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Marine mesocosm system: A reliable tool for testing bioaccumulation and effects of seawater enrichment with dissolved iron in reef organisms ☆

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☆ **Related research article:** G. Duarte, E.N. Calderon, C.M. Pereira, L.F. Marangoni, H.F. Santos, R.S. Peixoto, A. Bianchini, C.B. Castro, A novel marine mesocosm facility to study global warming, water quality, and ocean acidification, Ecol. Evol. 5 (2015) 4555–4566, doi: 10.1002/ece3.1670.

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Marine mesocosm system as a reliable tool to test the effect of aquatic contaminants in reef organisms

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ABSTRACT

In 2015, a marine mesocosm facility was designed and implemented by the Coral Vivo Project in its research station (Porto Seguro, Bahia State, Brazil) to initially study the effects of global impacts, especially ocean warming and acidification, on coral reefs. However, local impacts, including seawater contamination with metal(loid)s, are considered as a major threat to coral reefs. Also, in 2015, the largest disaster involving a mining dam occurred in Brazil. Iron (Fe) mining tailings originated from the dam failure affected not only freshwater ecosystems (rivers, lakes and lagoons), but also adjacent beaches, mangroves, restingas, reefs and other marine systems. Seawater, sediments and biota were contaminated with metal(loid)s, especially Fe, arsenic (As), mercury (Hg) and manganese (Mn). Therefore, we aimed to adapt the marine mesocosm facility of the Coral Vivo Project to evaluate the bioaccumulation and biological impacts of increasing concentrations of dissolved Fe on a diversity of reef organisms. Results obtained indicate a great versatility and reliability of the marine mesocosm system for application in biological and ecological studies on the isolated effect of seawater dissolved Fe on reef organisms of different functional groups simultaneously.

- Studies involving seawater enrichment with dissolved Fe can be performed using a marine mesocosm system.
- The marine mesocosm is a reliable tool to study the isolated effects of metal(loid)s on reef organisms.

Specifications table

Subject area:	Environmental Sciences
More specific subject area:	Marine Pollution
Name of your method:	Marine mesocosm system as a reliable tool to test the effect of aquatic contaminants in reef organisms
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Resource availability:	Not applicable

Background

The contamination of aquatic environments is one of the main challenges of the 21st century due to the large amount of chemical substances arising from human activities [1]. One of the most striking disasters in Brazil was undoubtedly the collapse of the Fundão dam (Mariana, Minas Gerais State, Brazil), in November 2015. It is considered the most notorious socio-environmental disaster in Brazil and one of the most extensive related to the mining activity in the world [2]. Laboratory analyses of water, sediment and biological samples collected in the affected area have indicated increased levels of metal(loid)s, especially iron (Fe), arsenic (As), mercury (Hg) and manganese (Mn) [3–5]. In addition to the impacts on freshwater ecosystems, the iron mining tailings originated from the dam collapse reached the Doce River mouth and consequently the Atlantic Ocean. Costal and marine ecosystems such as beaches, mangroves, restingas, rhodolith and algal beds, and coral reefs, were also affected [5,6]. The impacts of the mud plume extended to organisms from different levels of marine food webs (microbiota, plankton, benthic macrofauna, corals, shrimps, fish, birds, turtles and mammals), with unprecedented damage to biodiversity, functions and services [7–15].

Recent studies indicate that some material released from the dam collapse have reached the Abrolhos Archipelago Region, off the south coast of the state of Bahia (northeastern Brazil) and the north coast of the state of Espírito Santo (southeastern Brazil) [16]. Therefore, reef organisms have been chronically exposed to increased levels of metal(loid)s dissolved in seawater and associated with particulate matter from multiple sources, including tailings originated from the Fundão dam failure [16–18]. It is important to note that the Abrolhos Region harbors the largest known marine biodiversity in the entire South Atlantic Ocean [19]. In this context, it is imperative to investigate how reef organisms would respond to the acute and chronic exposure to increased levels of metal(loid)s in seawater, especially Fe. Indeed, Brazilian reef organisms have been shown to be negatively affected when exposed to excessive concentrations of some essential metals alone or in combination with global stressors (seawater increasing temperature and/or acidification). However, studies reported are still limited to Cu and Zn in foraminifers and scleractinian corals [20–31].

Considering this background, we aimed to develop studies focused on the biological and ecological effects of increasing levels of dissolved Fe in seawater using a great diversity of reef organisms by adapting the original version of the marine mesocosm system of the Coral Vivo Project [32]. The marine mesocosm facility implemented by the Coral Vivo Project in its research station (Porto Seguro, Bahia State, Brazil) in 2015 was designed to study the effects of global impacts, especially ocean warming and acidification, on Brazilian coral species. Before its first use, this system was adapted to also run toxicological experiments. However, this adaptation permitted the use of forty-eight 10-L aquaria disposed in a second feeding line of the main structure of the mesocosm system. This method worked well but was limited to performing experiments using only one species at a time [22–31]. Therefore, we have adapted the marine mesocosm system to allow ecotoxicological experiments using a diversity of reef organisms simultaneously.

Method details

Procedures

The Coral Vivo marine mesocosm system is located at the Arraial d'Ajuda District, Porto Seguro City, Bahia State, in northeastern Brazil (Fig. 1). In this mesocosm system seawater is pumped from the beach in front of the Coral Vivo Project facilities (Mugucê reef) through an extensive piping and pumping system into four 5000-L cisterns. Each one of these cisterns is used to keep the control seawater or to prepare seawater at one of the desired experimental conditions. The marine mesocosm system allows to test a control and three experimental treatments. The cisterns are connected to four 310-L tanks installed in the control room attic, which are also separated by experimental treatment. In turn, each one of these tanks is connected to four plastic troughs, thus allowing to test the control or each experimental condition in quadruplicate (Fig. 2). Organisms to be tested in the marine mesocosm system are then randomly distributed among sixteen plastic 130-L raceways (4 experimental units per experimental condition) containing running seawater under the desired experimental conditions (Fig. 3).

In the present study, our main goal was to adapt the original design of the Coral Vivo Project marine mesocosm system to test the biological and ecological effects of increasing levels of dissolved Fe in seawater. For this, dosing pumps were used in which their pulse frequencies per minute were finely adjusted to obtain the desired final dissolved Fe concentrations in seawater (Fig. 4A). The hoses of these pumps were submerged inside a plastic tank containing 300 L of the Fe ($FeCl_3$) stock solution, which was replenished whenever necessary throughout the experiment (Fig. 4B).

The experiment was carried out in quadruplicate, in which four treatments were used: one control (without Fe addition) and three increasing levels of dissolved Fe in seawater (100, 300 and 900 µg/L), thus totaling 16 experimental units (4 control units and 4 units per experimental treatment). Each experimental condition was identified by one color (green, red, yellow and blue). The number of individuals (n) tested for each reef organism species and their respective distribution in the marine mesocosm system depended on the study to be carried out and its specific objectives. In all cases, at least 3 individuals were tested per experimental unit. Also, sediment traps were introduced into the marine mesocosm system to analyze Fe accumulation in sediments. Therefore, the following studies were simultaneously performed in the marine mesocosm system: (a) Fe bioaccumulation in all reef organisms tested (turf algae, fleshy macroalgae, calcareous algae (crustose and rhodolith), hydrocorals, scleractinian corals, brittle stars and fish),; (b) Fe effect on calcareous algae calcification and growth; (c) Fe effect on contact-competition interactions between corals and other benthic reef organisms; (d) Fe influence on habitat use, aggressiveness and foraging patterns of the dusky damselfish *Stegastes fuscus*; (e) Fe effects on physiological and behavioral responses of the brittle star *Ophioderma apressum*; and (f) Fe accumulation in sediments (Fig. 5).

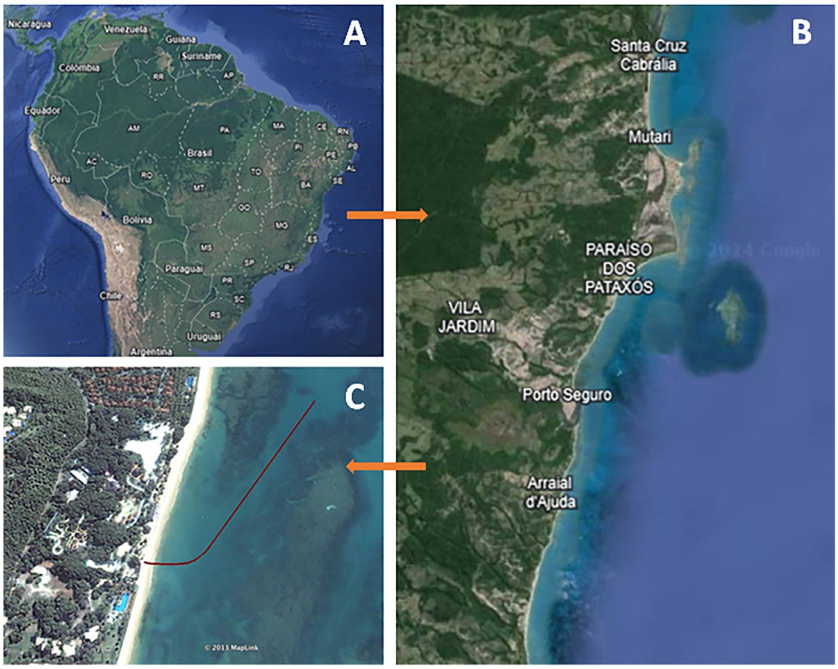

Fig. 1. Location of the Coral Vivo Project marine mesocosm system: (A) map of Brazil; (B) map of the south coast of the Bahia State (northeastern Brazil) indicating the Arraial d'Ajuda District (Porto Seguro City); and (C) Mucugê Beach with the red line representing the pumping and pipeline system for seawater collection from the Mucugê reef system located at 500 m distance from the marine mesocosm system. Images source: Google Earth.

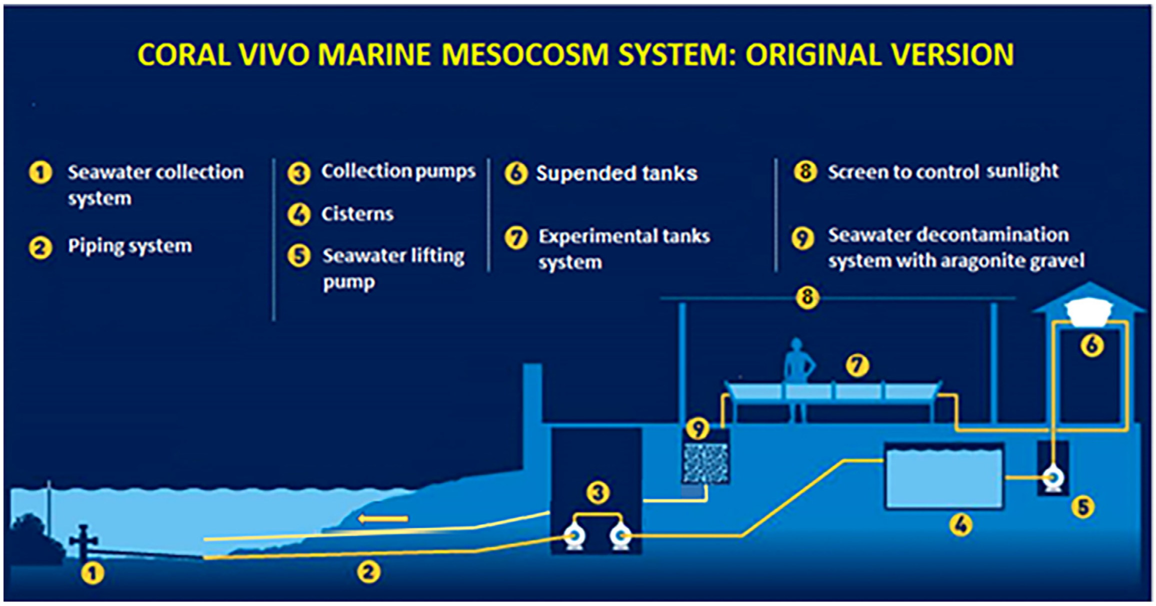

Fig. 2. General layout of the Coral Vivo Project marine mesocosm system indicating the main parts of the structure.

Fig. 3. Sixteen plastic raceways (100×40×30 cm, length x width x height; 130 L) where organisms to be tested are randomly housed according to the control and the desired experimental conditions. Therefore, each experimental condition is run in quadruplicate (4 troughs per experimental condition).

Seawater flow running into the sixteen experimental units was monitored using a digital water flow meter (TM050, Great Plains Industries, Wichita, KS, USA) mounted on the end of the seawater intake pipe. The seawater inflow in each experimental unit was then adjusted whenever necessary to maintain a constant flow of 8–10 L/min. Regarding seawater quality monitoring, four measurements of seawater temperature, pH, salinity and dissolved oxygen content were daily (06h00, 12h00, 18h00, and 00h00) performed using mercury-in-glass thermometer, portable pH-meter (PG1300, Gehaka, São Paulo, SP, Brazil), hand refractometers (SRH10-ATC - Soma, São Paulo, SP, Brazil), and portable digital dissolved oxygen meter (DO-5519, Lutron, Taiwan), respectively. Seawater temperature in one experimental unit of each experimental condition was also monitored by using four underwater dataloggers (MX2202, HOBO pendant temperature/light sensor, Onset, Bourne, MA, USA) placed in the four plastic troughs located at the corners of the mesocosm

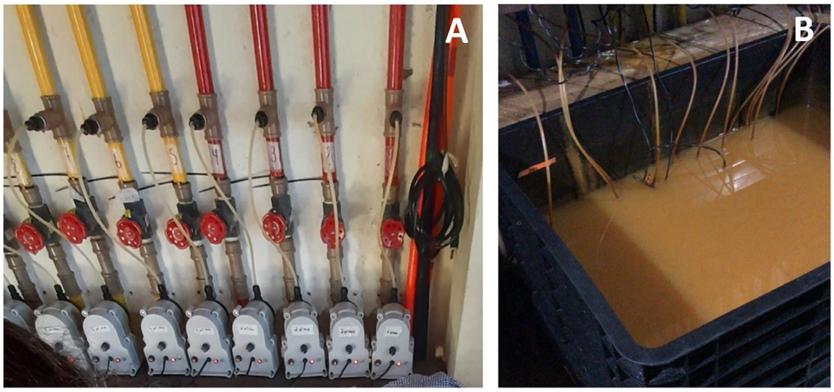

Fig. 4. Pumping and piping system adapted to increase the levels of dissolved Fe in seawater running into the marine mesocosm system: (A) electromagnetic dosing pumps used to dilute the $FeCl_3$ stock solution at the desired final concentration of dissolved Fe to be tested in each one of the sixteen experimental units (plastic troughs); and (B) plastic tank used to house 300 L of $FeCl_3$ stock solution.

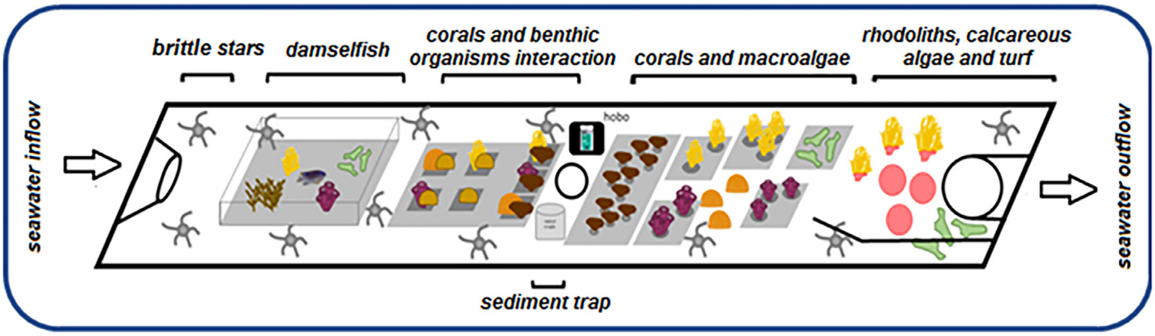

Fig. 5. Layout of the experimental unit (plastic trough) showing the distribution of the different reef organisms employed in the biological and ecological studies performed using the Coral Vivo Project marine mesocosm system.

system. Solar radiation on the tanks was reduced by using shade cloth to maintain levels like to those generally recorded in the adjacent reefs (200 µmol photons m^{-2} s^{-1}). Dissolved Fe concentration in seawater was measured twice a day using a portable multiparameter (eXact Micro 20, Industrial Test Systems, Rock Hill, SC, USA) and immediately adjusted by regulating the respective dosing pumps whenever needed. In addition, 10-mL seawater samples from each experimental unit were collected twice a day for further quantification of total (non-filtered sample) and dissolved (filtered sample; 0.5 µm-mesh filter) Fe concentrations. Non-filtered and filtered seawater samples were acidified (1% final concentration) with ultrapure (Suprapur®) 65% HNO_3 (Merck, Darmstadt, Germany) and kept refrigerated (1.7–3.3 °C) until analysis. Every 5 days, sediments retained in the traps were collected and frozen (−20 °C) for further quantification of Fe. After 14 and 28 days of exposure to the experimental conditions, samples of all reef organisms tested were collected and frozen (−80 °C) for further analysis of Fe bioaccumulation. In the laboratory, all collected samples were prepared for Fe quantification by Inductively Coupled Plasma Spectrometry (ICP-MS, PlasmaQuant MS Q, Analytik Jena, Jena, Germany) as previously described [5] (Fig. 6).

Statistical analysis

Data for each parameter measured were expressed as mean ± standard error (SE). A mean value for the 28-day period of experiment was calculated for each replicate. Mean values obtained for the four replicates of the same experimental condition were compared. Taken together the results obtained for the four replicates of each experimental condition, mean daily values for each parameter were calculated. Finally, a general mean value for each experimental condition was calculated. Mean values obtained for the four experimental conditions tested were then compared. In all cases, mean values were compared by one-way analysis of variance (ANOVA) followed by the Tukey HSD test. In all cases, the level of significance adopted was 95% ($\alpha = 0.05$).

Method validation

Mean values of seawater temperature showed some variation over the 28-day period of experiment. These variations were very similar among the experimental conditions. Also, results were quite similar for data on seawater temperature obtained using the mercury-in-glass thermometer and underwater datalogger. The same finding was observed when data were analyzed considering the

Fig. 6. Daily routine to monitor the physical and chemical parameters of seawater in the marine mesocosm system: (A) portable pH meter (seawater pH measurement); (B) hand refractometer (seawater salinity measurement); (C) portable multiparameter (*in loco* seawater Fe concentration measurement); (D) portable digital dissolved oxygen meter (seawater dissolved oxygen content measurement); (E) seawater samples collection for laboratory quantification of total and dissolved Fe concentrations by Inductive Coupled Plasma Mass Spectrometry (ICP-MS).

four replicates separately (Fig. 7) or together (Fig. 8). It is worth noting that the marine mesocosm is an open system. Therefore, it would be expected that the temperature changes naturally occurring in the coastal reef from which seawater is pumped would be reflected in the temperature of the seawater flowing in the marine mesocosm system. Considering data obtained with mercury-in-glass thermometer, mean values of seawater temperature were not different among replicates for each experimental condition (Fig. 9).

As observed for temperature, mean values of seawater pH, salinity and dissolved oxygen content also showed some variation over the 28-day period of experiment. These variations were very similar among the experimental conditions when all replicates for each experimental condition were taken together (pH: Fig. 10; salinity: Fig. 11; dissolved oxygen content: Fig. 12). The same finding was observed when data for each seawater parameter were analyzed considering the four replicates separately. Mean values of each seawater parameter analyzed were not different among replicates for each experimental condition (pH: Fig. 13; salinity: Fig. 14; dissolved oxygen content: Fig.15). As an open system, it was expected that changes naturally occurring in the physicochemical conditions of the seawater pumped from the coastal reef would be reflected in the conditions of the seawater flowing in the marine mesocosm system.

No significant difference was observed among the general mean values calculated for each seawater parameter analyzed (temperature, pH, salinity and dissolved oxygen content) when measurements made in the four replicates performed for each experimental condition were taken together over the 28-day period of study (Fig. 16).

Fe concentration in seawater varied along the 28-day period of experiment. This variation was more marked when measurements were performed using the portable multiparameter unit (Fig. 17). This would be expected since results produced by the portable multiparameter unit are subject to some seawater conditions such as turbidity, organic matter content, microorganisms and debris. Total (Fig. 18) and dissolved (Fig. 19) Fe concentration values varied less when measurements were performed using ICP-MS, especially at the lower concentrations of Fe. In this case, data generated are much less influenced by other factors associated with the seawater being analyzed. Measurements performed using ICP-MS are much more accurate than those using the portable multiparameter unit, as ICP-MS (chemical analysis) shows a much higher sensitivity than the portable multiparameter unit (physical analysis). Concentrations of Fe in the control condition (without Fe addition to seawater) were much lower and more similar to those

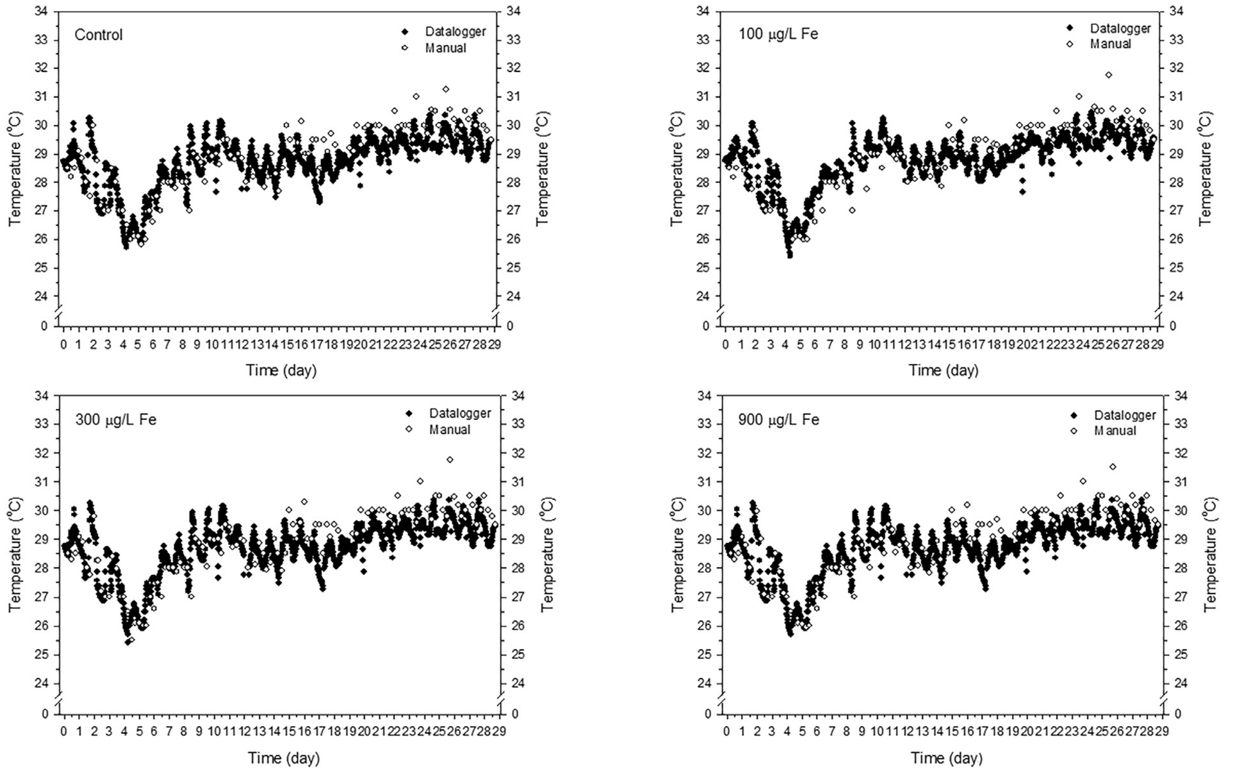

Fig. 7. Seawater temperature measured using underwater datalogger (closed circles) and mercury-in-glass thermometer (open circles) over the 28-day period of study for each experimental condition tested. Thermometer data are shown for all the four replicates performed in each experimental condition.

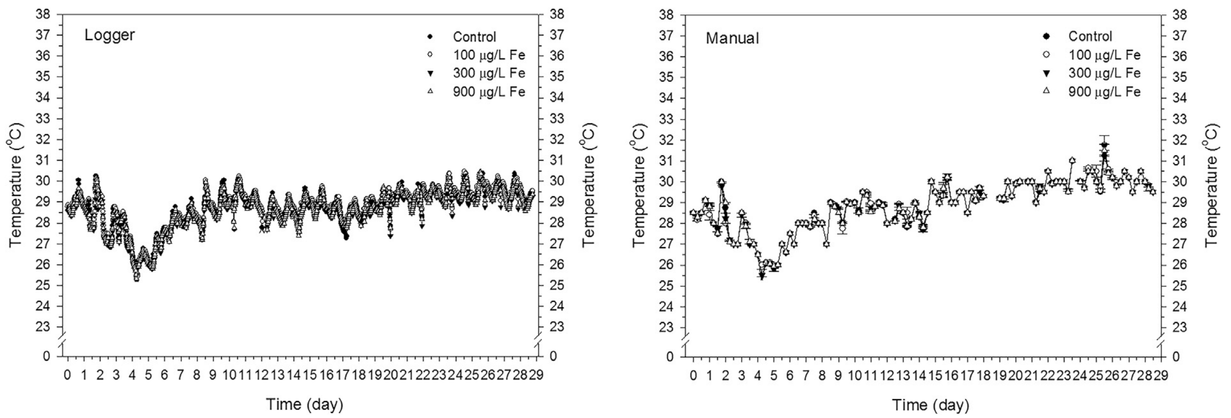

Fig. 8. Seawater temperature measured using underwater datalogger or mercury-in-glass thermometer over the 28-day period of study for each experimental condition tested. Thermometer (manual) data (mean ± SE) are shown considering the mean value for all replicates performed in each experimental condition.

found in non-contaminated sea water [33] when measured using ICP-MS (Figs. 18 and 19) than the portable multiparameter unit (Fig. 17). However, measurements performed with the portable multiparameter unit were quite important to monitor *in loco* the Fe concentration in seawater four times a day during the whole experimental period and adjust it whenever necessary.

Based on data generated by ICP-MS, no significant difference was observed among the replicates performed for each experimental condition, for both total Fe (Fig. 20) and dissolved Fe (Fig. 21) concentrations in seawater. However, in both cases, significant different general mean values were observed among the experimental conditions tested. For total Fe concentration, they corresponded to 22.46 ± 1.65, 117.62 ± 3.96, 322.88 ± 5.29, and 910.55 ± 10.42 µg/L for the control, 100, 300, and 900 µg/L treatments, respectively. For dissolved Fe concentration, they were 20.18 ± 0.74, 99.04 ± 2.75, 300.13 ± 4.51, and 870.55 ± 10.74 µg/L for the control, 100,

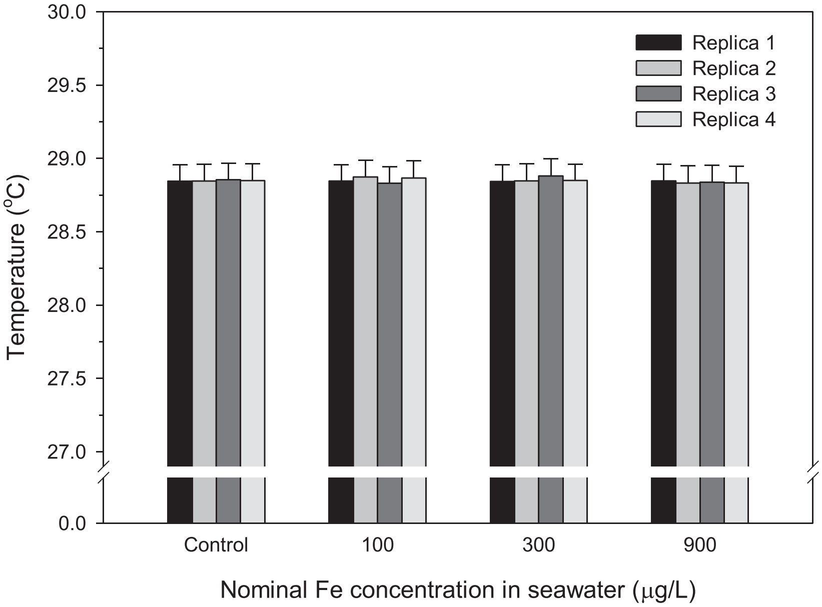

Fig. 9. Mean (± SE) values of seawater temperature measured in the four replicates of each experimental condition tested using the mercury-in-glass thermometer. Mean values were calculated based on data obtained over the 28-day period of study. No significant difference was observed among the replicates for each experimental condition ($P > 0.05$).

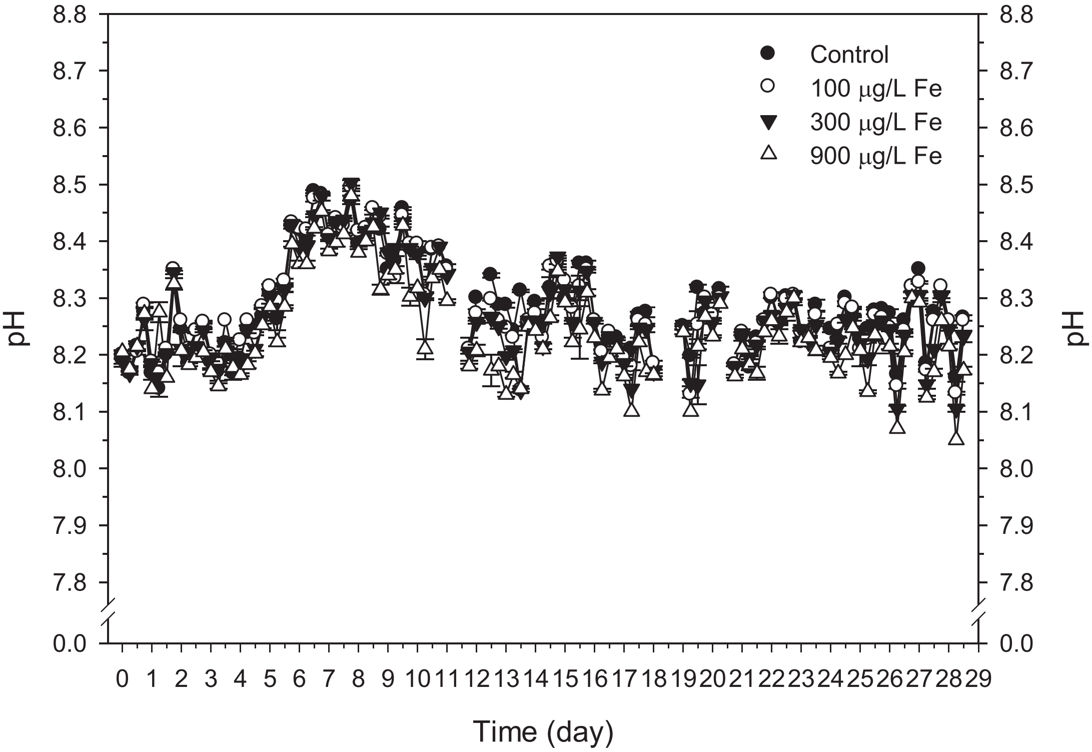

Fig. 10. Mean values (± SE) of seawater pH over the 28-day period of study for each experimental condition tested. Data are the mean value for all replicates performed in each experimental condition.

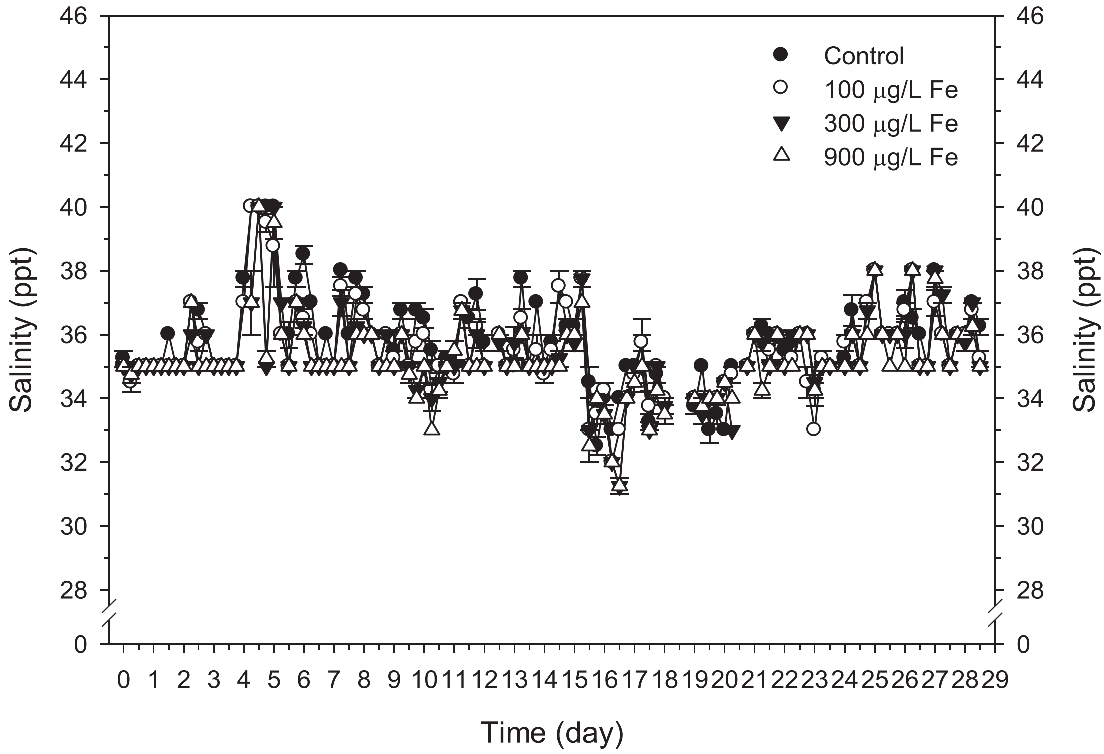

Fig. 11. Mean values (± SE) of seawater salinity over the 28-day period of study for each experimental condition tested. Data are the mean value for all replicates performed in each experimental condition.

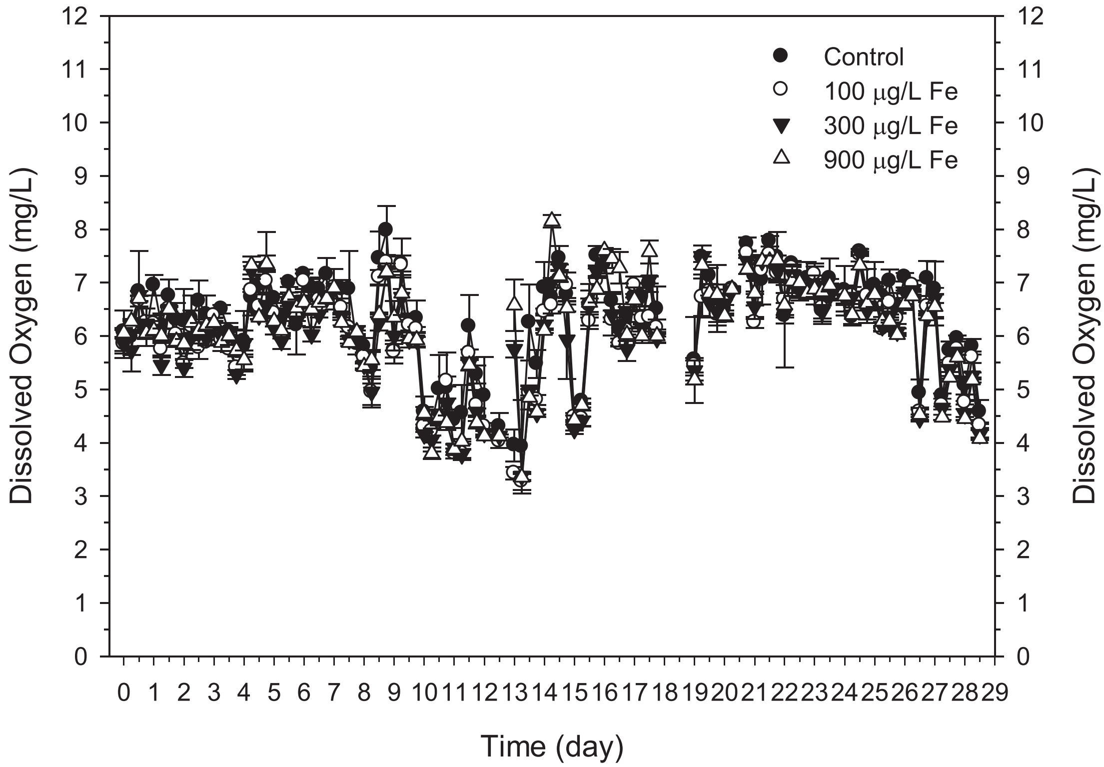

Fig. 12. Mean values (± SE) of dissolved oxygen content in seawater over the 28-day period of study for each experimental condition tested. Data are the mean value for all replicates performed in each experimental condition.

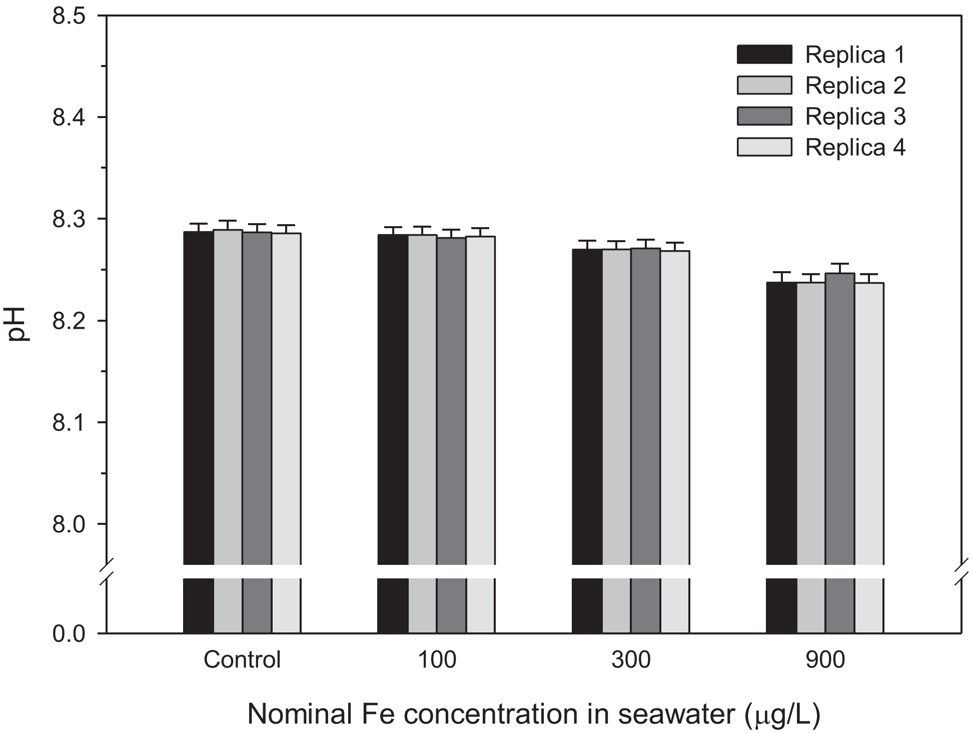

Fig. 13. Mean (± SE) values of seawater pH measured in the four replicates of each experimental condition tested. Mean values were calculated based on data obtained over the 28-day period of study. No significant difference was observed among the different replicates for each experimental condition ($P > 0.05$).

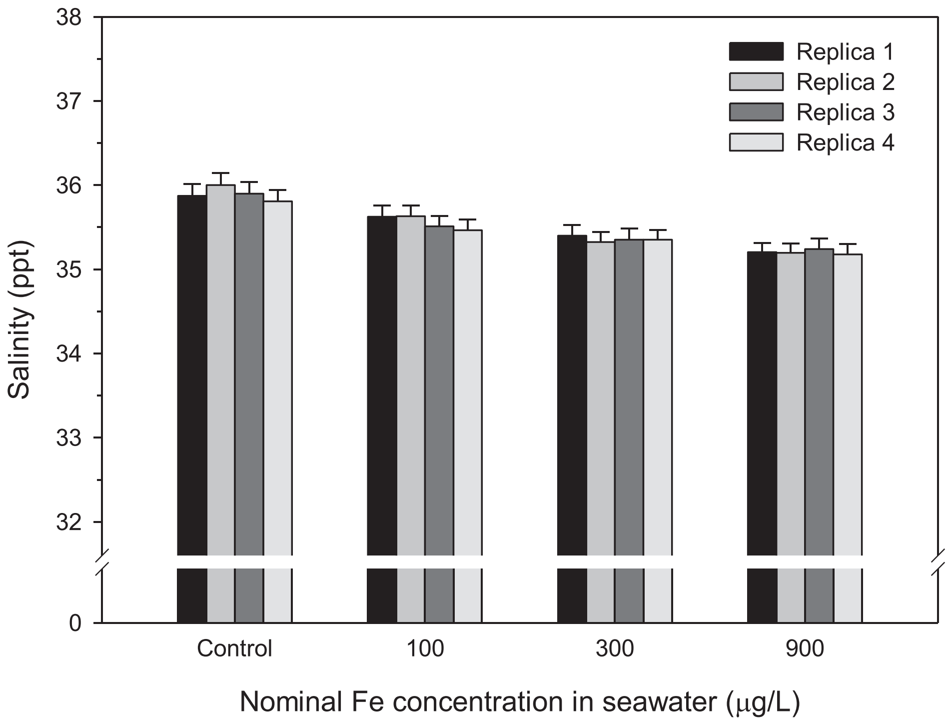

Fig. 14. Mean (± SE) values of seawater salinity measured in the four replicates of each experimental condition tested. Mean values were calculated based on data obtained over the 28-day period of study. No significant difference was observed among the different replicates for each experimental condition ($P > 0.05$).

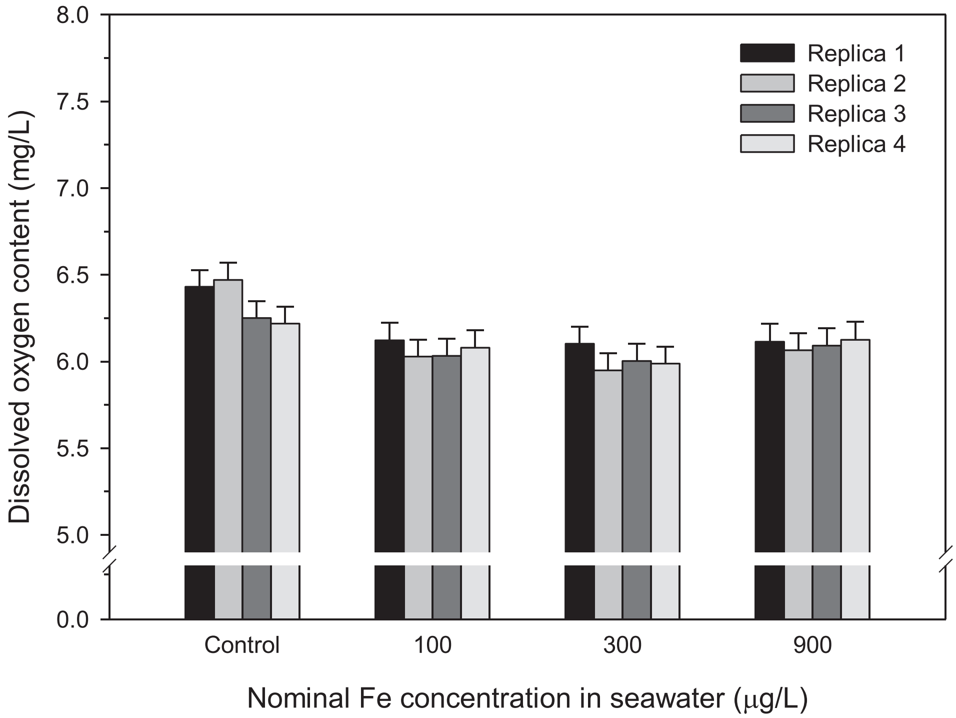

Fig. 15. Mean (± SE) values of dissolved oxygen content of seawater measured in the four replicates of each experimental condition tested. Mean values were calculated based on data obtained over the 28-day period of study. No significant difference was observed among the different replicates for each experimental condition ($P > 0.05$).

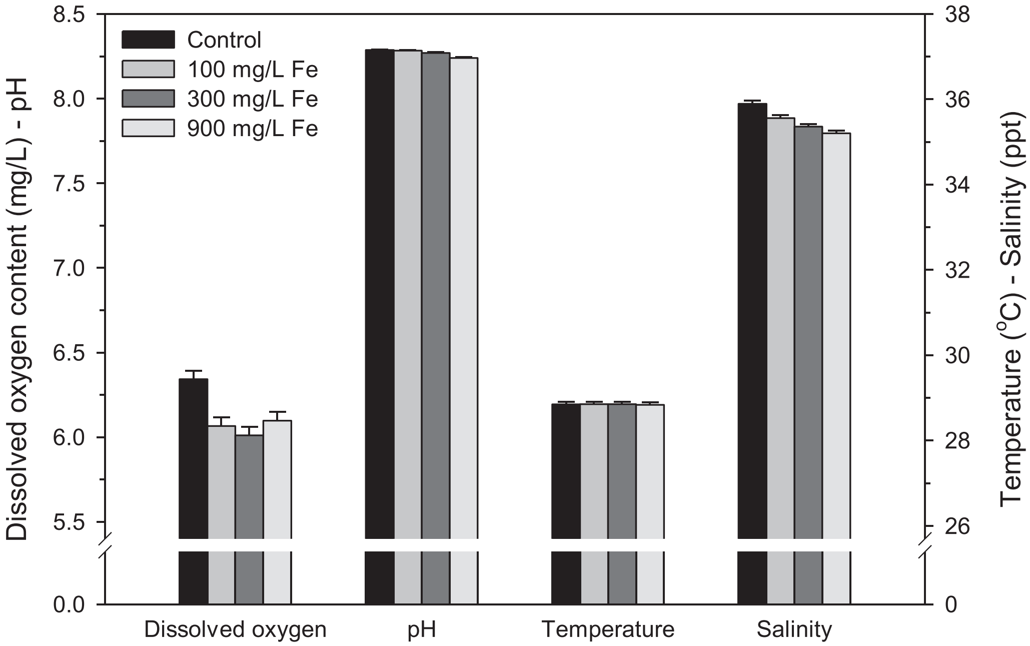

Fig. 16. Mean (± SE) values of dissolved oxygen content, pH, temperature and salinity of seawater for each experimental condition tested. Mean values were calculated based on data obtained along the 28-day period of study and for all the four replicates performed. For each seawater parameter analyzed, no significant difference was observed among the experimental conditions ($P > 0.05$).

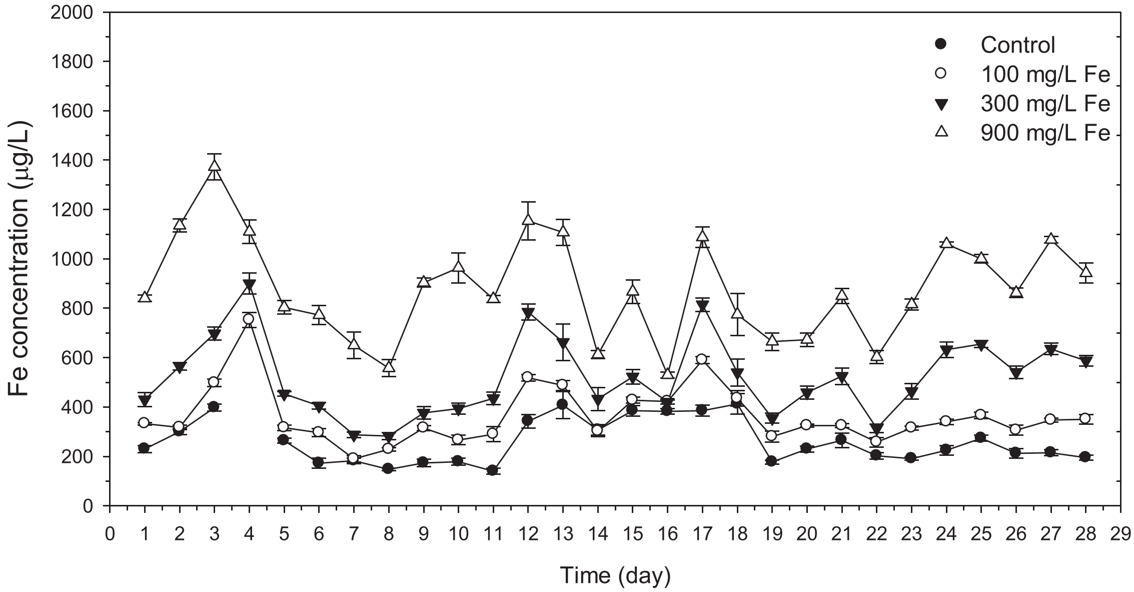

Fig. 17. Mean values (± SE) of Fe concentration in seawater over the 28-day period of study for each experimental condition tested. Data are the mean value for all replicates performed in each experimental condition. Measurements were performed using the portable multiparameter (eXact Micro 20, Industrial Test Systems, Rock Hill, SC, USA).

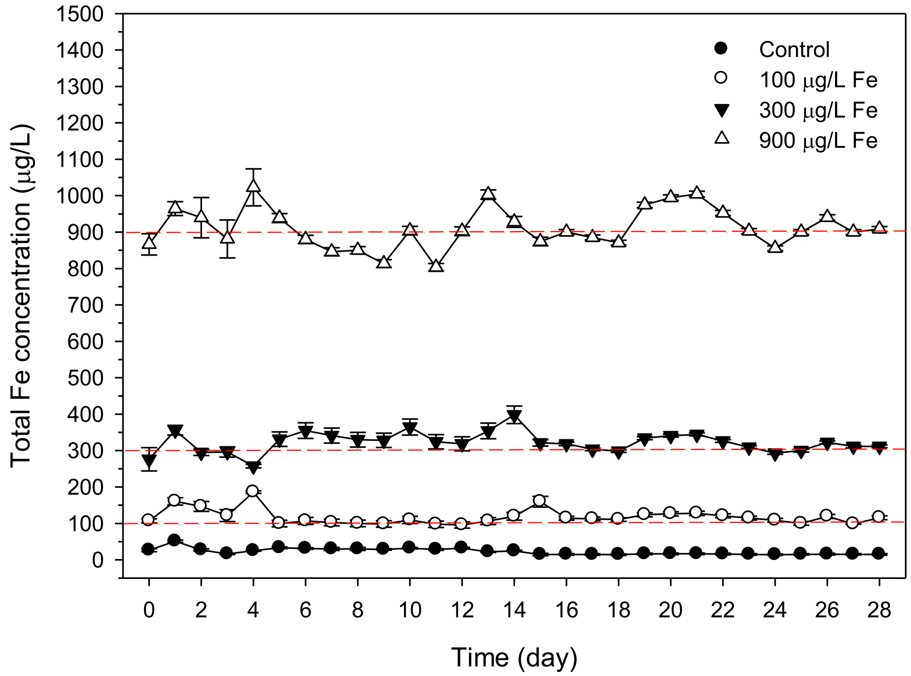

Fig. 18. Mean values (± SE) of total Fe concentration in seawater over the 28-day period of study for each experimental condition tested. Data are the mean value for all replicates performed in each experimental condition. Measurements were performed using ICP-MS. Red dashed lines represent the nominal Fe concentrations aimed.

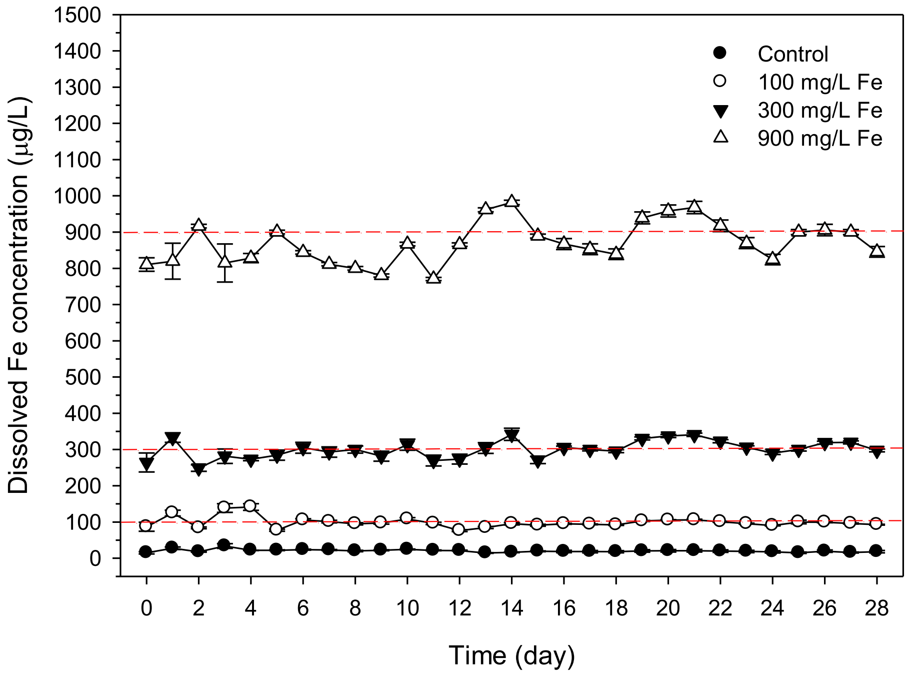

Fig. 19. Mean values (± SE) of dissolved Fe concentration in seawater over the 28-day period of study for each experimental condition tested. Data are the mean value for all replicates performed in each experimental condition. Measurements were performed using ICP-MS. Red dashed lines represent the nominal Fe concentrations aimed.

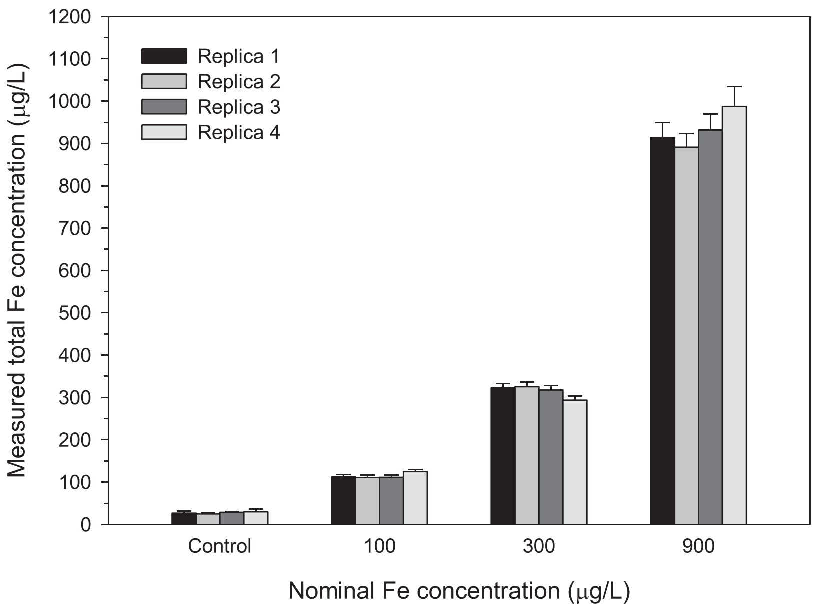

Fig. 20. Mean (± SE) values of total Fe concentration in seawater measured in the four replicates of each experimental condition tested. Mean values were calculated based on data obtained over the 28-day period of study. Measurements were performed using ICP-MS. No significant difference was observed among the different replicates for each experimental condition ($P > 0.05$).

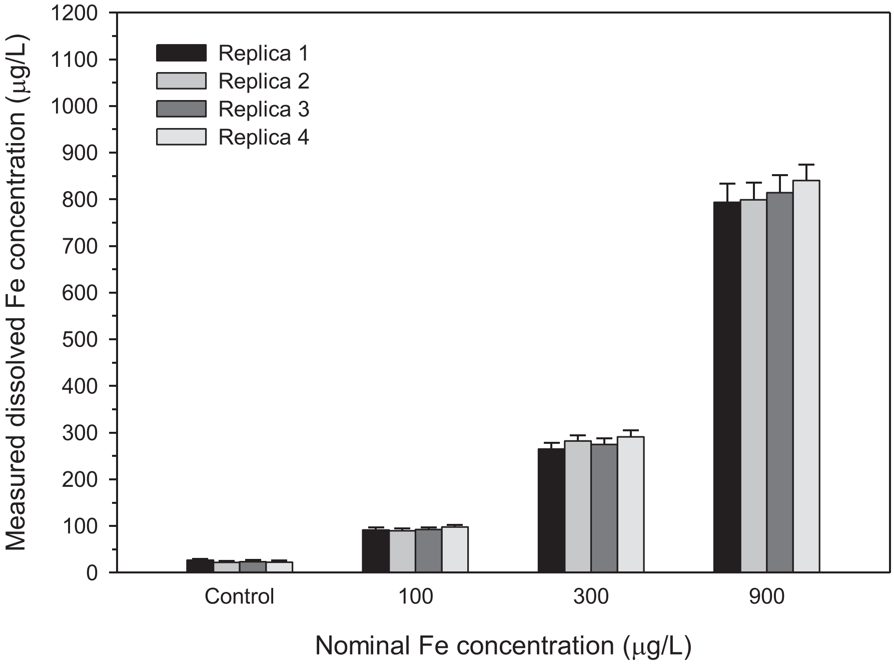

Fig. 21. Mean (± SE) values of dissolved Fe concentration in seawater measured in the four replicates of each experimental condition tested. Mean values were calculated based on data obtained over the 28-day period of study. Measurements were performed using ICP-MS. No significant difference was observed among the different replicates for each experimental condition ($P > 0.05$).

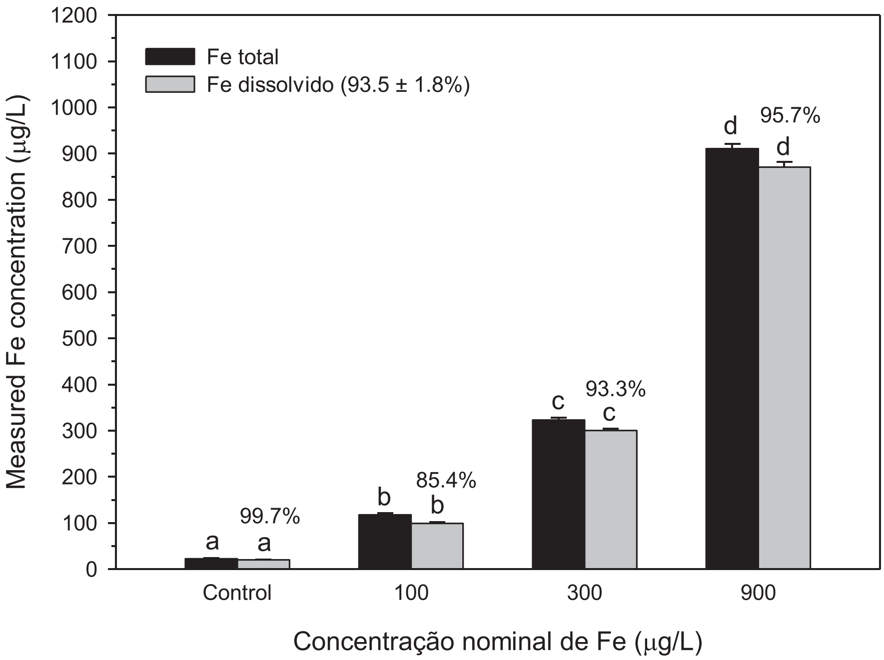

Fig. 22. General mean values (± SE) of total and dissolved Fe concentration in seawater for each experimental condition tested. Values were calculated considering the four replicates performed for each experimental condition and data measured over the 28-day period of study using ICP-MS. Values shown at the top of the vertical bars represent the percentage of dissolved Fe respect with total Fe. Different letters represent different general mean values among the experimental conditions, for total or dissolved Fe concentration ($P < 0.05$).

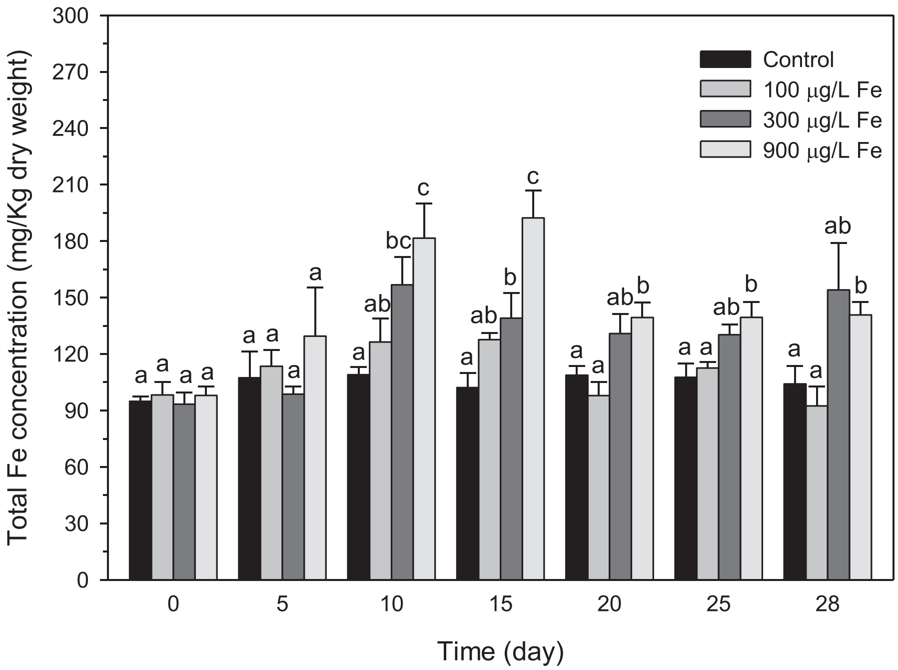

Fig. 23. Mean values (± SE) of total Fe concentration in sediments trapped every 5 days (except for the last sampling: 3 days) over the 28-day period of study for each experimental condition tested. Data are the mean value for all replicates performed in each experimental condition. Measurements were performed using ICP-MS. Different letters indicate significant different mean values among the experimental conditions for each sampling time.

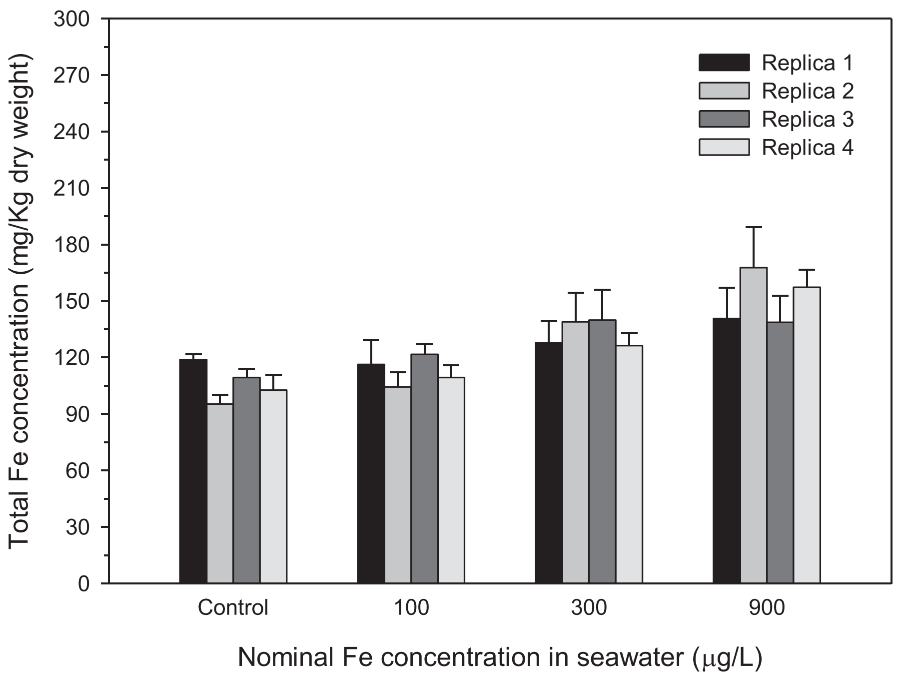

Fig. 24. Mean values (± SE) of total Fe concentration in sediments trapped every 5 days (except for the last sampling: 3 days) for each experimental condition tested. Data are the mean value for each replicate performed in each experimental condition over the 28-day period of study. Measurements were performed using ICP-MS. No significant difference was observed among replicates for each experimental condition ($P > 0.05$).

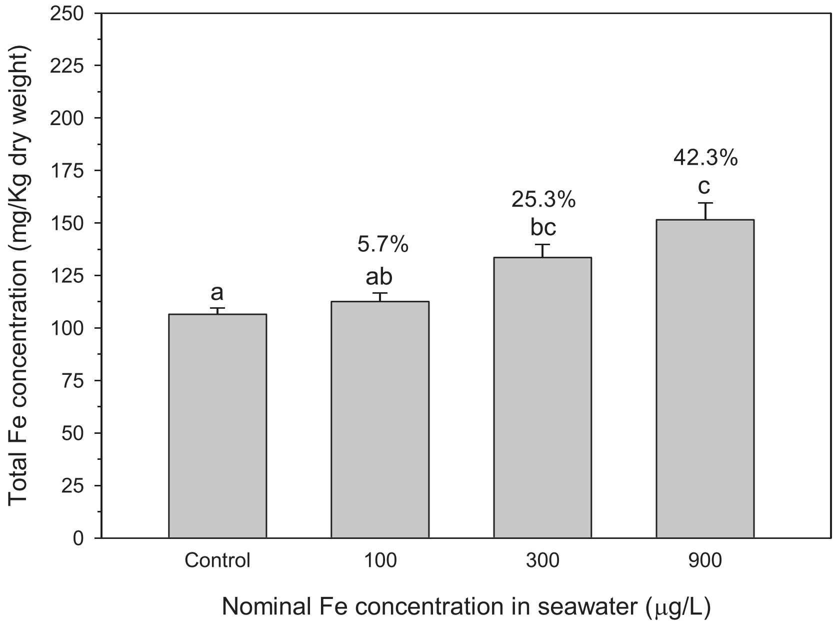

Fig. 25. General mean values (± SE) of total Fe concentration in sediments trapped every 5 days (except for the last sampling: 3 days) for each experimental condition tested. Values were calculated considering the four replicates performed for each experimental condition and data measured over the 28-day period of study using ICP-MS. Values shown at the top of the vertical bars represent the percentage of sediment enrichment with Fe respect with the control condition. Different letters represent different general mean values among the experimental conditions ($P < 0.05$).

300, and 900 µg/L treatments, respectively. The percentage of dissolved Fe respect with the total Fe in seawater corresponded to 93.5 ± 1.8% (Fig. 22).

Sediment trapped in the experimental tanks every five days (except for the last sampling: 3 days) were also analyzed for total Fe concentration (Fig. 23). No significant difference was observed among the four replicates performed for each experimental condition (Fig. 24). In general, sediments trapped in the experimental tanks subjected to seawater with dissolved Fe increments were enriched with this metal every 5 days, especially after the first week of experiment and at the higher levels of Fe increment in seawater (Fig. 23). General mean values of sediment enrichment with Fe significantly increased with the increasing levels of dissolved Fe increment in seawater, reaching 42.3% at the higher Fe concentration tested (Fig. 25).

Based on the findings described in the present study, we conclude that adaptations made in the original version of the mesocosm system of the Coral Vivo Project [32] were sucessfull. An adequate number ($n = 4$) of real biological replicates could be performed withouth significant changes in the main physical and chemical parameters of seawater among experimental units. Also, an adequate number of levels of Fe increment in seawater (0, 100, 300 and 900 µg/L) was possible to be tested. Furthermore, the levels tested remained relatively constant and very close to the desired nominal concentrations for 28 days. Therefore, studies involving the exposure of coral reef organisms to seawater contamination with dissolved Fe or other metal(loid)s can be performed with a great reliability. In addition, the adapted version of the marine mesocosm system allows now the exposure of a great diversity of reef organisms such as turf algae, fleshy macroalgae, rodoliths, brittle stars, hydrocorals, scleractinian corals, and fishes. As mentioned earlier, only one species could be exposed at a time using the previous version of the marine mesocosm of the Coral Vivo Project. Another important aspect to be considered is the much higher amount of biological material that can be exposed simultaneoulsy in the new adapted version of this system. Studies aiming the evaluation of Fe and other metal(loid)s bioaccumulation, as well as the consequent biological impacts of increasing concentrations of these contaminants in seawater can now be performed on a diversity of reef organisms of different functional groups simultaneously.

CRediT authorship contribution statement

Adalto Bianchini: Conceptualization, Methodology, Investigation, Formal analysis, Resources, Data curation, Validation, Visualization, Writing - Original draft preparation, Writing - Reviewing and Editing, Supervision, Project administration, Funding acquisition. **Letícia May Fukushima**: Methodology, Investigation, Formal analysis, Data curation, Validation, Writing - Reviewing and Editing. **Ana Carolina Grillo**: Methodology, Investigation, Formal analysis, Data curation, Validation, Writing - Reviewing and Editing. **Kelly Yumi Inagaki**: Methodology, Investigation, Formal analysis, Data curation, Validation, Writing - Reviewing and Editing. **Juliana de Andrade Souza**: Methodology, Investigation, Formal analysis, Data curation, Validation, Writing - Reviewing and Editing. **Ligia Salgado Bechara**: Methodology, Investigation, Formal analysis, Data curation, Validation, Writing - Reviewing and Editing. **Giovanna Destri**: Methodology, Investigation, Formal analysis, Data curation, Validation, Writing - Reviewing and Editing. **Vitor André Passos Picolloto**: Methodology, Investigation, Formal analysis, Data curation, Validation, Writing - Reviewing and Editing. **Thales Jean Vidal**: Methodology, Investigation, Formal analysis, Data curation, Validation, Writing - Reviewing and Editing. **Alessandra Lopes de Araújo**: Methodology, Investigation, Formal analysis, Data curation, Validation, Writing - Reviewing and Editing. **Juliana da Silva Fonseca**: Methodology, Investigation, Formal analysis, Data curation, Validation, Writing - Reviewing and Editing. **Emiliano Nicolas Calderon**: Methodology, Resources, Writing - Reviewing and Editing, Project administration, Funding acquisition. **Kely Paula Salvi**: Methodology, Investigation, Formal analysis, Resources, Data curation, Validation, Writing - Reviewing and Editing. **Carlos Henrique Figueiredo Lacerda**: Methodology, Investigation, Formal analysis, Resources, Data curation, Validation, Writing - Reviewing and Editing. **Patrícia Gomes Costa**: Methodology, Investigation, Formal analysis, Resources, Data curation, Validation, Writing - Reviewing and Editing. **Miguel Mies**: Conceptualization, Methodology, Investigation, Formal analysis, Resources, Data curation, Validation, Writing - Reviewing and Editing, Supervision, Project administration, Funding acquisition.

Limitations

None.

Ethics statements

No ethical considerations were required.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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