9 mg L^{-1}, respectively. In turn, the dissolved Fe concentrations corresponded to 0.020 ± 0.0007 mg L^{-1}, 0.099 ± 0.0028 mg L^{-1}, 0.300 ± 0.0045 mg L^{-1}, and 0.871 ± 0.0107 mg L^{-1}, respectively. Regarding speciation, the measured concentration of dissolved Fe corresponded to 99.7%, 85.4%, 93.3%, and 95.7% of the measured total concentration of Fe in seawater, respectively. At the lower concentrations of Fe tested, higher levels of dissolved Fe were observed in the mesocosm (chronic) experiment than in the laboratory (acute) experiment, suggesting that a possible accumulation or loss can be occurred in the acute experiment.

3.2. Effects of Fe on Biomarkers

Mean values for all biological parameters analyzed in each coral species and experimental condition tested in the laboratory (acute) experiment are shown in Table S1. The maximum quantum yield of the photosystem II was not influenced by the acute exposure to seawater containing increased levels of Fe. This was observed for the three species of tested corals (Figure 1A; Table S2). Also, CA activity was not affected in *Siderastrea* sp. and *M. alcicornis*. In *M. harttii*, CA activity was reduced by 31.7% after exposure to 0.3 mg L^{-1} Fe and increased by 102.4% after being exposed to 0.9 mg L^{-1} Fe (Figure 1B; Table S2). The Ca^{2+}-ATPase activity of the three tested coral species was not altered in any of the Fe concentrations tested (Figure 1C; Table S2).

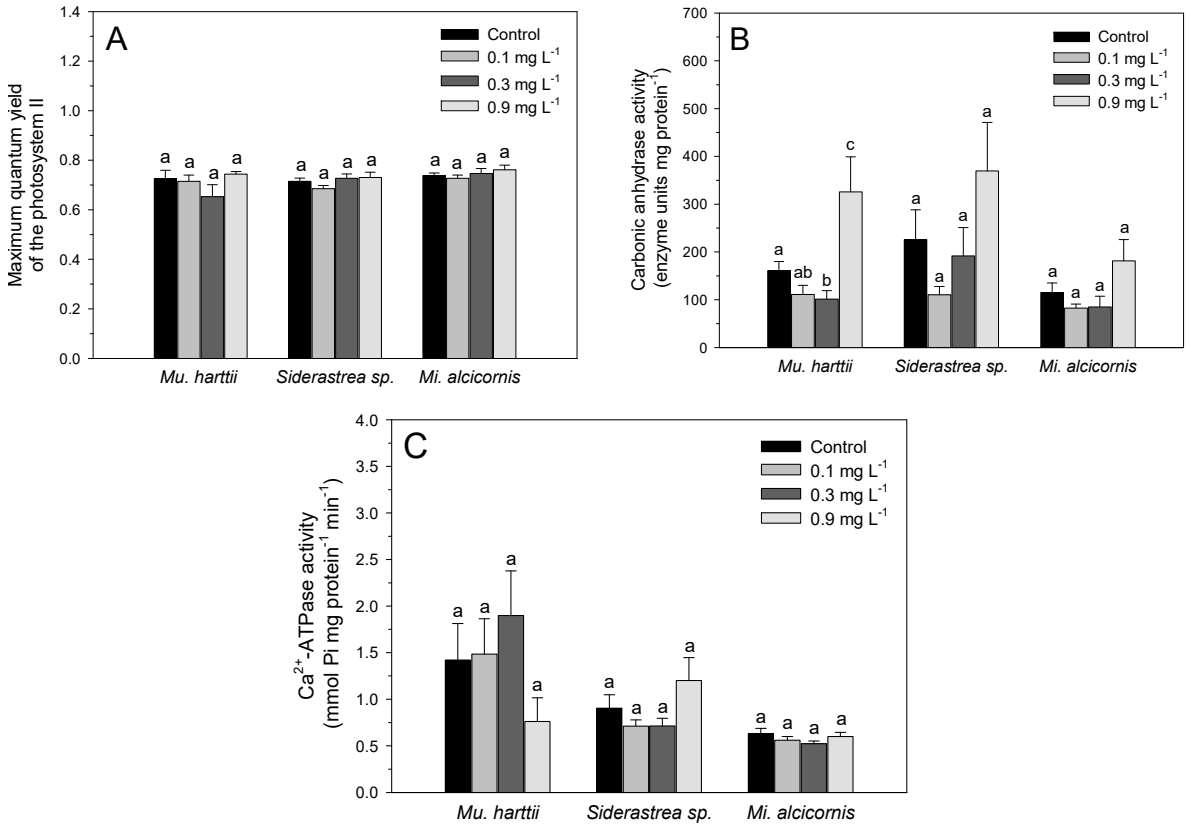


Figure 1. Biomarkers in the corals *Mussismilia harttii*, *Siderastrea* sp., and *Millepora alcicornis*: (**A**) maximum quantum yield of the photosystem II, (**B**) carbonic anhydrase activity, and (**C**) Ca^{2+}-ATPase activity. Corals were exposed to different levels of increments of Fe concentration in the aquarium seawater under controlled laboratory conditions for 4 days. Vertical bars and lines represent mean values and standard errors (n = 3), respectively. For each coral species, different letters indicate significant differences ($p < 0.05$) between the experimental groups.

Mean values for all biological parameters analyzed in each coral species and experimental condition tested in the mesocosm (chronic) experiment are shown in Table S3. The maximum quantum yield of the photosystem II was not affected after chronic exposure of *Mu. harttii* (Figure 2A; Table S4), *Siderastrea* sp (Figure 2B; Table S4), and *Mi. alcicornis* (Figure 2C; Table S4) to the increased levels of Fe. Only a 20.5% reduction was observed in *Mi. alcicornis* exposed for 17 days to 0.1 mg L^{-1} Fe, when compared to the control condition at the same experimental time (Figure 2C; Table S4). Also, *Mu. harttii* exposed to seawater with increased levels of Fe tended to display reduced values of the maximum quantum yield of the photosystem II along the experimental time. In this case, lower values were observed 24 days after the coral exposure to 0.3 mg L^{-1} Fe (19.8% reduction) and 0.9 mg L^{-1} Fe (20.9% reduction) (Figure 2A; Table S4).

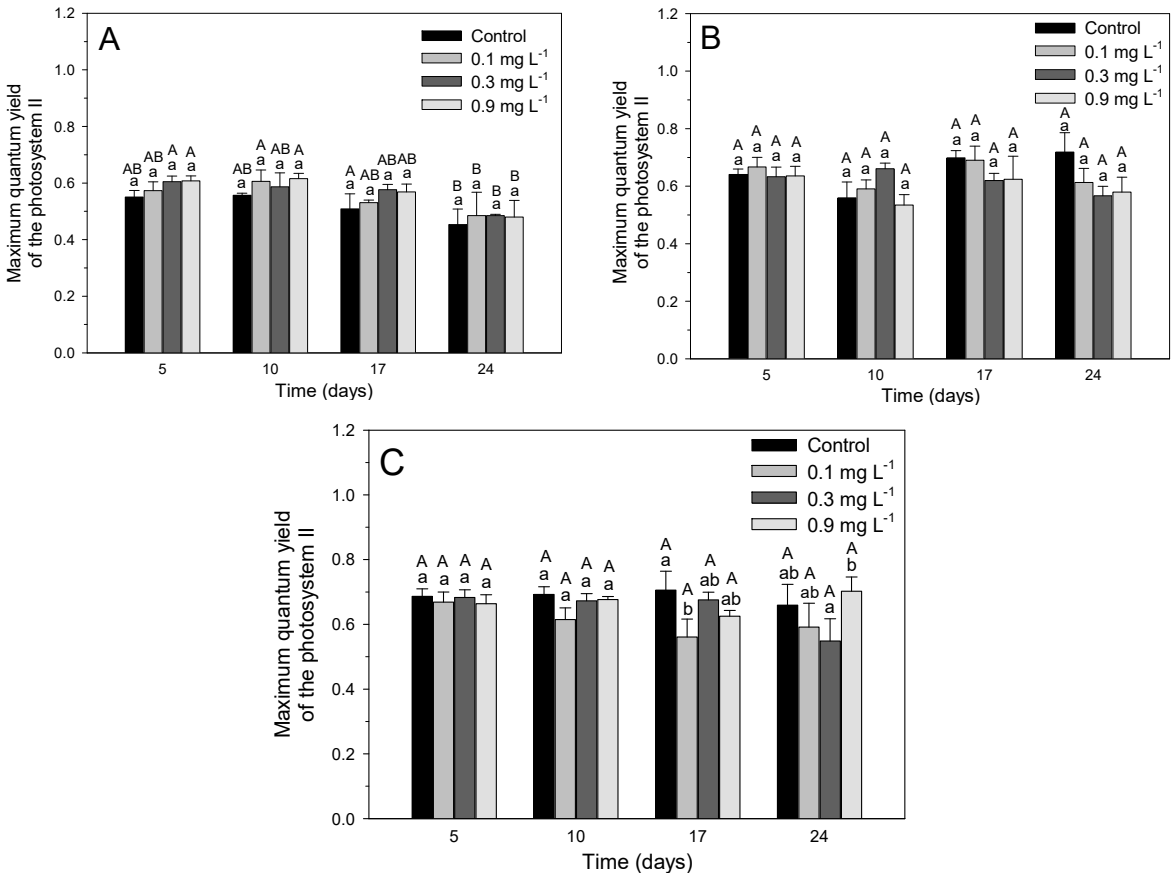


Figure 2. Maximum quantum yield of the photosystem II in reef-building corals: (**A**) *Mussismilia harttii*, (**B**) *Siderastrea* sp., and (**C**) *Millepora alcicornis*. Corals were exposed to different levels of increments of Fe concentration in the mesocosm seawater for up to 24 days. Vertical bars and lines represent mean values and standard errors (n = 4), respectively. Different small letters indicate significant differences ($p < 0.05$) between the experimental groups for the same exposure time. Different capital letters indicate significant difference ($p < 0.05$) between the exposure times for the same experimental condition.

Regarding enzyme activities, CA activity was not affected after chronic exposure of *Mu. harttii* (Figure 3A; Table S4) and *Siderastrea* sp. (Figure 3C; Table S4) to seawater containing increased levels of Fe. However, it was reduced in *Mi. alcicornis* exposed for 14 days to 0.3 mg L^{-1} Fe (62.1% reduction) and 0.9 mg L^{-1} Fe (54.5% reduction) (Figure 3B; Table S4). In the three coral species, Ca^{2+}-ATPase activity was not influenced by the exposure to seawater with increased levels of Fe (Figure 4; Table S4).

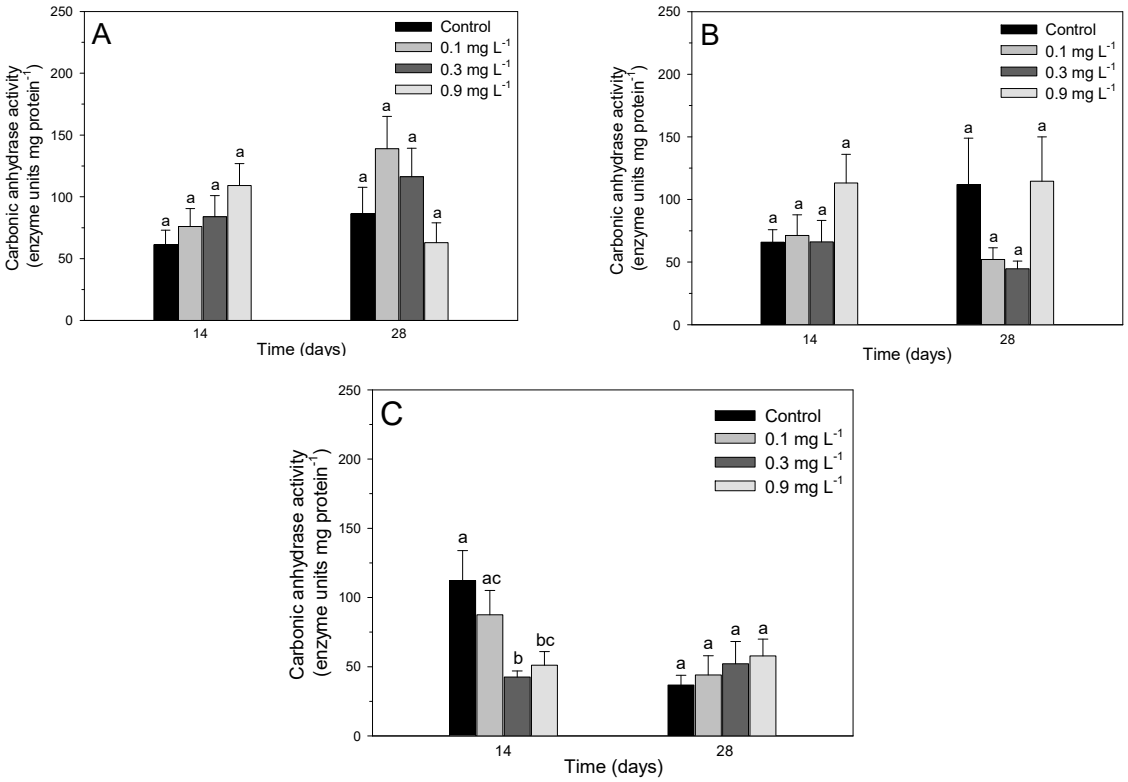


Figure 3. Carbonic anhydrase activity in reef-building corals: (**A**) *Mussismilia harttii*, (**B**) *Siderastrea* sp., and (**C**) *Millepora alcicornis*. Corals were exposed to different levels of increments in Fe concentration in the mesocosm seawater for 14 and 28 days. Vertical bars and lines represent mean values and standard errors (n = 4), respectively. For each coral species, different letters indicate significant differences ($p < 0.05$) between the experimental groups for the same exposure time.

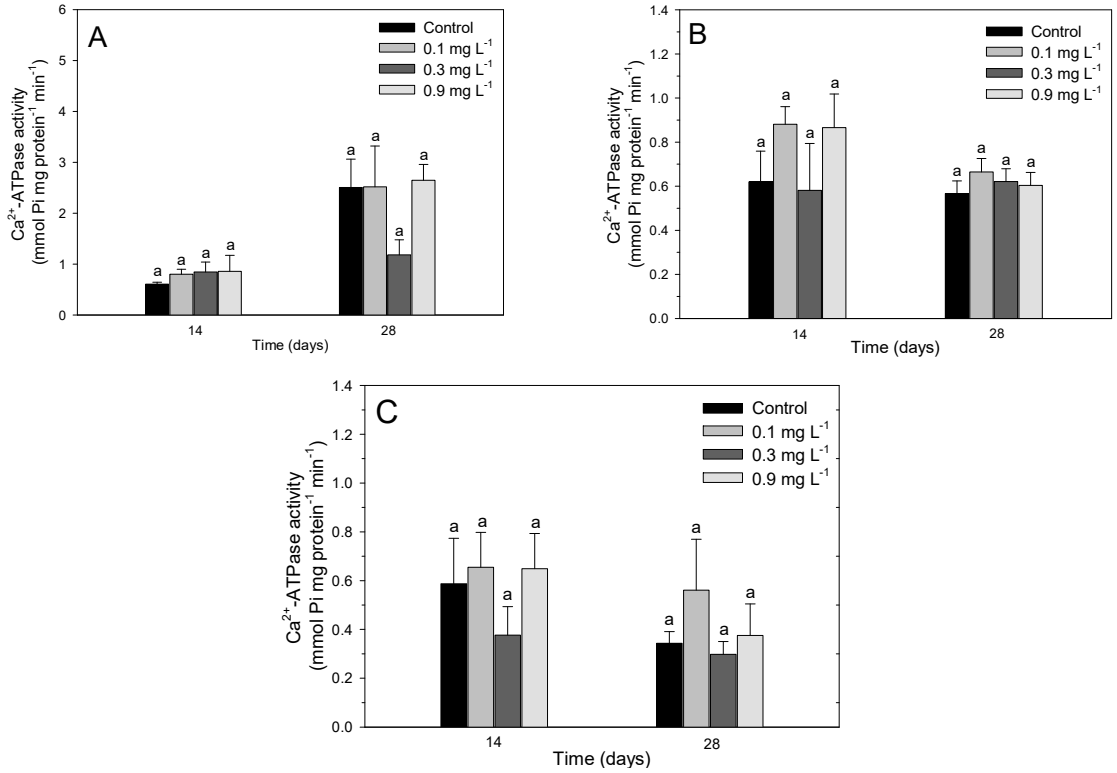


Figure 4. Ca^{2+}-ATPase activity in reef-building corals: (**A**) *Mussismilia harttii*, (**B**) *Siderastrea* sp., and (**C**) *Millepora alcicornis*. Corals were exposed to different levels of increments in Fe concentration in the mesocosm seawater for 14 and 28 days. Vertical bars and lines represent mean values and standard errors (n = 4), respectively. For each coral species, different letters indicate significant difference ($p < 0.05$) between the experimental groups for the same exposure time.

4. Discussion

4.1. Acute versus Chronic Effects

Integration of findings from the acute (laboratory) and chronic (mesocosm) experiments performed here clearly indicate that the major effects of incremented levels of Fe in seawater on coral health- and growth-related parameters are observed only after an exposure period longer than 4 days. Indeed, significant changes in the maximum yield of the photosystem II was only observed after the longer period of exposure tested (28 days). In this case, a significant reduction over the exposure time was observed at the higher concentrations of Fe tested (0.3 and 0.9 mg L^{-1}). However, this response was only observed in one of the three species of corals tested (*Mu. harttii*).

The influence of excessive levels of Fe on the photosynthetic process remains contradictory. For instance, Fe exposure has been shown to reduce symbiont density and nitrogen fixation in corals [84,85]. In plants, excessive Fe concentrations may lead to deficiencies of other essential nutrients for photosynthesis, such as Mn, P, K, Ca, and Mg [86,87], along with reductions in chlorophyll and carotenoid contents [88]. On the other hand, other reports have pointed out the importance of Fe in the primary producer's metabolism and growth [89]. This metal play an essential role as enzyme cofactor in the photosynthesis and respiration processes [90,91], resulting in increased growth rate of Symbiodiniaceae dinoflagellates [92–94] and respiration rate in corals [95].

Regarding the growth-related biomarkers, the only acute effect observed was an increased CA activity in the scleractinian coral *Mu. harttii*. However, it is worth noting that this inhibitory effect was only observed in this species and only in one of the three increments in Fe concentration tested (0.3 mg L^{-1}). Furthermore, it is important to stress that this inhibitory effect was not observed after a long period of exposure (14 and 18 days) in the mesocosm system. Also, a transitory significant change in CA activity was observed in the hydrocoral *Mi. alcicornis* chronically exposed to the higher increments of Fe concentration tested.

The findings reported above, clearly indicate that the responses of the health- and growth-related biomarkers evaluated in the present study are species-specific and markedly dependent on the time of exposure to incremented levels of Fe in seawater. Therefore, future field-relevant studies considering longer periods of time (>28 days) are key to better evaluate the potential ecological effects of elevated concentrations of Fe in seawater in corals and hydrocorals.

4.2. Enzyme-Specific Effects

Results from both acute and chronic experiments performed in the present study indicate that only CA had its activity affected by the incrementing levels of Fe in seawater. Tissue bioaccumulation of Fe can generate free radicals and lead to a condition of oxidative stress [59–62]. This essential metal is capable of generating free radicals due its role in oxidation and reduction cycles, mainly in the presence of H_2O_2, generating even more unstable and toxic reactive species. It is well known that ROS can cause several biological injuries, including enzyme activity inhibition [60,63]. Unfortunately, measurement of ROS and oxidative damage (lipid peroxidation and protein oxidation) were not evaluated in the present study. Therefore, a direct link between Fe-induced oxidative stress and CA activity inhibition could not be demonstrated here. Future work considering the analysis of these parameters under scenarios of incrementing concentrations of Fe in seawater would help to confirm the possible direct relationship between oxidative stress and CA activity inhibition in corals.

Interestingly, a recovery of the CA was observed in the hydrocoral *Mi. alcicornis* after 28 days of exposure to Fe. In fact, no influence of Fe exposure was observed in the three coral species after 28 days of experiment, regardless the Fe concentration tested. In corals, the activities of enzymes involved in energy metabolism (glycolytic pathway, Krebs cycle, and electron transport chain) were shown to be inhibited after acute exposure to Cu [58], an essential metal like Fe. However, as observed for CA, enzyme activities were fully recovered after a long period of exposure to the metal [58]. A recovery of energetic conditions could induce antioxidant defense mechanisms in corals [84]. In fact, a prolonged exposure (12 days) of *M. harttii* to an essential metal (Cu) increased the activity of antioxidant enzymes [96]. Such physiological response could thus explain the reduced oxidative damage observed in this coral species exposed to increments of Fe in seawater [97], as well as the recovery of CA activity observed here.

In reef-building corals, Ca^{2+}-ATPase plays a crucial role in calcification. It transports Ca^{2+} to the sites of calcification and thus contributing to the formation of $CaCO_3$ for the skeleton [13,16]. This enzyme has been used as a tool to assess the health of the biomineralization process in calcifying invertebrates [54,67,98,99]. The effects of Cu, an essential metal like Fe, on the activity of ATPases, including Ca^{2+}-ATPase, have been documented for several groups of organisms [98,100–102], including corals [23,67]. Ca^{2+}-ATPase activity of the coral species tested here was not affected by the increments of Fe in seawater. This result was observed after either acute or

chronic exposure to the metal. This finding indicates a low sensitivity of Ca^{2+}-ATPase activity to Fe, contrary to what is observed for Cu, an essential metal like Fe.

4.3. Ecological Implications

The three coral species studied here are known to display different growth morphology [103,104] and exhibit varying degrees of tolerance to anthropogenic stressors. Within the South Atlantic context, *Mi. alcicornis* and *Mu. harttii* may be considered more sensitive, while *Siderastrea* spp. are often considered stress-tolerant [105–109]. However, in general, our findings show that the coral species evaluated in the present study were differentially and only transitorily affected with the increments of Fe in seawater. This may be attributed to the fact that Brazilian reefs develop under a marginal oceanographic setting [110]. The numerous river discharges along the continental shelf result in elevated turbidity and nutrient concentrations, creating challenging conditions for reef establishment [103,104,111–113].

Our findings with Fe agree with the fact that seawater contamination with Cu, another essential metal, negatively influenced the photosynthetic apparatus in *Mu. harttii* [114]. Furthermore, it has been recently demonstrated in corals that photosynthesis, calcification enzymes, and oxidative status parameters were negatively affected by exposure to Fe and Cu [81,115–117]. Also, coral competing with macroalgae and zoantharians was also reported to influenced by incremented concentrations of Fe in seawater [118]. It is important to stress that these effects can lead to major ecological impacts associated with the health and growth of corals and hydrocorals. As they are main reef builders and structural complexity providers [28–31], negative impacts of essential metals can lead to marked changes in reef structure and biodiversity (Figure 5). This is especially concerning if we consider that the increasing threats from global stressors can exacerbate the toxicity of essential metals such as Cu and Fe in corals [119–122]. Therefore, future studies focused on this matter are key to further elucidate the physiological and ecological effects of increased levels of essential metals (Fe and Cu) in seawater on corals under environmentally relevant actual and future scenarios. Information from these studies, integrated with those already reported, will certainly help to implement and/or improve the coral management and conservation strategies.

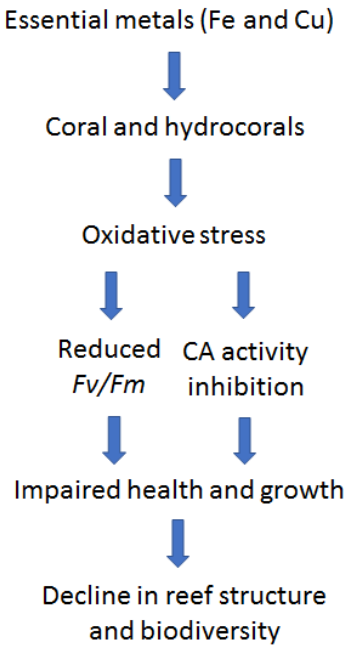


Figure 5. Conceptual figure summarizing the mechanistic links among exposure to increased concentrations of essential metals (Fe and Cu) in seawater, oxidative stress, photosynthetic efficiency (*Fv/Fm*), carbonic anhydrase (CA) activity, and potential ecological effects in corals and hydrocorals.

5. Conclusions

The dissolved Fe concentrations tested in the present study reflect both natural levels of this metal commonly found in marine environments and those reported following large-scale contamination events. Our findings indicate that, under the current experimental conditions, Fe exposure induced limited but measurable changes in photosystem efficiency and carbonic anhydrase activity, suggesting tolerance within short-term exposures yet

highlighting the need for multi-stressor investigations. Also, our findings indicate that the responses of the health- and growth-related biomarkers affected by the incrementing levels of Fe in seawater are species-specific and dependent on the exposure time. Therefore, future field-relevant studies considering longer chronic periods of exposure and the evaluation of mixtures of metals (e.g., Fe and Cu) are key to better evaluate the potential ecological effects of seawater enrichment with essential metals in corals and hydrocorals.

Supplementary Materials

The additional data and information can be downloaded at: https://media.sciltp.com/articles/others/251219093646073 0/EESUS-25100053-SM-FC1.pdf. Table S1: Mean values (±standard error; n = 3) for all biological parameters analyzed in each coral species and experimental condition tested in the laboratory (acute) experiment: maximum quantum yield of the photosystem II (*Fv/Fm*), carbonic anhydrase (CA) and Ca^{2+}-ATPase activity. Table S2: One-way analysis of variance results for data on the maximum quantum yield of the photosystem II and the activity of enzymes involved in the calcification process in the three coral species exposed to increments in the Fe concentration of the aquarium seawater under laboratory conditions for 4 days. Significant *p* values are marked in bold. Table S3: Mean values (±standard error; n = 4) for all biological parameters analyzed in each coral species and experimental condition tested in the mesocosm (chronic) experiment: maximum quantum yield of the photosystem II (*Fv/Fm*), carbonic anhydrase (CA) and Ca^{2+}-ATPase activity. Table S4: One-way analysis of variance results for data on the maximum quantum yield of the photosystem II and the activity of enzymes involved in the calcification process in the three coral species exposed to increments in the Fe concentration of the mesocosm seawater for 14 and 28 days. Significant *p* values are marked in bold.

Author Contributions

J.d.S.F.: investigation, formal analysis, writing—original draft preparation, writing—reviewing and editing; L.M.F.: investigation, writing—reviewing and editing; J.V.L.B.: investigation, writing—reviewing and editing; T.J.V.: investigation, writing—reviewing and editing; K.P.S.: methodology, resources, writing—reviewing and editing; C.H.F.L.: methodology, resources, writing—reviewing and editing; P.G.C.: investigation, formal analysis, writing—reviewing and editing; M.M.: conceptualization, methodology, writing—reviewing and editing, project administration, funding acquisition; A.B.: conceptualization, methodology, investigation, formal analysis, data curation, writing—reviewing and editing, supervision, project administration, funding acquisition. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by Projeto Coral Vivo and its sponsors Petrobras (Programa Petrobras Socioambiental—PETRO V; grant number 5850.0104469.17.2) and Arraial d'Ajuda Eco Parque. Adalto Bianchini is a research fellow of the Brazilian Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; grant number 311410/2021-9).

Institutional Review Board Statement

The authors state that the present study was performed following the Declaration of Helsinki guidelines.

Informed Consent Statement

Not applicable.

Data Availability Statement

Authors will make the data available upon reasonable request.

Conflicts of Interest

The authors declare no conflict of interest. The authors declare that the funders had no involvement in any stage of this study.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

References

1. Hatcher, B.G. Coral reef primary productivity. A hierarchy of pattern and process. *Trends Ecol. Evol.* **1990**, *5*, 149–155. https://doi.org/10.1016/0169-5347(90)90221-X.
2. Davis, K.L.; Colefax, A.P.; Tucker, J.P.; et al. Global coral reef ecosystems exhibit declining calcification and increasing primary productivity. *Commun. Earth Environ*. **2021**, *2*, 105. https://doi.org/10.1038/s43247-021-00168-w.
3. Fisher, R.; O'Leary, R.A.; Low-Choy, S.; et al. Species richness on coral reefs and the pursuit of convergent global estimates. *Curr. Biol.* **2015**, *25*, 500–505. https://doi.org/10.1016/j.cub.2014.12.022.
4. Costanza, R.; De Groot, R.; Sutton, P.; et al. Changes in the global value of ecosystem services. *Global Environ. Chang.* **2014**, *26*, 152–158. https://doi.org/10.1016/j.gloenvcha.2014.04.002.
5. Giglio, V.J.; Aued, A.W.; Cordeiro, C.A.; Eggertsen, L.; et al. A global systematic literature review of ecosystem services in reef environments. *Environ. Manag.* **2024**, *73*, 634–645. https://doi.org/10.1007/s00267-023-01912-y.
6. Leão, Z.M.A.N.; Kikuchi, R.K.P.; Ferreira, B.P.; et al. Brazilian coral reefs in a period of global change: A synthesis. *Braz. J. Oceanogr.* **2016**, *64*, 97–116. https://doi.org/10.1590/S1679-875920160916064sp2.
7. Pinheiro, H.T.; Rocha, L.A.; Macieira, R.M.; et al. South-western Atlantic reef fishes: Zoogeographical patterns and ecological drivers reveal a secondary biodiversity centre in the Atlantic Ocean. *Divers. Distrib.* **2018**, *24*, 951–965. http://dx.doi.org/10.1111/ddi.12729.
8. Castro, C.B.; Pires, D.O. Brazilian coral reefs: What we already know and what is still missing. *Bull. Mar. Sci.* **2001**, *69*, 357–371.
9. Pereira, P.H.; Lima, G.V.; Araujo, J.C.; et al. Mesophotic reefs of the largest Brazilian coastal protected area: Mapping, characterization and biodiversity. *Diversity* **2022**, *14*, 760. https://doi.org/10.3390/d14090760.
10. Coni, E.O.; Ferreira, C.M.; Moura, R.L.; et al. An evaluation of the use of branching fire-corals (*Millepora* spp.) as refuge by reef fish in the Abrolhos Bank, eastern Brazil. *Environ. Biol. Fishes* **2013**, *96*, 45–55. https://doi.org/10.1007/s10641-012-0021-6.
11. Luza, A.L.; Quimbayo, J.P.; Ferreira, C.E.; et al. Low functional vulnerability of fish assemblages to coral loss in Southwestern Atlantic marginal reefs. *Sci. Rep.* **2022**, *12*, 17164. https://doi.org/10.1038/s41598-022-20919-9.
12. Allemand, D.; Ferrier-Pagès, C.; Furla, P.; et al. Biomineralisation in reef-building corals: From molecular mechanisms to environmental control. *C. R. Palevol.* **2004**, *3*, 453–467. https://doi.org/10.1016/j.crpv.2004.07.011.
13. Allemand, D.; Tambutté, E.; Zoccola, D.; et al. Coral calcification, cells to reefs. In *Coral Reefs: An Ecosystem in Transition*; Dubinsky, Z., Stambler, N., Eds.; Springer: Dordrecht, The Netherlands, 2011; pp. 119–150. https://doi.org/10.1007/978-94-007-0114-4.
14. Bertucci, A.; Moya, A.; Tambutté, S.; et al. Carbonic anhydrases in anthozoan corals e a review. *Bioorg. Med. Chem.* **2013**, *21*, 1437–1450. https://doi.org/10.1016/j.bmc.2012.10.024.
15. Rollion-Bard, C.; Blamart, D. Possible controls on Li, Na, and Mg incorporation into aragonite coral skeletons. *Chem. Geol.* **2015**, *396*, 98–111. https://doi.org/10.1016/j.chemgeo.2014.12.011.
16. Al-Horani, F.A.; Al-Moghrabi, S.M.; De Beer, D. The mechanism of calcification and its relation to photosynthesis and respiration in the scleractinian coral *Galaxea fascicularis*. *Mar. Biol.* **2003**, *142*, 419–426. http://dx.doi.org/10.1007/s00227-002-0981-8.
17. Cohen, A.L.; McConnaughey, T.A. A geochemical perspective on coral mineralization. *Rev. Mineral. Geochem.* **2003**, *54*, 151–187. http://dx.doi.org/10.2113/0540151.
18. LaJeunesse, T.C.; Parkinson, J.E.; Gabrielson, P.W.; Jeong, H.J.; Reimer, J.D.; Voolstra, C.R.; Santos, S.R. Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. *Curr. Biol.* **2018**, *28*, 2570–2580. https://doi.org/10.1016/j.cub.2018.07.008.
19. Colombo-Pallotta, M.F.; Rodríguez-Román, A.; Iglesias-Prieto, R. Calcification in bleached and unbleached *Montastraea faveolata*: Evaluating the role of oxygen and glycerol. *Coral Reefs* **2010**, *29*, 899–907. https://doi.org/10.1007/s00338-010-0638-x.
20. Davy, S.K.; Allemand, D.; Weis, V.M. Cell biology of cnidarian-dinoflagellate symbiosis. *Microbiol. Mol. Biol.* **2012,** *76*, 229–261. https://doi.org/10.1128/mmbr.05014-11.
21. Muscatine, L.; Porter, J.W. Reef corals: Mutualistic symbioses adapted to nutrient-poor environments. *Bioscience* 1**977**, *27*, 454–460. https://doi.org/10.2307/1297526.
22. Stanley, G.D. Photosymbiosis and the evolution of modern coral reefs. *Science* **2006**, *312*, 857–858. https://doi.org/10.1126/science.1123701.
23. Fonseca, J.S.; Marangoni, L.F.; Marques, J.A.; et al. Effects of increasing temperature alone and combined with copper exposure on biochemical and physiological parameters in the zooxanthellate scleractinian coral *Mussismilia harttii*. *Aquat. Toxicol.* **2017**, *190*, 121–132. https://doi.org/10.1016/j.aquatox.2017.07.002.

24. Jones, R.J.; Kildea, T.; Hoegh-Guldberg, O. PAM chlorophyll fluorometry: A new in situ technique for stress assessment in scleractinian corals, used to examine the effects of cyanide from cyanide fishing. *Mar. Pollut. Bull.* **1999**, *38,* 864–874. https://doi.org/10.1016/S0025-326X(98)90160-6.
25. Silverstein, R.N.; Cunning, R.; Baker, A.C. Change in algal symbiont communities after bleaching, not prior heat exposure, increases heat tolerance of reef corals. *Glob. Chang. Biol.* **2014**, *21*, 236–249. https://doi.org/10.1111/gcb.12706.
26. van Dam, J.W.; Negri, A.P.; Uthicke, S.; Mueller, J.F. Chemical pollution on coral reefs: Exposure and ecological effects. In *Ecological Impacts of Toxic Chemicals*; Sanchez-Bayo, F., van den Brink, P.J., Mann, R.M., Eds.; Bentham Science Publishers: Amsterdam, The Netherlands, 2011; Chapter 9, pp. 187–211. https://doi.org/10.2174/978160805121210187.
27. McLean, M.; Cuetos-Bueno, J.; Nedlic, O.; et al. Local stressors, resilience, and shifting baselines on coral reefs. *PLoS ONE* **2016**, *11*, 0166319. https://doi.org/10.1371/journal.pone.0166319.
28. Harborne, A.R.; Rogers, A.; Bozec, Y.M.; et al. Multiple stressors and the functioning of coral reefs. *Ann. Rev. Mar. Sci.* **2017**, *9*, 445–468. https://doi.org/10.1146/annurev-marine-010816-060551.
29. Ellis, J.I.; Jamil, T.; Anlauf, H.; et al. Multiple stressor effects on coral reef ecosystems. *Glob. Chang. Biol.* **2019**, *25*, 4131–4146. https://doi.org/10.1111/gcb.14819.
30. Hughes, T.P.; Anderson, K.D.; Connolly, S.R.; et al. Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. *Science* **2018**, *359*, 80–83. https://doi.org/10.1126/science.aan8048.
31. Dietzel, A.; Bode, M.; Connolly, S.R.; et al. Long-term shifts in the colony size structure of coral populations along the Great Barrier Reef. *Proc. R. Soc. B* **2020**, *287*, 20201432. https://doi.org/10.1098/rspb.2020.1432.
32. Tebbett, S.B.; Connolly, S.R.; Bellwood, D.R. Benthic composition changes on coral reefs at global scales. *Nat. Ecol. Evol.* **2023**, *7*, 71–81. https://doi.org/10.1038/s41559-022-01937-2.
33. Prouty, N.G.; Cohen, A.; Yates, K.K.; et al. Vulnerability of coral reefs to bioerosion from land-based sources of pollution. *J. Geophys. Res. Oceans* **2017**, *122*, 9319–9331. https://doi.org/10.1002/2017jc013264.
34. Silbiger, N.J.; Nelson, C.E.; Remple, K.; et al. Nutrient pollution disrupts key ecosystem functions on coral reefs. *Proc. R. Soc. B* **2018**, *285*, 20172718. https://doi.org/10.1098/rspb.2017.2718.
35. Dubinsky, Z.; Stambler, N. Marine pollution and coral reefs. *Glob. Chang. Biol.* **1996**, *2*, 511–526. https://doi.org/10.1111/j.1365-2486.1996.tb00064.x.
36. Jones, R.J. Zooxanthellae loss as a bioassay for assessing stress in corals. *Mar. Ecol. Prog. Ser.* **1997**, *149*, 163–171. https://doi.org/10.3354/meps149163.
37. Verma, R.; Dwivedi, P. Heavy metal water pollution—A case study. *Rec. Res. Sci. Technol.* **2013**, *5*, 98–99.
38. Francini-Filho, R.B.; Cordeiro, M.C.; Omachi, C.Y.; et al. Remote sensing, isotopic composition and metagenomics analyses revealed Doce River ore plume reached the southern Abrolhos Bank Reefs. *Sci. Total Environ.* **2019**, *697*, 134038. https://doi.org/10.1016/j.scitotenv.2019.134038.
39. Magris, R.A.; Marta-Almeida, M.; Monteiro, J.A.F.; et al. A modelling approach to assess the impact of land mining on marine biodiversity: Assessment in coastal catchments experiencing catastrophic events (SW Brazil). *Sci. Total Environ.* **2019**, *659*, 828–840. https://doi.org/10.1016/j.scitotenv.2018.12.238.
40. Coimbra, K.T.O.; Alcântara, E.; de Souza Filho, C.R. Possible contamination of the Abrolhos reefs by Fundao dam tailings, Brazil—New constraints based on satellite data. *Sci. Total Environ.* **2020**, *733*, 138101. https://doi.org/10.1016/j.scitotenv.2020.138101.
41. Evangelista, H.; de Paula, R.L.M.; Magalhães, N.; et al. Intake of trace contaminants by corals in Abrolhos reef bank (western South Atlantic) during two decades of coastal impacts. *Cont. Shelf Res.* **2023**, *255*, 104946. https://doi.org/10.1016/j.csr.2023.104946.
42. Leão, Z.M.A.N.; Kikuchi, R.K.P. The Abrolhos reefs of Brazil. In *Coastal Marine Ecosystems of Latin America. Ecological Studies*; Seeliger, U., Kjerfve, B., Eds.; Springer-Verlag: Berlin/Heidelberg, Germany, 2001; Volume 144, pp. 83–96. https://doi.org/10.1007/978-3-662-04482-7_7.
43. Dutra, G.F.; Santos, L.P.; Coutinho, B.H.; et al. Marine biodiversity hotspots in the Abrolhos Region and Vitória-Trindade Seamount Chain, Brazil, with implications for conservation. *Ocean Coast. Res.* **2025**, *73*, e25019. https://doi.org/10.1590/2675-2824073.24055.
44. Dal Pizzol, J.L.; Marques, J.A.; Fonseca, J.S.; et al. Metal accumulation induces oxidative stress and alters carbonic anhydrase activity in corals and symbionts from the largest reef complex in the South Atlantic ocean. *Chemosphere* **2022**, *290*, 133216. https://doi.org/10.1016/j.chemosphere.2021.133216.
45. Cardoso, G.O.; Falsarella, L.N.; Chiroque-Solano, P.M.; et al. Coral growth bands recorded trace elements associated with the Fundão dam collapse. *Sci. Total Environ.* **2021**, *807*, 150880. https://doi.org/10.1016/j.scitotenv.2021.150880.
46. Costa, P.G.; Marube, L.C.; Artifon, V.; et al. Temporal and spatial variations in metals and arsenic contamination in water, sediment and biota of freshwater, marine and coastal environments after the Fundão dam failure. *Sci. Total Environ.* **2022**, *806*, 151340. https://doi.org/10.1016/j.scitotenv.2021.151340.

47. Romero, J.; Tonetti Botana, M.; Elias, A.; et al. Effect of iron speciation on growth and heat resistance of Symbiodiniaceae. *Ocean Coast. Res.* **2022**, *70,* 22016. https://doi.org/10.1590/2675-2824070.21103jmdr.
48. Dellisanti, W.; Zhang, Q.; Ferrier-Pagès, C.; et al. Contrasting effects of increasing dissolved iron on photosynthesis and O_2 availability in the gastric cavity of two Mediterranean corals. *PeerJ*. **2024**, *12*, e17259. https://doi.org/10.7717/peerj.17259.
49. Tu, T.-H.; Hsieh, H.-Y.; Meng, P.-J.; et al. Physiological responses of scleractinian coral to trace metal enrichment and thermal stress. *Mar. Environ. Res.* **2025**, *207*, 107085. https://doi.org/10.1016/j.marenvres.2025.107085.
50. Grant, A.J.; Graham, K.; Frankland, S.; et al. Effect of copper on algal-host interactions in the symbiotic coral *Plesiastrea versipora*. *Plant Physiol. Biochem.* **2003**, *41*, 383–390. https://doi.org/10.1016/S0981-9428(03)00034-2.
51. Bielmyer, G.K.; Grosell, M.; Bhagooli, R.; et al. Differential effects of copper on three species of scleractinian corals and their algal symbionts (*Symbiodinium* spp.). *Aquat. Toxicol.* **2010**, *97*, 125–133. https://doi.org/10.1016/j.aquatox.2009.12.021.
52. Yost, D.M.; Jones, R.J.; Mitchelmore, C.L. Alterations in dimethylsulfoniopropionate (DMSP) levels in the coral *Montastraea franksi* in response to copper exposure. *Aquat. Toxicol.* **2010**, *98*, 367–373. https://doi.org/10.1016/j.aquatox.2010.03.005.
53. Schwarz, J.A.; Mitchelmore, C.L.; Jones, R.; et al. Exposure to copper induces oxidative and stress responses and DNA damage in the coral *Montastraea franksi*. *Comp. Biochem. Physiol. C* **2013**, *157*, 272–279. https://doi.org/10.1016/j.cbpc.2012.12.003.
54. Fonseca, J.S.; Marangoni, L.F.B.; Marques, J.A.; Bianchini, A. Carbonic anhydrase as a potential biomarker for acute exposure to copper in corals. *Chemosphere* **2019**, 227, 598–605. https://doi.org/10.1016/j.chemosphere.2019.04.089.
55. Nystrom, M.; Nordemar, I.; Tedengren, M. Simultaneous and sequential stress from increased temperature and copper on the metabolism of the hermatypic coral *Porites cylindrical*. *Mar. Biol.* **2001**, *138*, 1225–1231. https://doi.org/10.1007/s002270100549.
56. Negri, A.P.; Hoogenboom, M.O. Water contamination reduces the tolerance of coral larvae to thermal stress. *PLoS ONE* **2011**, *6*, e19703. https://doi.org/10.1371/journal.pone.0019703.
57. Nikinmaa, M. Climate change and ocean acidification interactions with aquatic toxicology. *Aquat. Toxicol.* **2013**, *126*, 365–372. https://doi.org/10.1016/j.aquatox.2012.09.006.
58. Fonseca, J.S.; Marangoni, L.F.B.; Marques, J.A.; et al. Energy metabolism enzymes inhibition by the combined effects of increasing temperature and copper exposure in the coral *Mussismilia harttii*. *Chemosphere* **2019**, *236*, 124420. https://doi.org/10.1016/j.chemosphere.2019.124420.
59. Winterbourn, C.C. Toxicity of iron and hydrogen peroxide: The Fenton reaction. *Toxicol. Lett.* **1995**, *82/83*, 969–974. https://doi.org/10.1016/0378-4274(95)03532-X.
60. Liu, R.; Liu, W.; Doctrow, S.R.; et al. Iron toxicity in organotypic cultures of hippocampal slices: role of reactive oxygen species. *Neurobiol. Aging* **2003**, *24*, 977–983. https://doi.org/10.1046/j.1471-4159.2003.01708.x.
61. Vijayavel, K.; Downs, C.A.; Ostrander, G.K.; et al. Oxidative DNA damage induced by iron chloride in the larvae of the lace coral *Pocillopora damicornis*. *Comp. Biochem. Physiol. C* **2012**, *155*, 275–280. https://doi.org/10.1016/j.cbpc.2011.09.007.
62. Lesser, M.P. Oxidative stress in marine environments: Biochemistry and physiological ecology. *Annu. Rev. Physiol.* **2006**, *68*, 253–278. https://doi.org/10.1146/annurev.physiol.68.040104.110001.
63. Vajreswari, A.; Rao, P.S.; Kaplay, S.S.; et al. Erythrocyte membrane in rats fed high erucic acid-containing mustard oil: Osmotic fragility, lipid composition, and (Na^+, K^+)- and (Ca^{2+}, Mg^{2+})-ATPases. *Biochem. Med.* **1983**, *29*, 74–84. https://doi.org/10.1016/0006-2944(83)90056-x.
64. Kaya, E.D.; Soyüt, H.; Beydemir, S. Carbonic anhydrase activity from the gilthead seabream (*Sparus aurata*) liver: The toxicological effects of heavy metals. *Environ. Toxicol. Pharmacol.* **2013**, *36*, 514–521. https://doi.org/10.1016/j.etap.2013.05.019.
65. Kaya, E.D.; Soyüt, H.; Beydemir, S. The toxicological impacts of some heavy metals on carbonic anhydrase from gilthead sea bream (*Sparus aurata*) gills. *Environ. Toxicol. Pharmacol.* **2015**, *39*, 825–832. https://doi.org/10.1016/j.etap.2015.01.021.
66. Lionetto, M.G.; Caricato, R.; Erroi, E.; et al. Potential application of carbonic anhydrase activity in bioassay and biomarker studies. *Chem. Ecol.* **2006**, *22*, S119–S125. https://doi.org/10.1080/02757540600670661.
67. Marangoni, L.F.B.; Marques, J.A.; Duarte, G.A.S.; et al. Copper effects on biomarkers associated with photosynthesis, oxidative status and calcification in the Brazilian coral *Mussismilia harttii* (Scleractinia, Mussidae). *Mar. Environ. Res.* **2017**, *130*, 248–257. https://doi.org/10.1016/j.marenvres.2017.08.002.
68. Ross, C.L.; DeCarlo, T.M.; McCulloch, M.T. Environmental and physiochemical controls on coral calcification along a latitudinal temperature gradient in Western Australia. *Glob. Chang. Biol.* **2019**, *25*, 431–447. https://doi.org/10.1111/gcb.14488.
69. Venn, A.A.; Tambutté, E.; Zoccola, D.; et al. Coral calcification at the cellular scale: Insight through the 'window' of the growing edge. In *Frontiers in Invertebrate Physiology: A Collection of Reviews*; Saleuddin, S., Leys, S.P., Roer, R.D., et al., Eds.; Apple Academic Press: New York, NY, USA, 2024; pp. 343–397. https://doi.org/10.1201/9781003403319-7.
70. De'ath, G.; Lough, J.M.; Fabricius, K.E. Declining coral calcification on the great barrier reef. *Science* **2009**, *323*, 116–119. https://doi.org/10.1126/science.1165283.
71. Weis, V.M.; Allemand, D. What determines coral health? *Science* **2009**, *324*, 1153–1155. https://doi.org/10.1126/science.1172540.

72. Grottoli, A.G.; Toonen, R.J.; van Woesik, R.; et al. Increasing comparability among coral bleaching experiments. *Ecol. Appl.* **2021**, *31*, e02262. https://doi.org/10.1002/eap.2262.
73. Armstrong, F.A.J. The iron content of sea water. *J. Mar. Biol. Assoc. U.K.* **1957**, *36*, 509–517. https://doi.org/10.1017/S0025315400025807.
74. Bianchini, A.; Silva, C.C.; Lauer, M.M.; et al. Avaliação do Impacto da Lama/Pluma Samarco sobre os Ambientes Costeiros e Marinhos (ES e BA) com Ênfase nas Unidades de Conservação: 1ª Expedição do Navio de Pesquisa Soloncy Moura do CEPSUL/ICMBio. 2016. Available online: https://www.gov.br/icmbio/pt-br/centrais-de-conteudo/publicacoes/documentos/documentos-rio-doce-espirito-santo/DCOM_relatorio_revisado_atualizado_29_04_2016_AB.pdf (accessed on 19 September 2025).
75. Sá, F.; Longhini, C.M.; Costa, E.S.; et al. Time-sequence development of metal (loid)s following the 2015 dam failure in the Doce river estuary, Brazil. *Sci. Total Environ.* **2021**, *769*, 144532. https://doi.org/10.1016/J.SCITOTENV.2020.144532.
76. Longhini, C.M.; Rodrigues, S.K.; Costa, E.S.; et al. Environmental quality assessment in a marine coastal area impacted by mining tailing using a geochemical multi-index and physical approach. *Sci. Total Environ.*, **2022**, *803*, 149883. https://doi. org/10.1016/J.SCITOTENV.2021.149883.
77. PMBA. *Programa de Monitoramento da Biodiversidade Aquática da Área Ambiental I—Porção Capixaba do Rio Doce e Região Marinha e Costeira Adjacente, RT-51/SET 25, RSE2025 PMBA/Fest-UFES*; Fundação Espírito-santense de Tecnologia: Vitória, ES, Brazil, 2025.
78. Duarte, G.; Calderon, E.N.; Pereira, C.M.; et al. A novel marine mesocosm facility to study global warming, water quality, and ocean acidification. *Ecol. Evol.* **2015**, *5*, 4555–4566. https://doi.org/10.1002/ece3.1670.
79. Bianchini, A.; Fukushima, L.M.; Grillo, A.C.; et al. Marine mesocosm system: A reliable tool for testing bioaccumulation and effects of iron in reef organisms. *MethodsX* **2024**, 13, 102949. https://doi.org/10.1016/j.mex.2024.102949.
80. Macedo, R.S.; Lombardi, A.T.; Omachi, C.Y.; et al. Effects of the herbicide bentazon on growth and photosystem II maximum quantum yield of the marine diatom *Skeletonema costatum*. *Toxicol. Vitr.* **2008**, *22*, 716–722. https://doi.org/10.1016/j.tiv.2007.11.012.
81. Downs, C.A.; Fouth, J.E.; Robinson, C.E.; et al. Cellular diagnostics and coral health: Declining coral health in the Florida Keys. *Mar. Pollut. Bull.* **2005**, *51*, 558–569. https://doi.org/10.1016/j.marpolbul.2005.04.017.
82. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.
83. Henry, R.P. Techniques for measuring carbonic anhydrase activity in vitro: The electrometric delta pH and pH stat methods. In *The Carbonic Anhydrases: Cellular Physiology and Molecular Genetics*; Dodgson, S.J., Tashian, R.E., Gros, G., et al., Eds.; Springer: New York, NY, USA, 1991; pp. 119–125. https://doi.org/10.1007/978-1-4899-0750-9.
84. Harland, A.D.; Brown, B.E. Metal tolerance in the scleractinian coral *Porites lutea*. *Mar. Pollut. Bull.* **1989**, *20*, 353–357. https://doi.org/10.1016/0025-326X(89)90159-8.
85. Rädecker, N.; Pogoreutz, C.; Ziegler, M.; et al. Assessing the effects of iron enrichment across holobiont compartments reveals reduced microbial nitrogen fixation in the Red Sea coral *Pocillopora verrucosa*. *Ecol. Evol.* **2017**, *7*, 6614–6621. https://doi.org/10.1002/ece3.3293.
86. Howeler, R.H. Iron induced ringing disease of rice in relation to physicochemical changes in a flooded soil. *Soil Sci. Soc. Am. J.* **1973**, *37*, 898–903. https://doi.org/10.2136/sssaj1973.03615995003700060030x.
87. Sahrawat, K. Iron Toxicity in Wetland Rice and the Role of Other Nutrients. *J. Plant Nutr.* **2005**, *27*, 1471–1504. https://doi.org/10.1081/PLN-200025869.
88. Arunachalam, R.; Paulkumar, K.; Ranjitsingh, A.J.A.; et al. Environmental assessment due to air pollution near iron smelting industry. *J. Environ. Sci. Technol.* **2009**, *2*, 179–186. https://doi.org/10.3923/jest.2009.179.186.
89. Rout, G.R.; Sahoo, S. Role of iron in plant growth and metabolism. *Rev. Agric. Sci.* **2015**, *3*, 1–24. https://doi.org/10.7831/ras.3.1.
90. Papanikolaou, G.; Pantopoulos, K. Iron metabolism and toxicity. *Toxicol Appl Pharmacol.* **2005**, *202*, 199–211. https://doi.org/10.1016/j.taap.2004.06.021.
91. Aisen, P.; Enns, C.; Wessling-Resnick, M. Chemistry and biology of eukaryotic iron metabolism. *Int. J. Biochem. Cell Biol.* **2001**, *33*, 940–959. https://doi.org/10.1016/s1357-2725(01)00063-2.
92. Ferrier-Pagès, C.; Schoelzke, V.; Jaubert, J.; et al. Response of a scleractinian coral, *Stylophora pistillata*, to iron and nitrate enrichment. *J. Exp. Mar. Biol. Ecol.* **2001**, *259*, 249–261. https://doi.org/10.1016/S0022-0981(01)00241-6.
93. Rodriguez, I.; Lin, S.; Ho, J.; et al. Effects of trace metal concentrations on the growth of the coral endosymbiont Symbiodinium. *Front. Microbiol.* **2016**, *7*, 82. https://doi.org/10.3389/fmicb.2016.00082.
94. Reich, H.G.; Tu, W.C.; Rodriguez, I.B.; et al. Iron availability modulates the response of endosymbiotic dinoflagellates to heat stress. *J. Phycol.* **2021**, *57*, 3–13. https://doi.org/10.1111/jpy.13078.
95. Leggat, W.; Marendy, E.M.; Baillie, B.; et al. Dinoflagellate symbiose: Strategies and adaptation for the acquisition and fixation of inorganic carbonic. *Funct. Plant Biol.* **2002**, *29*, 309–322. https://doi.org/10.1071/pp01202.

96. Fonseca, J.S.; Marangoni, L.F.B.; Marques, J.A.; et al. Elevated temperature and exposure to copper leads to changes in the antioxidant defense system of the reef-building coral *Mussismilia harttii. Front. Physiol.* **2021**, *12*, 804678. https://doi.org/10.3389/fphys.2021.804678.
97. Fukushima, L.M., Fonseca, J.S.; Vidal, T.J.; et al. Impact of iron exposure on Brazilian coral reefs: Acute vs. chronic stress responses. *Ecotoxicol. Environ. Saf.* **2025**, *298*, 118309. https://doi.org/10.1016/j.ecoenv.2025.118309.
98. Santini, O.; Chahbane, N.; Vasseur, P.; et al. Effects of low-level copper exposure on Ca^{2+}-ATPase and carbonic anhydrase in the freshwater bivalve *Anodonta anatina*. *Toxicol. Environ. Chem.* **2011**, *93*, 1826–1837. https://doi.org/10.1080/02772240903217598.
99. Prazeres, M.F.; Uthicke, S.; Pandolfi, J.M. Ocean acidification induces biochemical and morphological changes in the calcification process of large benthic foraminifera. *Proc. R. Soc. B* **2015**, *282*, 20142782. https://doi.org/10.1098/rspb.2014.2782.
100. Viarengo, A.; Mancinelli, G.; Pertica, M.; et al. Effects of heavy metals on the Ca^{2+}-ATPase activity present in gill cell plasma-membrane of mussels (*Mytilus galloprovincialis* Lam.). *Comp. Biochem. Physiol. C* **1993**, *106*, 655–660. https://doi.org/10.1016/0742-8413(93)90223-8.
101. Burlando, B.; Bonomo, M.; Capri, F.; et al. Different effects of Hg^{2+} and Cu^{2+} on mussel (*Mytilus galloprovincialis*) plasma membrane Ca^{2+}-ATPase: Hg^{2+} induction of protein expression. *Comp. Biochem. Physiol. C* **2004**, *139*, 201–207. https://doi.org/10.1016/j.cca.2004.11.001.
102. Sousa, L.; Pessoa, M.T.C.; Costa, T.G.; et al. Iron overload impact on P-ATPases. *Ann. Hematol.* **2018**, *97*, 377–385. https://doi.org/10.1007/s00277-017-3222-4.
103. Leão, Z.M.A.N.; Kikuchi, R.K.P.; Testa, V. Corals and corals reefs of Brazil. In *Latin America Coral Reefs*; Cortes, J., Ed.; Elsevier Science: Amsterdam, The Netherlands, 2003; pp. 9–52. https://doi.org/10.1016/B978-044451388-5/50003-5.
104. Mies, M.; Francini-Filho, R.B.; Zilberberg, C.; et al. South Atlantic coral reefs are major global warming refugia and less susceptible to bleaching. *Front. Mar. Sci.* **2020**, *7*, 514. https://doi.org/10.3389/fmars.2020.00514.
105. Tunala, L.P.; Tâmega, F.T.; Duarte, H.M.; et al. Stress factors in the photobiology of the reef coral *Siderastrea stellata*. *J. Exp. Mar. Bio. Ecol.* **2019**, *519*, 151188. https://doi.org/10.1016/j.jembe.2019.151188.
106. Longo, G.O.; Correia, L.F.; Mello, T.J. Coral recovery after a burial event: Insights on coral resilience in a marginal reef. *Mar. Biodivers.* **2020**, *50*, 92. https://doi.org/10.1007/s12526-020-01121-4.
107. Pereira, P.H.; Lima, G.V.; Pontes, A.V.; et al. Unprecedented coral mortality on Southwestern Atlantic coral reefs following major thermal stress. *Front. Mar. Sci.* **2022**, *9*, 725778. https://doi.org/10.3389/fmars.2022.725778.
108. Corazza, B.M.; Lacerda, C.H.; Güth, A.Z.; et al. No coral recovery three years after a major bleaching event in reefs in the Southwestern Atlantic refugium. *Mar. Biol.* **2024**, *171*, 114. https://doi.org/10.1007/s00227-024-04432-3.
109. Mies, M.; Destri, G.; Lacerda, C.H.F.; et al. Coral bleaching and mortality across a 24° latitudinal range in the Southwestern Atlantic during the fourth global bleaching event. *Coral Reefs* **2025**. https://doi.org/10.1007/s00338-025-02743-5.
110. Mies, M.; Banha, T.N.S.; Francini-Filho, R.B.; et al. Reef ecosystems in the Brazilian continental shelf. In *Oceanography, Biodiversity, Fisheries and Conservation of Brazilian Continental Shelf Habitats. Brazilian Marine Biodiversity*; Sumida, P.Y.G., Mies, M., Eds.; Springer: Cham, Switzerland, 2025; pp. 115–172. https://doi.org/10.1007/978-3-031-88338-5_5.
111. Leão, Z.M.; Kikuchi, R.K.P.; Oliveira, M.D.M.; et al. Status of Eastern Brazilian coral reefs in time of climate changes. *Pan-Am. J. Aquat. Sci.* **2010**, *5*, 224–235.
112. Santana, E.F.C.; Mies, M.; Longo, G.O.; et al. Turbidity shapes shallow Southwestern Atlantic benthic reef communities. *Mar. Environ. Res.* **2023**, *183*, 105807. https://doi.org/10.1016/j.marenvres.2022.105807.
113. Patrocinio, G.T.; Lopes, F.C.; Ciotti, Á.M.; et al. The Southwestern Atlantic coral *Mussismilia hispida* exhibit remarkable tolerance to nitrate enrichment. *Coral Reefs* **2025**, *44*, 629–642. https://doi.org/10.1007/s00338-025-02631-y.
114. Fonseca, J.S.; Mies, M.; Paranhos, A.; et al. Isolated and combined effects of thermal stress and copper exposure on the trophic behavior and oxidative status of the reef-building coral *Mussismilia harttii*. *Environ. Pollut.* **2021**, *268*, 115892. https://doi.org/10.1016/j.envpol.2020.115892.
115. Tang, J.; Cai, W.; Yan, Z.; et al. Interactive effects of acidification and copper exposure on the reproduction and metabolism of coral endosymbiont *Cladocopium goreaui*. *Mar. Pollut. Bull.* **2022**, *177*, 113508. https://doi.org/10.1016/j.marpolbul.2022.113508.
116. Cheng, M.; Luo, Y.; Yu, X.L.; et al. Effects of elevated temperature and copper exposure on the physiological state of the coral *Galaxea fascicularis*. *Mar. Environ. Res.* **2024**, *193*, 106218. https://doi.org/10.1016/j.marenvres.2023.106218.
117. Cryer, S.E.; Schlosser, C.; Allison, N. The combined effects of ocean acidification and copper on the physiological responses of the tropical coral *Stylophora pistillata*. *Mar. Environ. Res.* **2022**, *176*, 105610. https://doi.org/10.1016/j.marenvres.2022.105610.
118. Grillo, A.C.; Inagaki, K.Y.; Costa, P.G.; et al. Differential effects of iron enrichment on corals competing with macroalgae and zoantharians. *Environ. Pollut.* **2025**, *371*, 125944. https://doi.org/10.1016/j.envpol.2025.125944.

119. Cui, L.; Cheng, C.; Li, X.; et al. Comprehensive assessment of copper's effect on marine organisms under ocean acidification and warming in the 21st century. *Sci. Total Environ.* **2024**, *927*, 172145. https://doi.org/10.1016/j.scitotenv.2024.172145.
120. Banc-Prandi, G.; Fine, M. Copper enrichment reduces thermal tolerance of the highly resistant Red Sea coral *Stylophora pistillata*. *Coral Reefs* **2019**, 38, 285–296. https://doi.org/ 10.1007/s00338-019-01774-z.
121. Hutchins, D.; Boyd, P. Marine phytoplankton and the changing ocean iron cycle. *Nat. Clim. Chang.* **2016**, *6*, 1072–1079. https://doi.org/10.1038/nclimate3147.
122. Altafim, G.L.; Alves, A.V.; Trevizani, T.H.; et al. Ocean warming and CO_2-driven acidification can alter the toxicity of metal-contaminated sediments to the meiofauna community. *Sci. Total Environ.* **2023**, *885*, 163687. https://doi.org/10.1016/j.scitotenv.2023.163687.