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RESEARCH ARTICLE

An aquaria set-up for long-term, multiple-stressor research in marine organisms

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Abstract

1. Multiple stressor research in aquaria is a useful approach to better understand the ecophysiology of marine species under different environmental conditions, including global change scenarios. Long-term experiments are helpful to detect the response of sustained exposure to selected environmental conditions. Here, we present an experimental set-up suitable to run long-term experiments, composed of a life support system, a cost-effective aquaria set-up and an open-source controller based on the use of a Raspberry Pi. In this set-up, temperature, pH and dissolved oxygen (DO) are individually manipulated and simultaneously controlled in eight different treatments.
2. To prove the efficacy of the set-up, we provide an assessment over a nine-month experiment on a deep-sea coral species, combining values from current in situ and IPCC AR5 RCP 8.5 scenarios for the aforementioned parameters. Recorded data from the controllers and independent measurements (e.g. cross-checking with portable multiparameter devices and laboratory analyses) throughout the experimental time have been analysed and results have been discussed.
3. Overall, the experimental set-up performed well, proving the stability of the parameters over time, both individually and in combination. On average, low and high-temperature treatments varied ~0.4 and 0.3°C, respectively. Low pH treatments were maintained within 0.05 pH units, whereas ambient pH treatments varied ~0.04 pH units. Low DO treatments had a variation of ~0.3 mg L^{-1}, and ambient DO treatments varied ~0.2 mg L^{-1}. No significant differences between scenarios for any parameter were detected ($p < 0.05$). The resulting programming

code to read, control and register the values for these parameters is provided to contribute to its replicability across institutions.

4. The set-up performed well over extensive periods while providing a customisable controller as a cost-effective alternative. The versatility of the system, allowing to work with different species, environments and scenarios makes it valuable for aquaria experiments where interactions of multiple environmental factors need to be tested.

KEYWORDS
aquaria set-up, cold-water corals, cost-effective controller, flow-through system, global change, marine organisms, multiple stressors, open source controller

1 | INTRODUCTION

Marine organisms are increasingly being exposed to several stressors derived from anthropogenic activities, which overall contribute to global change. Some of the main impacts of global change include warming, acidification and deoxygenation (Bindoff et al., 2019; Kwiatkowski et al., 2020; Mora et al., 2013). These stressors may happen simultaneously and their effects can be cumulative (Pörtner, 2012; Rossi, 2023; Todgham & Stillman, 2013).

Over the last decades, experimental studies assessing the effects of global change on marine organisms have significantly increased and focus has been put on identifying potential synergies among stressors (Bass et al., 2021).

However, multiple-stressor research implies additional challenges, including: (1) complex aquaria set-ups to allow for proper replication (Cornwall & Hurd, 2016); (2) reliable control of water parameters, individually and in combination, to simulate the targeted environmental conditions; and (3) high costs derived from the purchase and maintenance of the experimental system. As a result, only a few studies have assessed the effects of combined warming, acidification and deoxygenation in aquaria, where the seawater parameters were manipulated for 72 h (Donham et al., 2022) to 30 days (Khan et al., 2020), except for a very complex set-up that lasted 10 months by Bernhard et al. (2021). Besides, multiple-stressor experimentation over long terms usually requires large-scale infrastructures and significant financial resources, including mesocosm (e.g. Falkenberg et al., 2016; Pansch & Hiebenthal, 2019) or complex aquaria set-ups (e.g. Bockmon et al., 2013; Burrell et al., 2016). Therefore, there is still little knowledge on whether marine organisms can acclimatise to prolonged exposure to multiple stressors and how physiological compensatory mechanisms might affect biological processes in the long term. Long-term experiments can uncover hidden impacts due to physiological trade-offs that can affect the long-term survival of marine organisms to global change (e.g. Godbold & Solan, 2013; Hennige et al., 2015; Mardones et al., 2021). Understanding the long-term impacts and potential interactions of global change is vital for achieving an extensive and predictive insight into the potential consequences of global change on marine ecosystems.

To perform long-term, multiple-stressor experiments with marine organisms, an ad hoc experimental set-up was developed at the aquaria facilities of the public aquarium *Aquarium Finisterrae* (A Coruña, Spain). The set-up offers a cost-effective alternative to costly systems (e.g. Bellworthy & Fine, 2018; Falkenberg et al., 2016) for conducting long-time, multiple-stressor research. Further, we developed an open-source, cost-effective controller system to adjust and maintain stable conditions over long periods. Here, we describe in full detail the experimental set-up and provide data from a nine-month experiment run with a cold-water coral species to assess the stability and reliability of the set-up over time. In addition, we provide a description of the code and usage guide to program the controller system in Appendix A.

2 | TEST OF THE EXPERIMENTAL SET-UP

The performance of the experimental set-up was assessed throughout the duration of a multiple-stressor experiment aimed to assess the single and cumulative effects of warming, acidification and deoxygenation on the cold-water coral *Dendrophyllia cornigera* off the NW Iberian Peninsula (Spain). To achieve this, a full factorial design was set up based on two scenarios: current in situ conditions and projected future conditions under the RCP 8.5 scenario for the NE Atlantic (IPCC, 2014; Sweetman et al., 2017; Table 1). Treatment values were established according to available information: current in situ temperature was settled based on reports from the study area at the depth of the collection of the cold-water coral species (12°C, Lange et al., 2024; Lauvset et al., 2024; Padin et al., 2020). Ambient pH and DO in the experimental aquaria were established as current in situ values for the study area (~7.99 pH units and 8.63 mg O_2 L^{-1}, respectively). Therefore, pH and DO were not controlled in treatments with ambient target values. For the warming treatments, a temperature increase of +2°C was applied to the maximum natural value recorded in situ, according to the temperature records and the projections for the study region (15°C, Sweetman et al., 2017). Similarly, a DO decrease of −20 µmol kg^{-1} was applied to the minimum natural DO value recorded; this value was further converted to mg L^{-1} for each temperature and established as the DO target for deoxygenation treatments (6.45 and 6.46 mg L^{-1} at 12 and 15°C, respectively). Finally, pH value for acidified treatments was

TABLE 1 Water parameters measured in the experimental aquaria during the 9 months of the experiment.

Treatment	Temperature (°C)		pH_T		Dissolved oxygen ($mg\,L^{-1}$)	
	Target	Observed	Target	Observed	Target	Observed
T1 (Control)	12	12.36 ± 0.43 (3.4)	7.99	7.98 ± 0.04 (0.5)	8.63	8.77 ± 0.19 (2.1)
T2 (↑Temp)	15	15.07 ± 0.21 (1.4)	7.99	7.94 ± 0.04 (0.5)	8.63	8.52 ± 0.18 (2.1)
T3 (↓pH)	12	12.42 ± 0.44 (3.5)	7.69	7.69 ± 0.08 (1.0)	8.63	8.75 ± 0.20 (2.2)
T4 (↓DO)	12	12.28 ± 0.33 (2.7)	7.99	8.00 ± 0.04 (0.5)	6.45	6.50 ± 0.31 (4.8)
T5 (↑Temp ↓pH)	15	15.00 ± 0.24 (1.6)	7.69	7.68 ± 0.04 (0.5)	8.63	8.46 ± 0.15 (1.8)
T6 (↑Temp ↓DO)	15	15.01 ± 0.30 (2.0)	7.99	7.95 ± 0.04 (0.5)	6.46	6.46 ± 0.26 (4.1)
T7 (↓pH ↓DO)	12	12.16 ± 0.33 (2.7)	7.69	7.67 ± 0.05 (0.7)	6.45	6.51 ± 0.34 (5.3)
T8 (↑Temp ↓pH ↓DO)	15	15.04 ± 0.30 (2.0)	7.69	7.64 ± 0.03 (0.4)	6.46	6.51 ± 0.41 (6.3)

Note: Measures for each treatment (T1 to T8) are included. Measured data are expressed as mean ± standard deviation (% coefficient of variation). Temperature and dissolved oxygen concentration (DO) were measured five times per week (n = 642), whereas pH_T (total scale at in situ temperature) was measured every 1–2 months (n = 19 in the control treatment, n = 21 for the rest of the treatments).

established for each temperature to achieve 1000 µatm pCO_2 (7.69 pH units at both temperatures) according to IPCC scenarios (IPCC, 2014). Temperature, pH and DO were accordingly modified in each of the resulting eight treatments (Table 1). The experimental test ran for more than 9 months, from the 6 May 2022 to 24 February 2023.

Independent measurements were conducted before the start and during the experiment to establish the accuracy and reliability of the system. Temperature and DO were measured daily with a YSI ProODO dissolved oxygen instrument. Salinity was measured weekly with a WTW 350i multiparameter device equipped with a ConOx probe. The pH was measured by spectrophotometry using a Sigma Aldrich impure indicator (Clayton & Byrne, 1993) every 1–2 months by a laboratory specialised in inorganic chemical oceanography at the Oceanographic Centre of A Coruña (IEO-CSIC). Total alkalinity from the aquaria system (single sample point) was measured using the double-ended potentiometric technique (Pérez et al., 2000; Perez & Fraga, 1987) every 1–2 months (Bramanti et al., 2013; Movilla, Gori, et al., 2014; Movilla, Orejas, et al., 2014). Additional parameters of the carbonate system were further calculated by using the R package *seacarb* (Gattuso et al., 2023; Table 2). Dissolved inorganic nutrient concentrations in the experimental aquaria were measured after 1, 6 and 9 months. Nitrites, nitrates, silicates and phosphates concentrations were determined using colorimetric methods (Becker et al., 2020; Hydes et al., 2010; Table B1). Finally, prokaryotic abundance in the input water, as well as in the set-up tanks and aquaria was quantified monthly using a CytoFLEX S flow cytometer (Gasol et al., 1999) (Table B2). All statistical analyses were performed using the software R (version 4.3.0; R Core Team, 2023).

3 | DESCRIPTION OF THE EXPERIMENTAL SET-UP

The experimental set-up is composed of two main parts: the life support system and the aquaria system (Figure 1). The life support system mainly relies on the facilities where the experimental set-up is placed. However, a brief description of specific measures to ensure the supply of filtered and cold seawater to the aquaria system is provided. The aquaria system is a cost-effective set of tanks where the environmental parameters (temperature, pH, and DO) are adjusted by an open-source and cost-effective controller.

3.1 | Life support system

Water supply is pumped from the sea (17 m depth, 500 $L\,h^{-1}$) and pre-cooled by a flow-through chiller (SIMAFI, ~ −2°C, Figure 1a). Input water is collected in a 1000 L isothermal tank (Figure 1b), where it is filtered by a layer of filter wool for mechanical filtration and several layers of bio-balls that enhance bacterial growth, allowing for biological filtration. The filtration tank is connected to an industrial chiller (Kosner KMCi-10) that cools the seawater to 9°C (Figure 1c) and to a protein skimmer (Aqua Medic Turboflotor 5000 Twin, Figure 1d). Water from the filtration tank is pumped through a cartridge filter system (50, 20 and 5 µm, AQUA PRO, Figure 1e) into a reservoir tank (1400 L), that is located above the aquaria system (Figure 1f). The location of the reservoir tank allows for a constant flow of seawater to the aquaria system by gravity, passing through a UV lamp (95 W, Wedeco Rex, Figure 1g) to prevent microbial growth. This tank also acts as a reservoir in case of power shutdown, since it will keep supplying seawater to the tanks of the aquaria system by gravity until the depletion of its volume (which would take up to 4 h, in this specific case). Overflowing water from the reservoir tank returns by gravity to the filtration tank (Figure 1b), where it is constantly recirculated through the filtration and cooling equipment.

To detect any failure in the water supply, temperature probes (DS18B20) are installed inside the main supply pipes: one in the input water pipe and another inside the pipe that distributes the water into the aquaria system (Figure 1h). In case of a malfunction that interrupts the supply of cold water, the pipes would get emptied, leading to a temperature increase to ambient values inside them and consequently triggering the alarm system (see Section 3.4).

TABLE 2 Variables for the seawater carbonate system in the experimental aquaria for each treatment over the experimental time.

Treatment	pH_T	TA ($\mu mol\,kg^{-1}$)	Sal	Temp (°C)
T1 (Control)	7.98 ± 0.04	2332 ± 32	35.0 ± 0.5	12.3 ± 0.2
T2 (↑Temp)	7.94 ± 0.04	2333 ± 30	35.1 ± 0.5	15.1 ± 0.1
T3 (↓pH)	7.69 ± 0.08	2333 ± 30	35.1 ± 0.5	12.3 ± 0.3
T4 (↓DO)	8.00 ± 0.04	2333 ± 30	35.0 ± 0.4	12.2 ± 0.3
T5 (↑Temp ↓pH)	7.68 ± 0.04	2333 ± 30	35.1 ± 0.4	14.9 ± 0.1
T6 (↑Temp ↓DO)	7.95 ± 0.04	2333 ± 30	35.1 ± 0.5	15.0 ± 0.1
T7 (↓pH ↓DO)	7.67 ± 0.05	2333 ± 30	35.1 ± 0.4	12.1 ± 0.3
T8 (↑Temp ↓pH ↓DO)	7.64 ± 0.03	2333 ± 30	35.1 ± 0.5	15.0 ± 0.1

Treatment	pCO_2 (µatm)	DIC ($\mu mol\,kg^{-1}$)	HCO_3^- ($\mu mol\,kg^{-1}$)	CO_3^{2-} ($\mu mol\,kg^{-1}$)	Ω_{Ca}	Ω_{Ar}
T1 (Control)	483 ± 51	2160 ± 31	2011 ± 32	129 ± 10	3.1 ± 0.2	2.0 ± 0.1
T2 (↑Temp)	546 ± 54	2162 ± 31	2011 ± 33	130 ± 10	3.1 ± 0.2	2.0 ± 0.1
T3 (↓pH)	1013 ± 171	2266 ± 38	2153 ± 40	72 ± 13	1.7 ± 0.3	1.1 ± 0.2
T4 (↓DO)	462 ± 54	2153 ± 32	2000 ± 35	134 ± 10	3.2 ± 0.2	2.0 ± 0.2
T5 (↑Temp ↓pH)	1061 ± 100	2261 ± 35	2145 ± 35	76 ± 6	1.8 ± 0.1	1.2 ± 0.1
T6 (↑Temp ↓DO)	527 ± 60	2156 ± 34	2003 ± 38	133 ± 10	3.2 ± 0.2	2.0 ± 0.2
T7 (↓pH ↓DO)	1072 ± 133	2276 ± 34	2165 ± 34	67 ± 7	1.6 ± 0.2	1.0 ± 0.1
T8 (↑Temp ↓pH ↓DO)	1159 ± 89	2272 ± 35	2159 ± 34	70 ± 4	1.7 ± 0.1	1.1 ± 0.1

Note: pH_T (total scale at in situ temperature), total alkalinity (single sample point), salinity and temperature of every experimental aquarium were used to calculate additional parameters. Mean values for the concentration of silicate and phosphate in the aquaria experiments were used in the calculations (2.1 and 0.31 $\mu mol\,kg^{-1}$, respectively). Parameter data are expressed at in situ temperature, as mean ± standard deviation, $n = 19$ in the control treatment, and $n = 21$ for the rest of the treatments.

Abbreviations: Ω_{Ar}, saturation state of seawater with respect to aragonite; Ω_{Ca}, saturation state of seawater with respect to calcite; CO_3^{2-}, carbonate ion concentration; DIC, dissolved inorganic carbon; HCO_3^-, bicarbonate ion concentration; pCO_2, partial pressure of CO_2; pH_T, pH in total scale; Sal, salinity; TA, total alkalinity; Temp, in situ temperature.

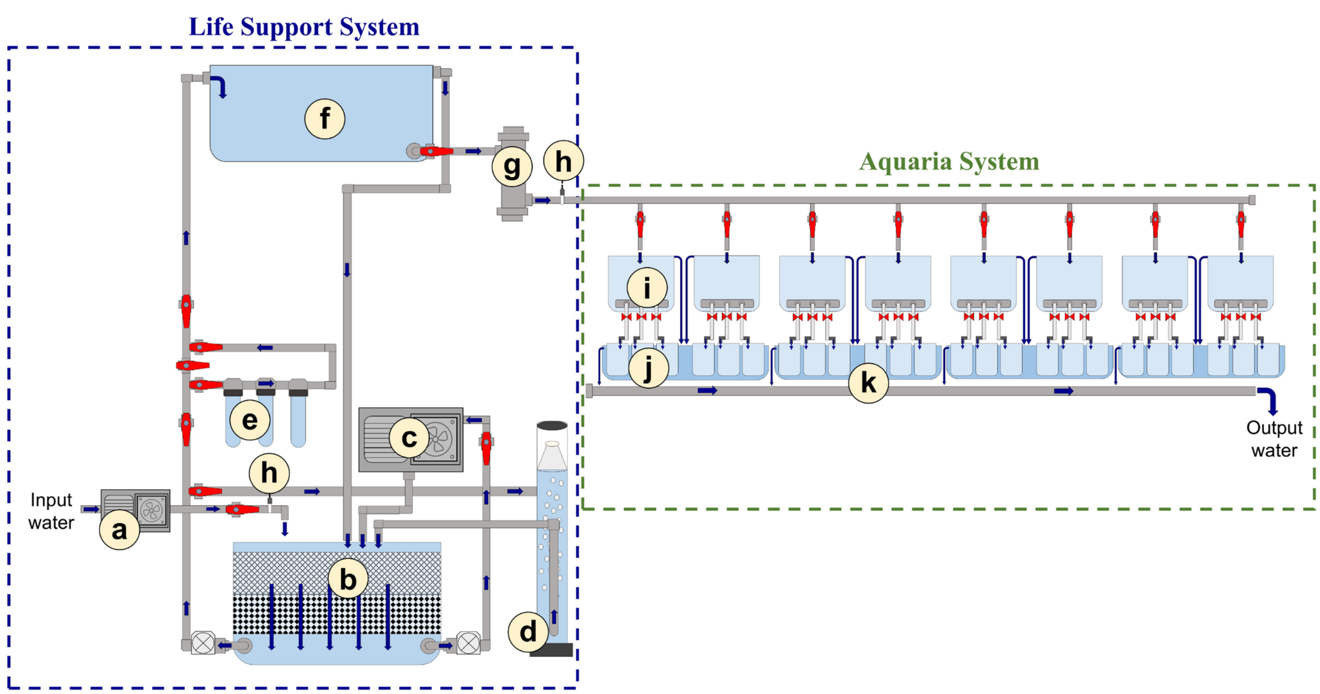

FIGURE 1 Schema of the life support system (blue-dashed section) and aquaria system (green-dashed section) of the experimental set-up. Arrows indicate the direction of water movement; (a) flow-through chiller, (b) filtration tank, (c) industrial chiller, (d) protein skimmer, (e) filter cartridge system, (f) reservoir tank, (g) UV lamp, (h) temperature probes, (i) header tanks, (j) experimental aquaria, and (k) water bath.

3.2 | Aquaria system

The aquaria system is installed in a room equipped with an air conditioning unit that keeps the room at 16–18°C (close to the experimental temperature target values), a dehumidifier that keeps the room dry to prevent damage to electronic devices and a carbon dioxide detector equipped with an alarm (1000 ppm CO_2) to ensure safety in case of any CO_2 gas leak. Modular shelves are installed to fit the header tanks and experimental aquaria (Figure 2). The room was kept in darkness to simulate the field conditions for the tested cold-water coral. Lights were turned on when maintenance or experimental measurements were conducted.

The seawater from the reservoir tank (Figure 1f) flows into the room through a pipe and is distributed to eight 80 L polycarbonate header tanks (Figures 1i and 2a), in which temperature, pH and DO are individually manipulated and monitored to achieve the set points for each treatment. Each header tank is connected through independent pipes to three 5 L polypropylene experimental aquaria (Figures 1j and 2b), which are the treatment replicates, holding the experimental organisms. The header tanks are located on the upper shelf and the experimental aquaria on the lower shelf, so that seawater is distributed into the experimental aquaria by gravity (Figure 2). The pipes that distribute the seawater from the header tanks to the experimental aquaria are equipped with a valve to control the flow rate and a pipe elbow (90° angle) that is placed next to the water surface to prevent injection of air bubbles (Figure 2c). The turnover rate of the seawater supply to the experimental aquaria is five times h^{-1}. Each header tank and aquarium are equipped with a lid to minimise gas exchange and maintain low pH and/or DO conditions, if necessary.

The experimental aquaria are placed inside 60 L propylene containers (Figures 1k and 2d) that collect the overflowing water from them and act as water baths ensuring the maintenance of the experimental temperature in the aquaria. The experimental aquaria are distributed into the different warming baths according to their target temperature. To increase the cooling capacity of the water baths, the overflowing water from the header tanks is connected to the water bath with a hose. The water inside each experimental aquarium overflows from an 8 mm hole next to the upper rim. Therefore, if the water supply is interrupted, the experimental aquarium will remain full of seawater and the water bath will buffer temperature changes inside the experimental aquarium. Each water bath is connected to a drain line by a hose to discard the overflow water. The height of the drain hole of the water bath, and therefore the water level inside it, is slightly below (1 cm) those of the experimental aquaria to avoid any water mixing.

Every header tank, water bath and aquarium in the system is equipped with a submersible pump to keep the water in constant movement (Figures 2e and 3k). The power of the pumps is determined by the volume of the tank or aquarium. Header tanks and water baths are equipped with Sicce Syncra 1.5 pumps (maximum rate of 1350 L h^{-1}), whereas experimental aquaria have Sicce Syncra Nano pumps (maximum rate of 430 L h^{-1}).

3.3 | Control and manipulation of parameters (temperature, pH and DO)

The design of the experimental set-up is a flow-through system of cold seawater, with ambient pH and DO, constantly supplied to the header tanks. This allows for the unidirectional manipulation of the water parameters: raising the temperature, and lowering pH and DO. Therefore, the fine-tuning of the parameters to the desired experimental conditions, simulating different environmental scenarios, is achieved by reaching an equilibrium between the manipulation of each environmental parameter and the seawater renewal rate in the header tanks (Figure 3).

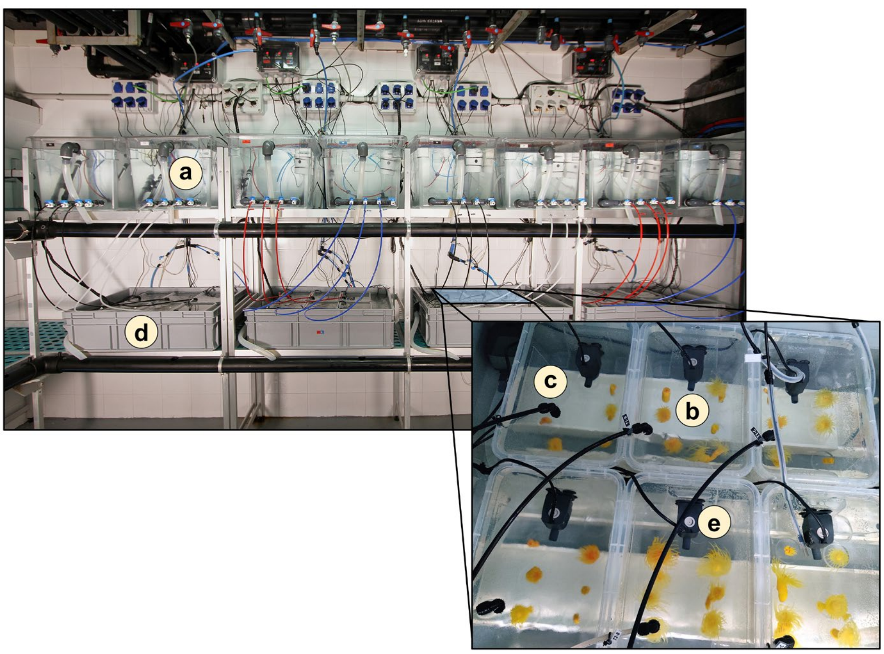

FIGURE 2 Aquaria system of the experimental set-up placed on the modular shelves. (a) Header tanks, (b) experimental aquaria, (c) seawater supply pipes equipped with a pipe elbow, (d) water baths and (e) recirculating pumps.

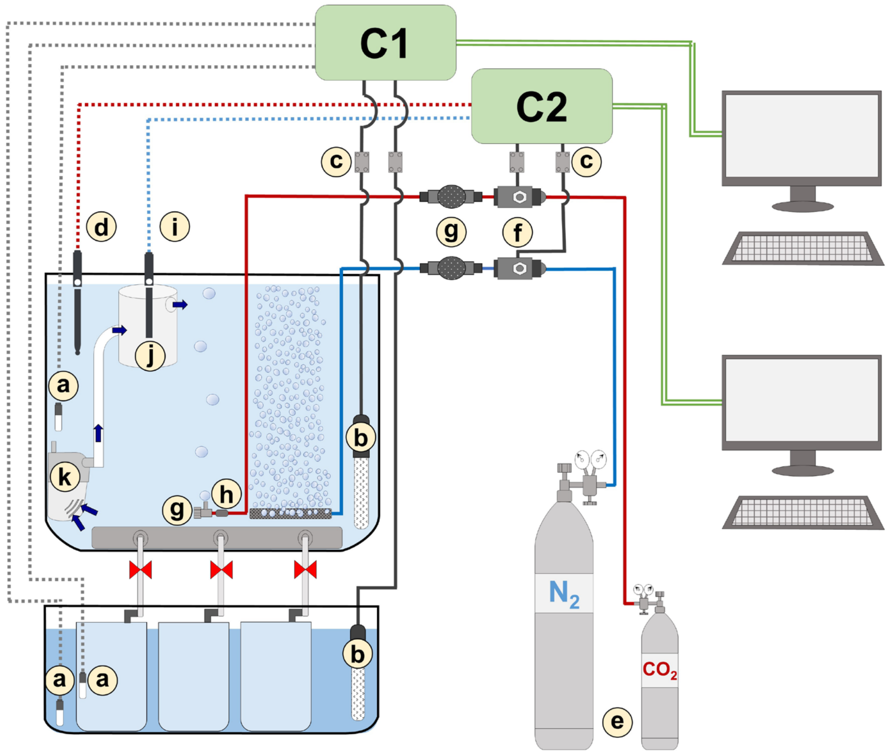

FIGURE 3 Schema of the manipulation and control of the parameters in a header tank (above) and its related experimental aquaria and water bath (below) by the controllers C1 and C2. Arrows indicate the direction of water movement: (a) Temperature probes, (b) heaters, (c) relay modules, (d) pH probe, (e) N_2 and CO_2 gas cylinders, (f) solenoid valves, (g) needle valves, (h) check valve, (i) DO probe, (j) cylindrical container, and (k) recirculating pump. Grey, red and blue lines indicate the connections between devices for the control of temperature, pH and DO, respectively. Dashed lines indicate signal from the probes, solid lines indicate hoses and cables, and double lines indicate the connection from the controllers to the control hardware.

To control and register the environmental parameters, two sets of controllers (Raspberry Pi 3, Figure 3, C1 and C2) are installed in a splash-free cabinet. C1 regulates and logs the temperature, whereas C2 regulates and logs pH and DO. A set of probes that measure temperature, pH and DO is installed in each header tank and connected to the controllers. Furthermore, an additional temperature probe (not depicted in Figure 3) is set in each header tank and connected to C2 to allow for the temperature compensation of the pH and DO measurements. A programming code allows the controllers to measure the values for each parameter every 40s and use relay modules to turn on/off the devices for the manipulation of the parameters (submersible heaters that raise the temperature, and solenoid valves that allow gas input inside each tank) after every reading, based on the selected set points. A second programming code records parameter values every 15min in a database for subsequent analyses (see Gutiérrez-Zárate et al., 2024a). A manual calibration value for each probe can be set on the controllers (i.e. displayed value = measured value by the probe + calibration value) to allow for the fine-tuning of the control of parameters. On the other hand, slight variations in parameter values occur between the header tanks and the experimental aquaria, as seawater may be warmed and reoxygenated during its transfer (due to the room air temperature and gas exchanges). To correct this, it is important that the parameter set points on the header tanks are adjusted to achieve the target values in the experimental aquaria.

Temperature is measured by digital temperature probes (DS18B20, ±0.5°C, Figure 3a) and regulated using heaters (Marina 300W, Figure 3b) placed in each header tank and water bath, and connected through relay modules (Figure 3c) to the temperature controller (Figure 3, C1). The temperature probes have been adapted to avoid potential water damage by enclosing them in silicone tubes, sealed with silicon (Figure B1). If the measured temperature of a header tank or water bath is lower than the set point, the controller turns on the respective relay module, activating the heater. An additional temperature probe is placed inside one of the experimental aquaria from each treatment to allow for continuous temperature monitoring inside them (Figure 3a).

The pH is measured using Atlas Scientific Lab Grade probes (±0.002 pH units) installed inside each header tank (Figure 3d) and connected to C2 using EZO™ pH Circuits and Electrically Isolated EZO™ Carrier Boards. If the measured pH value is higher than the set point, a solenoid valve for the CO_2 supply is activated, allowing the gas injection into the tank. The pH probes are monthly calibrated using WTW 4.01, 7.00 and 10.01 NBS calibration fluids at 25°C. The acclimation of these probes to the first calibration fluid (pH 7.00 NBS) at 25°C is slow, and accurate pH values are achieved after 4h acclimation. Consequently, two extra pH probes are also connected to C2, so that they can replace those being calibrated, preventing any interruption in the control of pH during the calibration procedure. To lower the pH, a pure CO_2 gas cylinder (13.4L volume, Figure 3e) with a pressure reducer and flow meter is connected through gas hoses to eight solenoid valves, corresponding to each header tank. Once the solenoid valve is activated by C2 (Figure 3f), the CO_2 gas flows through two sets of needle valves that lower the flow rate (Figure 3g) into the header tank. A check valve before the end of the hose is set to avoid gas back-flow and water entering the hose once the injection of CO_2 is stopped (Figure 3h). This is also important to facilitate the immediate bubbling of CO_2 once the gas supply is required. The best way to achieve a precise and stable control of the pH is to bubble individual and large (~3 mm in diameter) bubbles of CO_2 (~2 bubbles s^{-1}) without any gas diffuser. The pH is controlled and registered in the NBS scale and further transformed to the total scale for subsequent analyses using the R package *AquaEnv* (Hofmann et al., 2010).

Dissolved oxygen concentration (DO) is measured using Atlas Scientific Lab Grade probes (±0.05 mg L^{-1}) installed inside each header tank (Figure 3i) and connected to C2 using EZO™ Dissolved Oxygen Circuit and Electrically Isolated EZO™ Carrier Boards. Dissolved oxygen is measured and logged in % air saturation and mg L^{-1} (by manually providing the water salinity). Since the probes are polarographic DO sensors that consume oxygen while measuring, they are placed in a cylindrical container (10 cm diameter, Figure 3j) receiving the discharge of the recirculating pump of the header tank (Figure 3k) to be directly exposed to a strong water current through the probe membrane and achieving stable measurements. Probe calibration and electrolyte refill are performed monthly by one-step air calibration. To lower the DO of a header tank, its corresponding solenoid valve is activated by C2, allowing for the supply of pure N_2 gas from a 50 L gas cylinder into the header tank. N_2 injection is achieved in the form of abundant small bubbles through an elongated porous diffuser. The C2 controller controls DO based on the values of % air saturation measured by the probes.

3.4 | Alarm system and remote control

The controllers are programmed to detect possible malfunctions in the experimental set-up and notify them via email. Maximum and minimum values of temperature, pH and DO for each tank/aquarium are set in the controllers (±0.5°C, ±0.05 pH units, ±5% air saturation from their set point). If the measured parameter values are outside these ranges, a warning email is sent to established email addresses. The email contains details about the tank or aquarium whose measurements triggered the alarm.

The controllers also react to a possible loss of connection to the probes, being programmed to refresh the connections with the probes after five failed attempts and to immediately restart the control of parameters system afterwards. If the control system is interrupted for more than 5 min, the controllers will send an email notifying the malfunction to the established email addresses. Additionally, controllers are automatically restarted daily and the control of parameters system is programmed to run automatically after every start. This ensures the correct functioning of the controllers by optimising the use of their memory resources.

Finally, the internet connection of the controllers also allows for remote access and control of the whole system using computers or portable electronic devices via remote desktop software (AnyDesk Software GmbH, v7.0.5).

4 | PERFORMANCE ASSESSMENT OF THE EXPERIMENTAL SET-UP

4.1 | Performance of the life support system

Overall, the components of the life support system (i.e. chillers, skimmer, filter cartridges, UV lamp) worked without any malfunctions during the 9 months. As the assessment of the aquaria system lasted from May 2022 to February 2023, two specific adjustments were required according to the season to ensure stable temperatures in the header tanks: (1) the skimmer, which lacked thermal insulation, was removed from the circuit from June to August to avoid uncontrolled warming of the water during the summer months since the experimental set-up was used in a flow-through configuration with constant renewal, this did not affect water quality; (2) the additional chiller cooling the inflow water (Figure 1a) was removed from November to February since the remaining chiller (Figure 1c) was enough to ensure cold and stable temperatures during the winter months.

Each filter set (50, 20 and 5 μm) was replaced after 20 to 30 days to avoid filter clog. Filter replacement frequency varied depending on the weather conditions (e.g. heavy storms implied increased sediment in the inflow water). The UV lamp was functional throughout the whole experimental time, with no need for replacement, as showed in the results of the measurements of prokaryotic abundances in the experimental aquaria (see Section 4.3).

A power shortage affecting the water inflow pump took place on 1 December 2022 during the night, stopping water inflow into the filtration tank for 6 h. This resulted in the consecutive draining of the filtration tank first, followed by the reservoir tank, and, finally, the header tanks. The eventual exposure to air of the pH and DO probes in the header tanks resulted in abnormal readings, triggering the alarm system and compromising parameter control. However, the temperature probes, located at the bottom of the header tanks, remained submerged and continued to regulate the temperature accurately. During the time needed to fix the power shortage, all the corals remained in the corresponding treatment water and temperature in the experimental aquaria, although pH and DO inside them deviated significantly from their target values. This power shortage demonstrated the efficiency of the set-up and the alarm system to activate response actions while providing extra time to fix the malfunction.

4.2 | Performance of the control and manipulation of parameters

During the first months of the experiment, the temperature probes suffered from humidity damage that led to large temperature variations, further affecting the pH and DO control (Figure 5). After adapting the probes to waterproof conditions (Figure B1), they provided stable measurements throughout the rest of the experimental time, with no need for further calibrations. Beside this, no major issues affected the control of temperature, pH and DO. Wire connections were checked every 1–2 months to mitigate possible humidity damages. The pH probes showed offsets during the experiment (ΔpH > 0.05 pH units). Therefore, results from the independent pH measurements were used to calculate the offset from each probe and to manually correct their readings in the controller. The proper calibration of the DO probes was the most significant challenge and, at times, unachievable (e.g. discrepancies up to 3 mg L^{-1} and erratic peaks in the logged data).

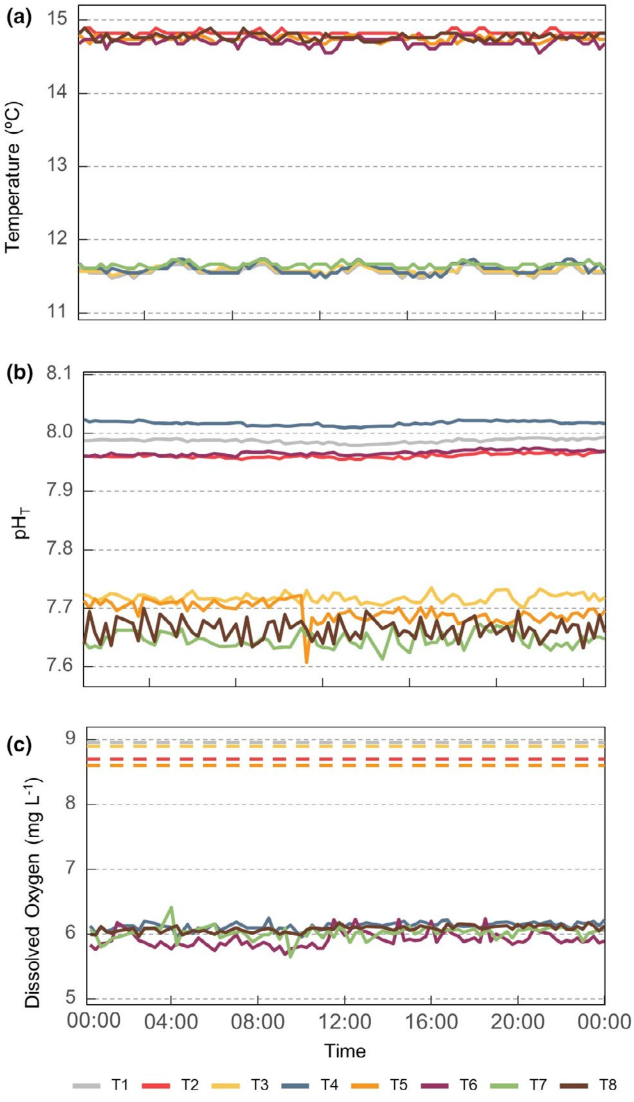

FIGURE 4 Parameters recorded over 24 h on the 31st of December 2022 for each of the eight header tanks (T1 to T8): (a) Temperature (°C), (b) pH$_T$ (total scale at in situ temperature) and (c) dissolved oxygen (DO, mg L^{-1}). Dashed horizontal lines represent the mean ambient DO values for the experimental aquaria from the same treatment, as measured independently (Figure 5). Parameters were recorded every 15 min (n = 83). Treatment conditions are (1) control (T1, grey); (2) warming (T2, red); (3) acidification (T3, yellow); (4) deoxygenation (T4, blue); (5) combined warming and acidification (T5, orange); (6) combined warming and deoxygenation (T6, purple); (7) combined acidification and deoxygenation (T7, green) and (8) combined warming, acidification, and deoxygenation (T8, brown).

Consequently, efforts were focused on calibrating the DO probes from the low DO treatments. Ambient DO treatments were maintained without probes, with DO values in the experimental aquaria checked daily through independent measurements (therefore, not depicted in Figure 4c). There were weekly minor calibration offsets (~0.3 mg L^{-1}), which were manually corrected in the controller.

Gas injection (CO_2 and N_2) into the header tanks was adjusted periodically. The need for adjustments of the gas supply was detected by the probes (pH and DO, respectively) and reported via the alarm system. Throughout the 9 months of the experiment, the average CO_2 gas consumption (at atmospheric pressure) was ~19 L d^{-1}. The N_2 gas consumption largely varied due to the weather conditions

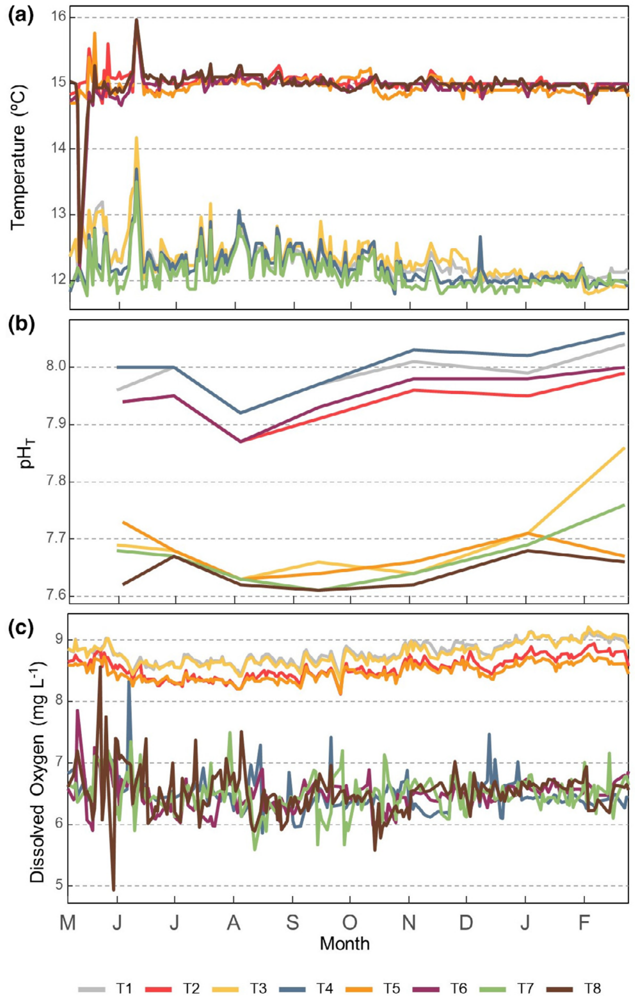

FIGURE 5 Mean parameter values from every treatment (T1 to T8) measured over the whole duration of the experiment (nine months): (a) temperature (°C), (b) pH$_T$ (total scale at in situ temperature) and (c) dissolved oxygen (DO, mg L^{-1}). Temperature and DO were measured five times per week (n = 642) and pH was measured every 1–2 months (n = 19 for the control treatment, n = 21 for the rest of the treatments). Treatment conditions are (1) control (T1, grey); (2) warming (T2, red); (3) acidification (T3, yellow); (4) deoxygenation (T4, blue); (5) combined warming and acidification (T5, orange); (6) combined warming and deoxygenation (T6, purple); (7) combined acidification and deoxygenation (T7, green) and (8) combined warming, acidification and deoxygenation (T8, brown).

(e.g. when the sea was rough, the inflow water was highly oxygenated and more N_2 was needed to reach the DO set point), with an average consumption of 947 L d^{-1} in spring and summer months and 1312 L d^{-1} in autumn and winter months.

As an example, the 24 h register (31 December 2022) of temperature, pH and DO displayed in Figure 4, shows the stability of the controlled parameters. Temperature variability for both the 'cold' (T1, T3, T4 and T7) and 'warm' (T2, T5, T6 and T8) header tanks (see Table 1) was below 0.07°C. The pH from acidified (T3, T5, T7 and T8) header tanks was maintained below 0.02 pH units, whereas measurements from the ambient pH header tanks (T1, T2, T4 and T6) varied within 0.004 pH units. Finally, DO for the deoxygenated (T4, T5, T6 and T8) header tanks varied within 0.12 mg L^{-1}. A slight temperature decrease occurred daily at 8:00 due to the automatic reset of the controllers, which stopped the control for a few minutes until the system fully restarted. Deviations in pH and DO were sporadically observed due to punctual malfunctions of the temperature probes that were used for the temperature compensation, due to humidity damages (see previously in the text). Further 24 h registers from each month during the experiment can be found in Figure B2.

4.3 | Independent monitoring of the system performance

The results of the control measurements for temperature, pH and DO over the 9 months of the experiment showed that the system achieved the target values in each of the eight treatments, therefore allowing to conduct multi-factor experiments over long periods, with all the parameters significantly different between the settled scenarios (Mann–Whitney-Wilcoxon test, temperature: $W=7529$, $p<0.001$; pH: $W=0$, $p<0.001$; DO: $W=6{,}590{,}624$, $p<0.001$).

The high-temperature treatments (T2, T5, T6 and T8) achieved the 15°C target (mean difference <0.1°C), whereas low-temperature treatments (T1, T3, T4 and T7) displayed slightly higher values (~0.3°C) than the 12°C target (Figure 5a). This could be due to the selected air temperature in the room where the aquaria system was installed (16–18°C), which should have been lower (~15°C). Acidified treatments (T3, T5, T7 and T8) were, on average, 0.02 units below the 7.69 target and were maintained within ~0.05 pH units, whereas ambient pH treatments (T1, T2, T4 and T6) varied ~0.04 pH units (Figure 5b). The high variability of the pH values observed in the acidified treatments, and therefore in the carbonate system parameters (Table 2), was mainly due to the calibration offset of the probes, as previously explained, since the independent measurements were conducted after their calibration and, therefore, before their readings were manually corrected. Low DO treatments with low (T4 and T7) and high temperatures (T6 and T8) were maintained ~0.06 and 0.02 mg L^{-1} above the target (6.45 and 6.46 mg L^{-1}, respectively). The variability of the measurements for all deoxygenated treatments was ~0.3 mg L^{-1}, in contrast with ambient DO treatments, which varied ~0.2 mg L^{-1}, showing the previously explained difficulties in the stabilisation of the readings of the DO probes. Treatments with ambient DO (T1, T2, T3 and T7) showed significant differences among cold and warm treatments (Mann–Whitney-Wilcoxon test, $W=6{,}590{,}624$, $p<0.001$), driven by the influence of the temperature on the oxygen solubility (Figure 5c). Additionally, the pH values in the ambient pH treatments showed no significant differences (*t*-test, $t=-1.67$, $p=0.1$) between those with (T4 and T6) and without (T1 and T2) nitrogen bubbling, showing that nitrogen bubbling did not affect the pH in our experimental set-up, contrary to the findings of previous works (Cohen & Kirchmann, 2004; Fricke et al., 1973; Gobler et al., 2014). However, these effects might be detectable under larger input nitrogen rates, such as those necessary to achieve hypoxic conditions, and must be addressed by simultaneously controlling the carbonate system by CO_2 inputs.

No significant differences were detected for dissolved inorganic nutrients concentrations in the experimental aquaria (Kruskal–Wallis test, Nitrite: $H=1.37$ $p=0.99$; Nitrate: $H=1.33$, $p=0.99$; Phosphate: $H=5.87$, $p=0.55$; Silicate: $H=1.97$, $p=0.96$; Table B1). Similarly, there were no significant differences in bacterial abundances observed among the experimental aquaria (Kruskal–Wallis test, $H=9.90$, $p=0.36$; Table B2).

5 | DISCUSSION

The presented experimental set-up successfully simulated global change scenarios in a long-term experiment. The life support and aquaria systems proved to be adequate to guarantee the required performance and stability of the different settings for the experiment. Additionally, the Raspberry Pi-based controller presented successfully modified, controlled and registered temperature, pH and DO. The performance of the control of parameters was assessed over an extensive period, exceeding 9 months, while combining three stressors, whereas previous similar set-ups were only assessed for weeks and controlling single or two stressors (Low et al., 2020; McLean et al., 2021; Onthank et al., 2023). This set-up contributes to the increasing implementation of programmable physical circuit boards as microcontrollers in aquaria experiments. By reducing the costs to implement parameter control at the required accuracy and stability, multiple-stressor research can be affordable to more research institutions, including public aquaria. This will result in increasing the number of studies, scenarios as well as studied species, contributing to a broader knowledge of the consequences of global change in marine species.

5.1 | System versatility

The system has been designed to independently modify temperature, pH and DO values in each treatment by individually modifying the parameters inside each header tank. Therefore, it allows for conducting multi-factor experiments simulating many different scenarios. The presented experiment, run to test the performance of the set-up, used experimental aquaria of 5 L volume (based on the size of the cold-water coral used). However, their volume can be chosen depending on the size and biomass of the targeted organisms. In the case of using larger volumes, special attention should be put on gas and cartridge filter consumption, as well as on the spatial requirements to fit the experimental aquaria below the header tanks to enable the water supply to flow by gravity. To improve scalability, auxiliary pumps could be installed, allowing for larger volumes and higher flow rates without compromising the robustness of the system. Although the experimental aquaria were designed to host benthic organisms, a strainer filter with an adequate mesh size can be connected to the outflow holes to retain and conduct experiments on pelagic organisms. The customisation feature of the controllers also allows for the programming of daily cycles of the controlled water parameters, extending the versatility of the system and making it suitable to simulate a large diversity of marine environments. The set-up can also be easily changed to act as a semi-open circuit by connecting the drain pipe to the filtration tank (Figure 1b). In this configuration, the skimmer would play a crucial role in maintaining nutrient quality, making it essential to thermally insulate it to prevent uncontrolled warming during the summer months. Doing this, the life support system would be less affected by seasonal changes in temperature, daily pH variations or heavy storms affecting DO. However, keeping the circuit open, as the system described here, allows to conduct a unidirectional manipulation of the selected parameters, without requiring the modification of the previously used water.

5.2 | Shortcomings and improvements

The main limitation detected for the optimal performance of the aquaria system was the lack of a truly temperature-controlled room due to budget and space constraints, thus requiring additional water cooling during summer (see Section 3.1). Indeed, the maintenance of cold temperatures in the experimental system implied a high seawater renewal rate and the consequent remarkable consumption of gas, especially N_2, and filter cartridges. A potential improvement could be achieved by connecting a chiller to control temperature in the warming baths. On the other hand, acidification experiments conducted in aquaria have been using soda-lime filters to bubble CO_2-free air in the tanks (e.g. Movilla, Orejas, et al., 2014; Rathbone et al., 2022; Thyrring et al., 2023), increasing the pH values with no alteration to the total alkalinity (Gattuso et al., 2010). In our aquaria system, a soda-lime filter (3 kg of soda lime) was installed and connected to the air inflow of the skimmer pumps. This filter would help to achieve the atmospheric 400 ppm pCO_2 target. However, due to the large volume and renewal rate of the water in the whole system, the soda lime was fully consumed after only a few hours. Therefore, the current in situ scenario has been considered as the ambient pH conditions, with no control over its values. Consequently, in the performed experimental test of the system, we observed daily natural pH variation in the input water, which affected the variability of this parameter in the ambient pH treatments, as also observed by Low et al. (2020).

Pseudoreplication in our experimental design (Hurlbert, 1984) was associated with the individual (non-replicated) header tank per treatment, which feeds the experimental aquaria. Replication of header tanks per treatment (see Cornwall & Hurd, 2016) is possible if space is available, or if fewer treatments are studied. On the other hand, since two independent water baths were used for each temperature tested, there is not full independence among the four treatments resulting from the combination of the tested levels of pH and DO under the same temperature. To achieve full independence, a single and larger water bath should be used for each temperature. An alternative option would be setting up an individual water bath for each experimental aquarium. Additionally, to improve the randomisation of the design, the experimental aquaria with the same temperature could be haphazardly distributed across the warming baths, while connected to the header tanks seawater supply by extended hoses.

Overall, the accuracy and precision of the temperature, pH and DO probes affected the capability of the control systems to manipulate and control the parameters. In this sense, the lack of routine pH measurements affected the accuracy and variability of the pH in the experimental aquaria, as the manual adjustments could not be performed daily. Therefore, the variability of the pH measurements was similar (Bellworthy & Fine, 2018; Lunden et al., 2014) or higher (Bockmon et al., 2013; Burrell et al., 2016) than other described set-ups. We are confident that by performing daily measurements with a lab-grade pH meter, as it was done for temperature and DO, the accuracy of the pH measurements can be highly and easily improved. However, the pH probes provided very stable measurements without any significant drift (Figure 4b) and the independent pH analyses, using spectrophotometric techniques, provided very accurate and reliable values. The proper placement of the DO probes was essential to achieve stable measurements since their membrane had to be directly exposed to the strong water current from the discharge of the recirculating pump. We found that proper placement, enough electrolyte solution and clean sensors were key factors for the correct functioning of the DO control system. Beyond these, it remains unclear why some DO probes were unreliable and had to be removed, while others worked effectively throughout the experiment without causing major issues. Alternatively, process probes, which are designed for the continuous monitoring of water parameters, could be installed on the header tanks instead of lab-grade ones to reduce their maintenance work, although it can lead to increased costs.

6 | CONCLUSIONS

Due to the recognised importance of conducting research on multiple stressors to gain a more comprehensive understanding of the potential impact of global change on marine ecosystems, the presented set-up has been proven reliable over long experimental periods. The provided cost-effective and open-source controller system allows for manipulating water parameters at the accuracy and stability needed for scientific and technical standards. Additionally, the easy and wide customisation possibilities of the aquaria set-up make it suitable for simulating many scenarios, in diverse marine environments and studied organisms.

AUTHOR CONTRIBUTIONS

Cristina Gutiérrez-Zárate, Alfredo Veiga, Andrea Gori and Covadonga Orejas conceived the scope of the manuscript. Cristina Gutiérrez-Zárate, Alfredo Veiga, Andrea Gori, Fabián Gerpe, Leo Domínguez, Alberto Rodríguez, Noelia Arias, Juancho Movilla and Covadonga Orejas contributed to the design and set-up of the system. Cristina Gutiérrez-Zárate and Alfredo Veiga performed the daily maintenance and measurements of the water parameters. Fabián Gerpe and Leo Domínguez developed the code for the controllers. Marta Álvarez, Rubén Acerbi, Lucía Vázquez, Marta M. Varela, Rodrigo Alba-Salgueiro and Joaquín Valencia-Vila provided the laboratory technical equipment and performed the laboratory measurements. All authors contributed substantially to the manuscript and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

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.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/2041-210X.14488.

DATA AVAILABILITY STATEMENT

The programming codes to set up the open-source controllers are publicly available from GitHub: https://github.com/c-gutierrez-zarate/Multiple-Stressor-Aquaria-Controller and have been archived with Zenodo: https://doi.org/10.5281/zenodo.14358290 (Gutiérrez-Zárate et al., 2024a). Data generated and analysed in this study are available in the data repository PANGAEA. The datasets include the dissolved inorganic nutrient concentrations, https://doi.org/10.1594/PANGAEA.963072 (Gutiérrez-Zárate et al., 2023); carbonate chemistry parameter values, https://doi.org/10.1594/PANGAEA.966668 (Gutiérrez-Zárate et al., 2024b); prokariotic abundances, https://doi.org/10.1594/PANGAEA.966611 (Gutiérrez-Zárate et al., 2024d); water physico-chemical variables, https://doi.org/10.1594/PANGAEA.966673 (Gutiérrez-Zárate et al., 2024f) recorded over the experiment. Additionally, 24-h water parameter measurement from a single day, https://doi.org/10.1594/PANGAEA.966672 (Gutiérrez-Zárate et al., 2024e), and monthly 24-h water parameter measurements, https://doi.org/10.1594/PANGAEA.973983 (Gutiérrez-Zárate et al., 2024c), both registered by the open-source controllers.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix A. Script Description and Usage Guide to Set Up the Open-Source Controllers.

Appendix B. Supplementary Figures and Tables.

Figure B1. Temperature probes adapted for waterproof conditions.

Figure B2. Example of seawater parameters recorded by the controllers over 24 h for each of the eight header tanks (T1–T8, see Figure 1 in main manuscript) from June 2022 to February 2023.

Table B1. Dissolved inorganic nutrient concentrations in the experimental aquaria for each treatment over the experimental time.

Table B2. Prokaryotic abundances in the input water, reservoir tank, and experimental aquaria for each treatment.

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