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Effects of pH-equilibrated ocean alkalinization on macro-benthic calcifiers: A mesocosm study from the port of La Spezia (Italy)

Davide Calvi[a,*], Sara Groppelli[a], Federico Comazzi[b], Samira Jamali Alamooti[d], Arianna Azzellino[c], Piero Macchi[d], Daniela Basso[a]

[a] Department of Earth and Environmental Sciences (DISAT), University of Milano-Bicocca, Piazza della Scienza, 20126, Milano, Italy
[b] Limenet s.r.l., 23851, Galbiate, LC, Italy
[c] Civil and Environmental Engineering Department (DICA), Politecnico di Milano, Piazza Leonardo da Vinci 32, 20133, Milano, Italy
[d] Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Via Luigi Mancinelli 7, 20131, Milano, Italy

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ABSTRACT

Ocean alkalinity enhancement (OAE) is emerging as a promising carbon dioxide removal (CDR) strategy, yet its ecological implications for benthic communities remain poorly understood. This study presents the first experimental evaluation of pH-equilibrated ocean alkalinization ($pH_{eq}OA$), a technique designed to produce bicarbonate-enriched seawater without increasing pH, and investigates whether increasing alkalinity levels affect the recruitment, growth and biomass of macro-benthic calcifiers (MBCs), as well as whether excessive alkalinization can trigger abiotic carbonate precipitation with negative ecological consequences. A mesocosm experiment was conducted in the port area of La Spezia (Liguria, Italy) using five alkalinization levels (Control, Low, Medium, High, and Oversaturated), and limestone settlement plates were deployed to quantify recruitment, growth, surface cover, and biomass of MBCs. Colonization was dominated by the polychaete *Serpula* sp. Abundance and growth-related metrics did not differ significantly among the Control, Low, Medium, and High treatments, with maximum values generally observed under Medium and High alkalinization level. In contrast, the Oversaturated treatment exhibited negligible recruitment and growth, driven by extensive abiotic calcium carbonate precipitation on settlement surfaces. These results indicate that alkalinity enhancement doesn't affect the performance of MBCs when maintained below a system-specific saturation threshold, whereas excessive alkalinization suppresses settlement and growth. Overall, $pH_{eq}OA$ appears ecologically compatible with local macro-benthic communities within an optimal alkalinization range, underscoring the importance of defining site-specific saturation thresholds that balance carbon removal efficiency with ecological integrity when implementing OAE technologies.

1. Introduction

Addressing climate change effectively requires not only effective rapid reductions in greenhouse gas emissions but also the large-scale removal of CO_2 from the atmosphere. Carbon Dioxide Removal (CDR) strategies are essential for extracting significant quantities of CO_2 and securely storing it in long-term reservoirs for millennia (Rogelj et al., 2018; IPCC, 2022). However, only a few CDR approaches have the potential to operate at a globally relevant scale, and all of them carry possible environmental consequences for the Earth system (Fuss et al., 2018). One such approach, Accelerated Weathering of Limestone (AWL), including ocean liming, involves the reaction of CO_2 with seawater and calcium carbonate ($CaCO_3$), forming bicarbonate ions (HCO_3^-), which are then released into the ocean (Rau and Caldeira, 1999; Caldeira and Rau, 2000). A key challenge in AWL is preventing the re-release of CO_2 into the atmosphere due to both abiotic and biotic precipitation of carbonate minerals such as calcite and aragonite, counteracting storage efforts (Suitner et al., 2024; Riebesell et al., 2023). Abiotic precipitation may occur under supersaturated conditions (Moras et al., 2022), while biotic precipitation may be enhanced by alkalinity increase (Bach et al., 2019). Research also points to potential issues related to sudden pH increase and chemical imbalance caused by

* Corresponding author.
E-mail addresses: d.calvi2@campus.unimib.it (D. Calvi), s.groppelli1@campus.unimib.it (S. Groppelli), federico.comazzi@limenet.tech (F. Comazzi), samira.jamali@polimi.it (S.J. Alamooti), arianna.azzellino@polimi.it (A. Azzellino), piero.macchi@polimi.it (P. Macchi), daniela.basso@unimib.it (D. Basso).

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conventional ocean liming, underscoring the necessity of pH-adjusted solutions to mitigate undesirable reactions (Iglesias-Rodríguez et al., 2023). A refinement of this method, known as Buffered Accelerated Weathering of Limestone (BAWL), introduces an excess of CO_2 and calcium hydroxide ($Ca(OH)_2$, or slaked lime) to generate a pH-stabilized bicarbonate solution (Caserini et al., 2021; De Marco et al., 2023). Investigations into the technical viability of this approach have contributed to the development of the Limenet® process (Limenet, 2024), an innovative pH-equilibrated ocean alkalinization ($pH_{eq}OA$) method. $pH_{eq}OA$ technology directly combines CO_2 with $Ca(OH)_2$ and seawater in closed reactors to produce a bicarbonate-enriched solution with controlled seawater pH and increased alkalinity. The term "pH-equilibrated" refers to the preparation process of the bicarbonate-enriched seawater, in which the outlet pH is adjusted to closely match ambient seawater conditions, thereby minimizing the initial carbonate chemistry perturbation relative to direct liming approaches and enabling the ecological effects of alkalinity enhancement to be evaluated independently of abrupt pH changes. This approach enhances the ocean's ability to sequester carbon while minimizing the risk of CO_2 re-release and simultaneously counteracting ocean acidification (Varliero et al., 2024). Moreover, recent pilot experiments have tested the stability of bicarbonate-enriched seawater produced through this process. In natural seawater monitored over three months, CO_2 remained stably sequestered up to a carbon addition of 1500 μmol/L, corresponding to a total DIC of approximately 3800 μmol/L (Varliero et al., 2024).

The application of $pH_{eq}OA$ could have significant implications for benthic organisms, which play a fundamental role in marine ecosystems by contributing to nutrient cycling (Covich et al., n.d.; Stief, 2013), sediment stabilization (Montserrat et al., 2008; Probert, 1984), and overall biodiversity (Snelgrove, 1997). Unlike conventional ocean liming methods, $pH_{eq}OA$ does not alter pH but increases alkalinity, potentially affecting calcifying organisms (Gim et al., 2018). Changes in alkalinity may impact biomineralization processes (Kempe and Kazmierezak, 1994), metabolic rates (Marín-Samper et al., 2024), and the availability of dissolved inorganic carbon (Krumins et al., 2013), potentially generating cascading effects on marine communities, food-web structure and ecosystem functioning when alkalinity enhancement exceeds site-specific operational thresholds, as suggested by recent OAE mesocosm studies on phytoplankton and planktonic ecosystems (Ferderer et al., 2022; Sánchez et al., 2024; Riebesell et al., 2023). Assessing these effects is crucial to understand the broader ecological consequences of large-scale ocean alkalinity enhancement.

To evaluate the ecological compatibility of $pH_{eq}OA$, a mesocosm experiment was carried out in the Gulf of La Spezia (Liguria, Italy) to investigate the response of local MBCs across a gradient of alkalinity enhancement. Specifically, the study aimed to determine whether increasing concentrations of bicarbonate-enriched seawater influence recruitment, growth, surface cover and biomass of benthic calcifiers, and whether excessive alkalinity enhancement promotes abiotic carbonate precipitation capable of negatively affecting benthic colonization. This research represents the initial ecological assessment of $pH_{eq}OA$ within a broader experimental framework designed to evaluate the environmental implications of this CDR technology.

2. Methods

2.1. Study site and experimental design

The Gulf of La Spezia is a semi-enclosed basin characterized by a complex hydrographic system influenced by seasonal variability, freshwater inflows, and human activities (Rossi et al., 2021). Seawater temperature ranges from 12 to 14 °C in winter to 22–26 °C in summer, while conductivity averages 50–55 mS cm^{-1}, decreasing nearshore due to river inputs. pH values (7.8–8.2) indicate a slightly alkaline environment, and dissolved oxygen is generally 80–100% saturated, though localized declines may occur in poorly mixed areas. Nutrient concentrations fluctuate, with nitrate at 1–5 μM, nitrite <1 μM, phosphate 0.1–0.5 μM, and silicic acid 1–10 μM, often elevated near riverine sources (Ciuffardi et al., 2013).

Geographically, the Gulf extends between Punta Bianca to the east and the islands of Tino and Palmaria to the west, partially enclosed by a 2.2 km dam. The maximum depth is ~12 m, and the average water residence time is about three weeks, though greater exchange occurs in the western sector through the Portovenere channel and the dam opening (Gasparini et al., 2009).

The area hosts intense anthropogenic activity, including shipping, aquaculture, military use, and tourism. Mussel farming, a long-standing economic practice, significantly influences nutrient dynamics and benthic ecosystems (Ferreira et al., 2018). While it can alter seafloor communities, it also provides habitat for diverse marine organisms associated with both floating and fixed structures (Bridger et al., 2022; McKindsey et al., 2011).

The mesocosm experiment was conducted at Porto Mirabello (44°06′04.7"N, 9°49′40.6″E) in La Spezia, Italy. The experimental setup included fifteen land-based mesocosms, housed under a transparent cover designed to maintain a controlled environment, by minimizing external influences such as temperature fluctuations, light variation, and contamination (Alexander et al., 2016; Groppelli et al., 2026; Jamali Alamooti et al., 2026). The mesocosms consisted of 1000 L transparent LDPE bags, randomly arranged to ensure uniform light and temperature exposure and to facilitate sampling and maintenance. Groups of three were placed in containment pools (P1-P5) filled with seawater, which functioned as thermal buffers (Fig. 1). To avoid overheating, the pool water was renewed frequently, while salinity inside the bags was regulated against evaporation by adding distilled water, especially during the final experimental stages, when higher temperatures were recorded.

The mesocosms were filled on March 14, 2024, using a submersible

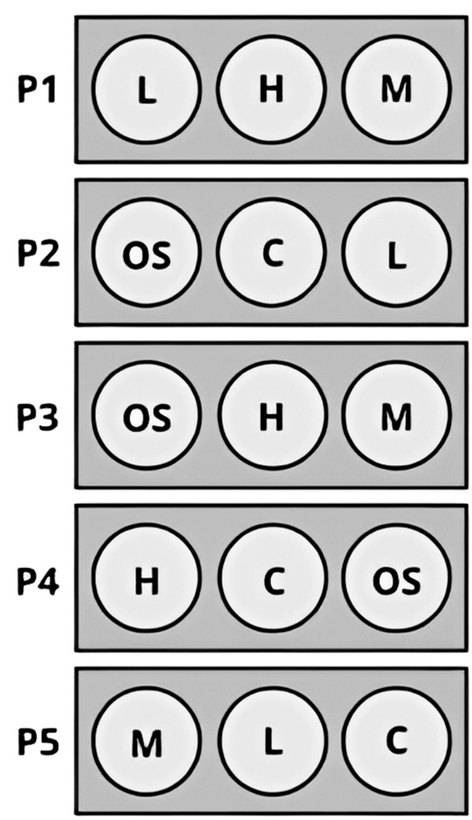

Fig. 1. Scheme of the experimental design. Control (C): P2C, P4C, P5C. Low (L): P1L, P2L, P5L. Medium (M): P1M, P3M, P5M. High (H): P1H, P3H, P4H. Oversaturated (OS): P2OS, P3OS, P4OS.

pump equipped with a flow meter, by diluting natural seawater with varying proportions of bicarbonate-enriched seawater (outlet solution), both sourced from the same spot in Porto Mirabello.

Five experimental conditions were established, ranging from an untreated control to an oversaturated level (Table 1), with progressive increases in dissolved inorganic carbon (DIC), alkalinity, and aragonite saturation state ($\Omega_{aragonite}$) (Groppelli et al., 2026; Jamali Alamooti et al., 2026).

The required amounts of bicarbonate-enriched seawater and the resulting DIC and $\Omega_{aragonite}$ values were calculated with CO_2SYS (Excel Macro v2.5; Pierrot et al., 2006), using seawater properties from La Spezia. Calculations were based on carbonate system equilibrium constants from Mehrbach et al. (1973), refitted by Dickson and Millero (1987), with corrections for $KHSO_4$ (Dickson, 1990) and total borate concentration (Uppström, 1974). Each condition was replicated three times (Groppelli et al., 2026; Jamali Alamooti et al., 2026).

$\Omega_{aragonite}$, defined as the ratio between $[Ca^{2+}][CO_3^{2-}]$ and the aragonite solubility constant (Zeebe and Wolf-Gladrow, 2001), is a key parameter used to evaluate the likelihood of carbonate mineral precipitation. Previous experimental studies have shown that the risk of abiotic $CaCO_3$ precipitation increases progressively with increasing saturation state, with substantial precipitation observed at $\Omega_{aragonite}$ values above approximately 7 under some experimental conditions (Moras et al., 2022). In the present study, $\Omega_{aragonite} \approx 5$ was not considered a site-specific precipitation threshold for the Gulf of La Spezia, but rather a conservative reference level approaching the precipitation-risk range. Accordingly, the treatment gradient was designed to span natural conditions (Control), moderate alkalinity enhancement levels below the precipitation-risk range (Low and Medium), a treatment approaching this conservative reference level (High), and an oversaturated worst-case scenario (Oversaturated) intended to evaluate the consequences of excessive alkalinization.

2.2. Environmental parameters

During the first two weeks of the experiment (March 14–28, 2024), temperature, pH, conductivity, and dissolved oxygen (DO) were measured three times per day with a Revio Giorgio Bormac multiparameter probe (Groppelli et al., 2026). Starting from March 28 (day 15), measurements were taken approximately every two weeks due to logistical constraints. However, the sampling frequency was increased during the final days of May due to the sudden and pronounced rise in temperature. The pH sensor (precision ± 0.02) was calibrated with three reference points (4.01, 7.00, 10.01) across its 0–14 range. The conductivity sensor (accuracy $\pm 2\%$ f.s.) was calibrated at 1413 μS and 12.88 mS. DO was assessed via an ORP sensor with 1 mV resolution, spanning -1000 to $+1900$ mV, and calibrated at 100% saturation. To ensure consistency, the probe was recalibrated prior to each sampling.

Alkalinity was determined using an automatic titrator (Hanna Instruments HI84531). The acid pump was calibrated before each session, and the integrated pH electrode was recalibrated every two weeks with certified buffers. Measurements were taken every two days from March 14 to 28, weekly until April 10 (day 27), and biweekly thereafter, all conducted at the Polytechnic University of Milan. Total alkalinity (TA) of natural seawater was defined as the control (TAC). Elevated TA levels in the treatments (TAT), resulting from $Ca(OH)_2$ additions, were expressed relative to the control as TAT – TAC (Jamali Alamooti et al., 2026).

Table 1
Volume of treated seawater over the total 1000 L volume, Total Alkalinity (TA) concentrations and the aragonite saturation state ($\Omega_{aragonite}$) for the 5 different treatments. C = control; L = low; M = medium; H = high; OS = Oversaturated.

Treatment	Treated volume (L - %)	TA addition (μeq L^{-1})	$\Omega_{aragonite}$
Control	0–0	0	3.4
Low	33–3.3%	262	3.9
Medium	67–6.7%	524	4.3
High	133–13.3%	1048	5.2
Oversaturated	263–26.3%	2096	7.1

Water samples from mesocosms were analyzed for ammonium nitrogen (NH_4^+-N) and orthophosphate phosphorus (PO_4^{3-}-P) approximately every two weeks. Ammonium was measured with Hach spectrophotometric kits after chloride removal using Ag_2O pretreatment. Orthophosphate was determined by the molybdenum blue method (Murphy and Riley, 1962), where phosphate forms a blue complex measured at 882 nm, suitable for concentrations between 0.001 and 0.3 mg L^{-1}. During the entire experiment, no nutrients were added to the mesocosms.

2.3. Sampling procedure: Counts and measurements

Each mesocosm contained six Perlato limestone plates, used as standardized substrates for colonization. Plates were suspended by nylon threads from a tubular structure, allowing colonization on both sides and preventing particle accumulation, while ensuring consistent exposure to environmental conditions across replicates. MBCs that colonized the limestone plates were sampled in two steps, three plates for each mesocosm per step, to assess ecological responses over time. The first sampling, conducted after 50 days, assessed early recruitment and settlement of MBCs, while the second and final sampling after 79 days evaluated subsequent growth and survival under prolonged exposure to the treatments. This duration was considered sufficient to detect early ecological responses while maintaining stable mesocosm conditions, as the progressive seasonal warming made longer experiments increasingly difficult to manage despite frequent water renewal in the surrounding pools. Immediately after removal, each plate was photographed on both sides, then hung on a suitable structure to dry, allowing for later counts under a stereomicroscope. At a later stage, length, surface area and percent cover of each organism were measured by analyzing the photographs of each plate face with the software ImageJ, while the mass of the MBCs was estimated by weighing a specimen of known surface area and applying proportional calculations to all the others. Estimating the mass of MBCs allows a quantitative assessment of biotic carbonate precipitation, providing an indirect measure of biomineralization under the different experimental conditions. Within the context of Ocean Alkalinity Enhancement (OAE) experiments, this metric could be particularly important for evaluating how increased alkalinity influences biologically mediated carbonate formation and its potential role in carbon sequestration.

2.4. Statistical analysis

Abiotic parameters (temperature, NH_4^+-N and PO_4^{3-}-P) were analyzed using two-way ANOVA, with treatment and experimental day as fixed factors. For biotic parameters, a preliminary correlation analysis was performed on abundance, length, surface area (including both surfaces s1 and s2, as well as total surface), percent cover, and biomass. Pearson's correlation coefficient was applied to identify significant correlations. A multivariate general linear model (Willig et al., 1986) analysis was performed utilizing abundance, length, surface area, percent cover, and biomass, as dependent variables. Treatment conditions, including Control, Low, Medium, High, and Oversaturated levels, and sampling time points were designated as independent factors in this analysis. Wilks' Lambda results were used to interpret the outcomes of the MANOVA. This multivariate statistic provides an overview of how the combined dependent variables are affected by the experimental factors, allowing us to assess the significance of group differences prior to examining the between-subjects effects in detail. The MANOVA results were subsequently confirmed using univariate ANOVA. A Tamhane test (Tamhane, 1979), commonly employed when group variances are unequal, which provides pairwise comparisons between group means while controlling for Type I error, was used for multiple comparisons between treatments.

3. Results

3.1. Abiotic parameters

Temperatures fluctuated between 15 and 20 °C at the beginning of the experiment until day 20 (Fig. 2a). A significant increase occurred between days 20 and 25, reaching approximately 25 °C, followed by a gradual rise with intermittent drops. Around day 40, a sharp decline was observed due to reduced illumination caused by bad weather, before temperatures recovered and continued to increase. The highest values, close to 35 °C, were recorded between days 70 and 79. These fluctuations were mainly due to rising external temperatures, while regular water renewal in the surrounding pools helped mitigate excessive warming and maintain comparable thermal conditions among mesocosms. Temperature varied significantly over time as a consequence of seasonal warming (Table S6, $p < 0.001$), whereas no significant differences among treatments were detected ($p = 0.970$).

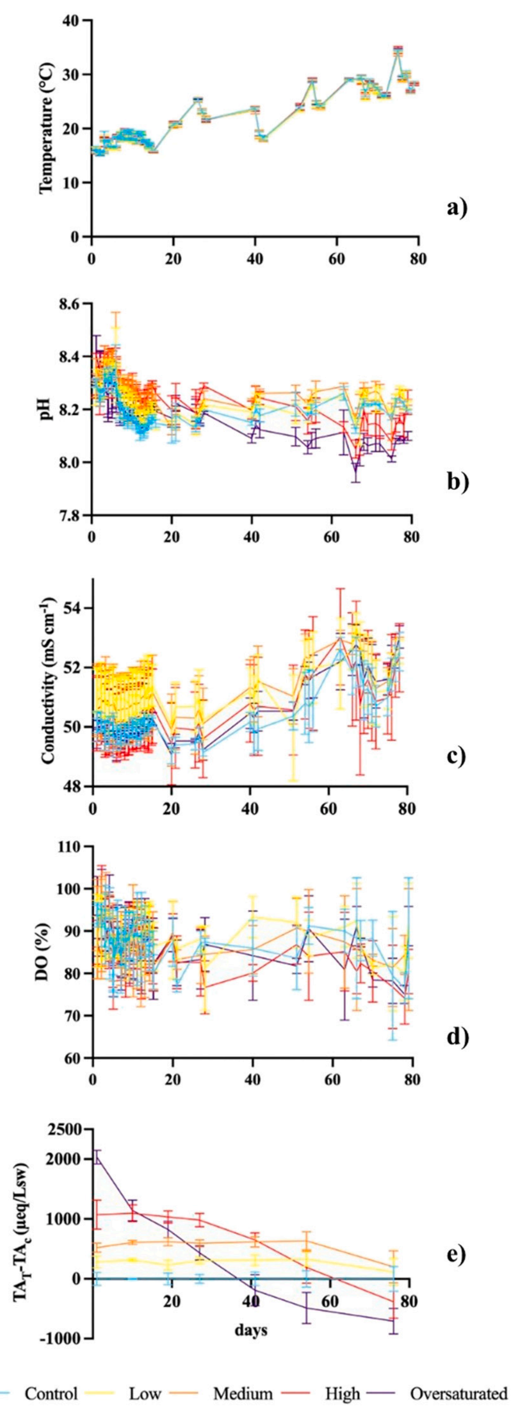

Fig. 2. Temporal variations of key environmental parameters over 79 days under the different alkalinization treatments: a) Temperature (°C); b) pH; c) Conductivity (mS cm^{-1}); d) Dissolved oxygen (% saturation); e) Total alkalinity differences between treated and control mesocosms (TA_T - TA_C).

pH initially increased, then stabilized around values between 8.1 and 8.3 (Fig. 2b). The different experimental conditions showed variations over time, with Oversaturated treatment displaying significantly lower pH values than Medium and High, while Low had a significantly lower pH than Medium but significantly higher than High.

Despite frequent additions of distilled water to keep salinity levels as stable as possible, conductivity (mS cm^{-1}) slightly increased over time, with all treatments starting at around 50 mS cm^{-1} and gradually rising, though with fluctuations due to rising temperatures and evaporation (Fig. 2c). In this case, the Low and Medium treatments maintained higher values, whereas the Control and Oversaturated treatments showed lower levels for most of the experimental period.

At the beginning of the experiment, dissolved oxygen (DO%) values for all treatments fluctuated between 80% and 100%, but on day 26, a sharp drop was recorded across all treatments (Fig. 2d). After this decrease, DO levels gradually recovered, with the Low and Medium treatments tending to maintain slightly higher values compared to the others, while the High and Oversaturated treatments consistently remained at lower levels.

The added TA of the Oversaturated and High treatments exhibited reductions of 134% and 135% relative to the initially added alkalinity, respectively (Fig. 2e). A decrease exceeding 100% indicates runaway precipitation of calcium carbonate, leading to a continued decline in TA to levels below those of the control seawater, resulting in net alkalinity loss (Moras et al., 2022). In contrast, the Low and Medium treatments showed negligible added TA reductions (approximately 4%) during the first 54 days of the experiment. However, from that point onward until the end of the experiment, these treatments experienced significant drops in added TA, 58% and 61%, respectively. This late-stage reduction is likely associated with the progressive temperature increase under the shelter (ranging from 15 °C to 36 °C between March and May), which decreased the solubility of calcium carbonate and promoted precipitation. A more detailed assessment of the carbonate chemistry dynamics of the same experimental system is provided by Jamali Alamooti et al. (2026).

Ultimately, mean NH_4^+-N and PO_4^{3-}-P concentrations remained around 78.8 and 5.4 µg L^{-1} (Table S1, S2), respectively, throughout the experiment. Two-way ANOVA revealed no significant treatment effects on either NH_4^+-N or PO_4^{3-}-P concentrations (Table S7), indicating comparable nutrient conditions among treatments.

3.2. Benthos response

In both sampling events, four distinct macro-benthic organisms were identified on the calcareous recruitment plates: two polychaete annelids belonging to the genera *Serpula* sp. (Fig. S1a) and *Janua* sp. (Fig. S1b), a bryozoan of the superfamily Buguloidea (Family Candidae) (Fig. S1c), and a barnacle of the family Balanidae (presumably *Amphibalanus amphitrite*) (Fig. S1d). Although some studies have reported benthic biodiversity in other Ligurian Sea areas, such as Portofino and the Gulf of Levanto (Vietti et al., 2010), information from the Gulf of La Spezia remains limited. Our observations revealed the widespread occurrence of these taxa, but only *Serpula* sp. was abundant enough to allow quantitative analyses. Community composition was strongly dominated by *Serpula* sp. across most treatments and sampling times, accounting for 76–96% of all recorded individuals at day 50 and 45–94% at day 79 (Fig. S2). In total, 332 individuals were recorded at day 50 and 349 at day 79. Settlement plates remained in good condition throughout the experiment, although those exposed to the Oversaturated treatment consistently exhibited a thin carbonate deposit resulting from abiotic calcium carbonate precipitation.

Recruitment of *Serpula* sp. was variable across treatments (Fig. 3a). At day 50, ten plates showed no colonization, including all nine plates from mesocosms P1L, P3OS, and P4OS, plus one plate from P1M. By day 79, eight plates remained uncolonized (one from P1L, one from P1M, and three from Oversaturated mesocosms). Abundance was generally low at day 50, and no significant differences were detected among treatments ($0.05 < p < 0.10$), consistent with an early colonization stage. Nevertheless, Oversaturated already showed the lowest values, with several plates completely devoid of individuals. Control mesocosms displayed relatively low variability, whereas Medium showed the widest

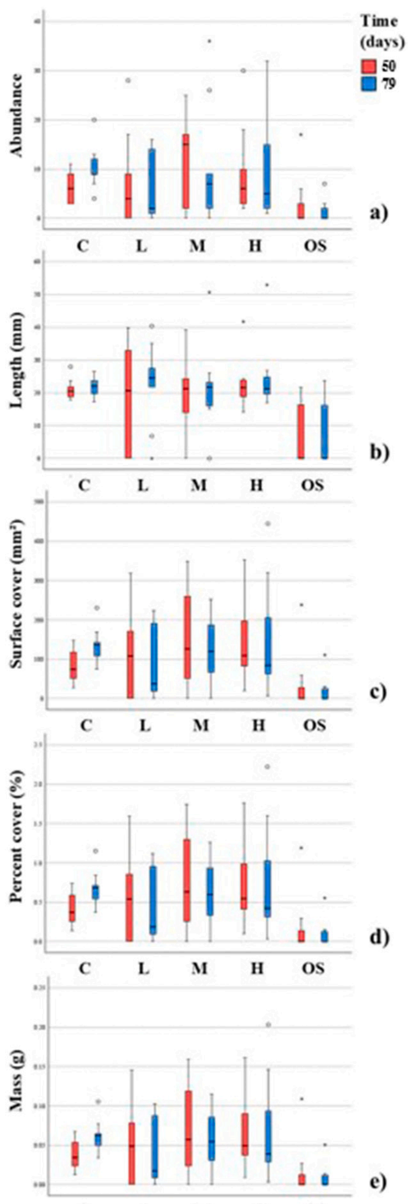

Fig. 3. Boxplots of (a) abundance, (b) length (mm), (c) surface cover (mm^2), (d) percent cover (%), and (e) biomass (g) across treatments (C, L, M, H, OS) at 50 (red) and 79 (blue) days. The central line in the boxes indicates the median, whiskers show data dispersion, and circles denote outliers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

range and the highest median. By day 79, differences were more pronounced, with significantly lower abundance in OS compared to Control ($p < 0.05$), while Medium and High supported higher numbers of recruits.

Patterns of individual length followed a similar trajectory (Fig. 3b). On day 50, significant differences were detected only between Low and Medium treatments ($p < 0.05$), with specimens in Medium being longer. By day 79, growth differences became more evident: individuals from Medium were significantly longer than those from all other treatments ($p < 0.05$), suggesting that this condition favored growth. The Low treatment also differed significantly from Control and High. Pairwise comparisons confirmed Medium as the most distinct treatment in terms of average length at the end of the experiment.

Both surface and percent cover, representing colony expansion, showed similar trends across treatments (Fig. 3c and d). At day 50, median surface cover ranged between 100 and 250 mm^2 in all treatments except Oversaturated, where values were near zero. By day 79, cover increased in all.

mesocosms except Oversaturated, with the highest medians in the Medium treatment (> 300 mm^2). Percent cover followed the same pattern, ranging from about 0.5 to 1.5% in Control, Medium, and High, and remaining close to zero in Oversaturated.

Biomass measurements provided an integrated perspective on both abundance and growth (Fig. 3e). Control, Medium, and High treatments showed comparable median masses (0.04–0.06 g), although variability was high, particularly in Medium and High. Low exhibited the widest dispersion, with both very low and relatively high values, while OS consistently displayed the lowest biomass, with median values close to zero and only a few outliers. This pattern confirmed the inhibitory effect of oversaturation on both settlement and growth.

Correlation analysis revealed strong associations among total length, mass, surface area, percent cover and organism count, supporting the use of MANOVA (Fig. 4 and Table S3). The multivariate test indicated a highly significant effect of treatment ($p < 0.001$), while neither sampling time nor treatment × time interaction were significant ($p > 0.50$) (Table 2).

When each variable was considered separately, consistent results emerged: total mass, surface area, and percent cover showed stronger treatment effects than length and abundance. Univariate ANOVA (Table S4) and a Tamhane post-hoc test (Table S5) confirmed these results for total mass, with Oversaturated differing significantly from all treatments except Low ($p = 0.12$).

4. Discussion

The physicochemical dynamics of the mesocosms were shaped by the interaction between seasonal environmental forcing and the imposed alkalinity treatments. The progressive rise in temperature throughout the experiment, consistent with seasonal warming, enhanced calcium carbonate precipitation (Mejri et al., 2014), thereby affecting both alkalinity and pH (Moras et al., 2022; Ruiz-Agudo et al., 2011). These conditions mimic those found in shallow and semi-enclosed port environments, where the $pH_{eq}OA$ technology is designed to operate and where strong thermal fluctuations, variable light availability, and limited water renewal naturally drive short-term changes in carbonate chemistry (Enochs et al., 2018; Hofmann et al., 2011) and oxygen conditions (Jarvis et al., 2023).

Alkalinity trends reflected these temperature-induced changes in carbonate solubility (Humphreys, 2024; Jamali Alamooti et al., 2026; Schulz et al., 2023). In the High and Oversaturated treatments, TA declined, and in the Oversaturated treatment even fell below initial seawater values, indicating runaway carbonate precipitation (Moras et al., 2022). Low and Medium treatments exhibited only a delayed and moderate reduction in TA, suggesting that moderate alkalinity enhancement can remain within the buffering capacity of the system. Temperature did not differ significantly among treatments throughout

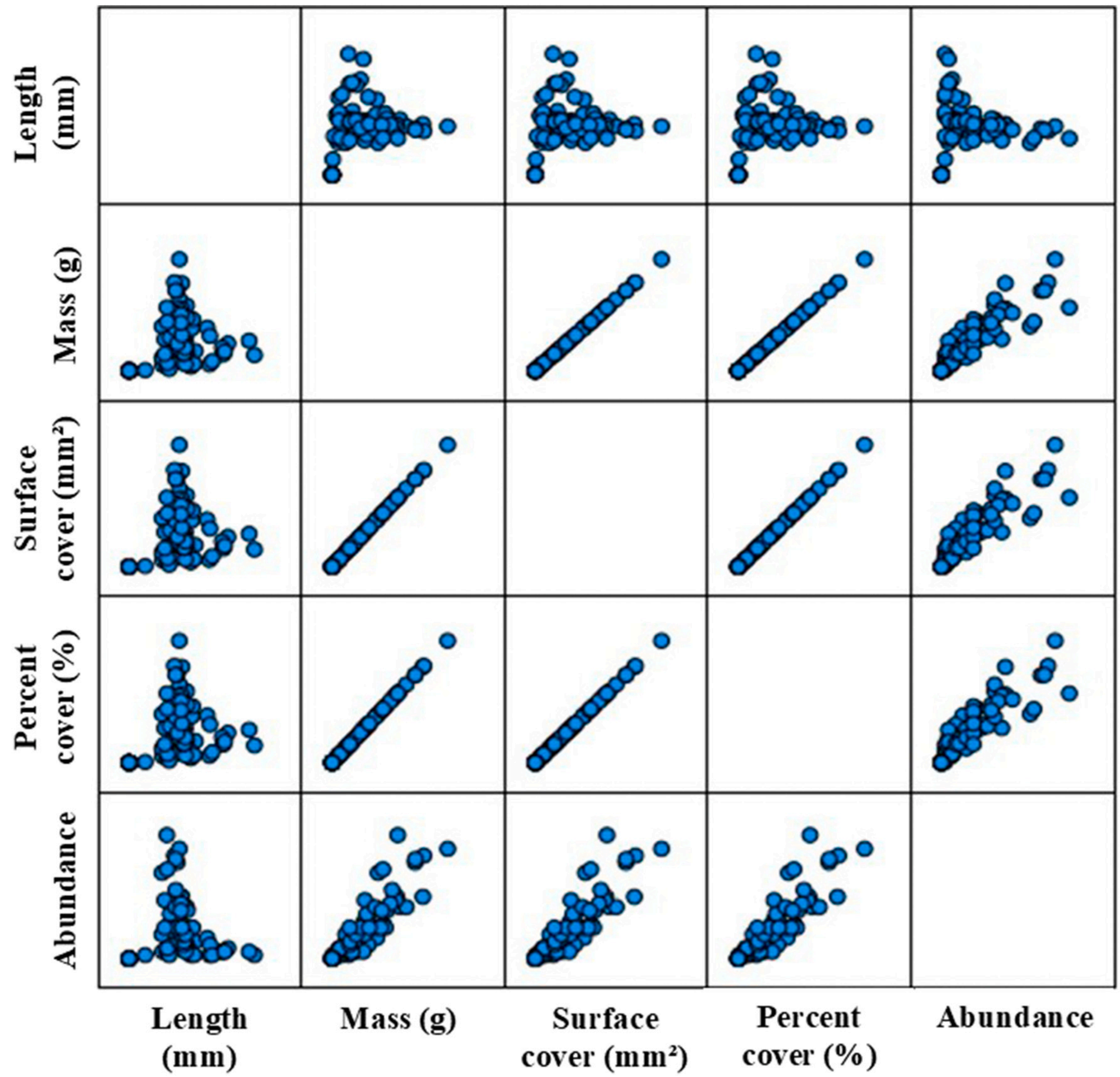

Fig. 4. Scatterplot matrix showing pairwise relationships among length (mm), mass (g), surface cover (mm^2), percent cover (%), and abundance. Each panel represents the bivariate distribution between two variables.

Table 2
Results of the multivariate analysis of variance (MANOVA) for the effects of Treatment, Sampling time, and their interaction (Treatment × Sampling time) on the dependent variables considered. Wilks' Lambda, F values, hypothesis and error degrees of freedom, and corresponding *p*-values are reported. Effects were considered significant at $p < 0.05$.

MANOVA						
Effect		Value	F	Hypothesis df	Error df	P-value
Intercept	Wilks' Lambda	0.417	36.276	3.000	78.0	<0.001
Treatment	Wilks' Lambda	0.587	3.847	12.000	206.7	<0.001
Sampling time	Wilks' Lambda	0.971	0.779	3.000	78.0	0.509
Treatment*Sampling	Wilks' Lambda	0.934	0.452	12.000	206.7	0.940

the experiment (Table S6), indicating that the observed treatment-specific responses cannot be attributed to differences in thermal conditions. Nevertheless, the progressive seasonal warming likely contributed to the enhanced carbonate precipitation observed during the final phase of the experiment by reducing $CaCO_3$ solubility. Corresponding shifts in pH supported this pattern: treatments with higher aragonite saturation ($\Omega_{aragonite}$) showed lower pH values, most likely due to CO_2 release associated with carbonate precipitation (Moras et al., 2022). These pH differences emerged progressively during the experiment and reflect treatment-specific carbonate chemistry dynamics, including carbonate precipitation, alkalinity loss and associated CO2 release, rather than differences in the initial preparation of the bicarbonate-enriched seawater. In the Oversaturated treatment, persistent precipitation and CO_2 degassing led to visible carbonate layer deposition on mesocosm surfaces and settlement plates, further destabilizing the chemical environment.

Conductivity and dissolved oxygen (DO) exhibited short-term variability linked to evaporation and changes in illumination. Despite this, the system showed notable resilience. Regular water renewal prevented extreme deviations and maintained physicochemical conditions within realistic coastal ranges. The temporary drop in DO following a period of low light was consistent with reduced photosynthetic activity rather than persistent hypoxia. Differences among treatments suggest that carbonate precipitation and associated CO_2 supersaturation influenced gas exchange processes (Bialik et al., 2022; Eisaman et al., 2023): in High and Oversaturated treatments, degassing and precipitation likely enhanced gas transfer and potentially stimulated microbial respiration, resulting in lower DO values. Conversely, the more moderate carbonate dynamics in Low and Medium treatments allowed DO to remain comparatively high.

Nutrient concentrations (NH_4^+-N and PO_4^{3-}-P) did not differ among treatments, demonstrating that the manipulations affected carbonate chemistry without altering nutrient availability. This ensures that biological responses observed in the experiment can be attributed to changes in carbonate equilibrium rather than trophic gradients.

The benthic response, particularly the settlement dynamics of *Serpula* sp., showed clear differences between the Oversaturated treatment and the others. During the first sampling, no statistically significant differences in abundance were observed, although a trend suggested early divergence. By day 79, abundance did not differ significantly among Control, Low, Medium, and High treatments, despite slightly higher values in Medium and High. The only treatment showing a significant difference was Oversaturated, as discussed below. These results indicate that Low-to-High bicarbonate-enriched conditions don't affect larval settlement and post-settlement success by increasing carbonate availability, thereby supporting biomineralization. This aligns with recent observations showing that moderate alkalinity enrichment can benefit calcifying organisms (Faucher et al., 2025). The variability in abundance within the Medium treatment suggests that settlement success may also depend on other unexplored biological or microhabitat factors.

In contrast, plates in the Oversaturated treatment showed almost no recruitment of *Serpula* sp. or other MBCs. This aligns with the extensive abiotic calcium carbonate precipitation occurring early in the experiment and persisting throughout. The formation of a carbonate layer likely created unsuitable settlement surfaces and may have altered the composition of microbial biofilms that provide settlement cues to larvae, as documented in other benthic invertebrates (Chase et al., 2016; Rippen et al., 2025). Excessive precipitation and the associated CO_2 release further decreased TA and pH, creating negative feedback that impaired settlement and survival. These outcomes demonstrate that extreme alkalinity conditions can shift the system from biologically favorable carbonate enrichment, where carbonate is incorporated into organismal skeletons, to chemically driven precipitation that suppresses benthic colonization and limits biogenic carbonate production.

In addition to abundance, size-related parameters including length, mass, surface area, and percent cover were measured and found to be closely correlated. Size estimates, particularly mass, integrate post-settlement growth and have already been used in calcifying organisms, such as foraminifera, to infer biologically mediated carbonate production (Barrett et al., 2023, 2025). In the context of OAE experiments, such metrics could be particularly informative when interpreted alongside TA, as biotic calcification converts alkalinity and inorganic carbon into solid carbonate (Bialik et al., 2022). At the same time, carbonate formation by *Serpula* sp. is associated with CO_2 release and alkalinity consumption, suggesting that biologically mediated precipitation could partially offset the intended effects of alkalinity enhancement (Bach et al., 2019). Although this contribution is likely small relative to bulk chemical fluxes, mass-based measurements could provide a cautious yet ecologically meaningful indication of benthic responses to altered carbonate chemistry and may help in evaluating the potential influence of biological calcification on OAE efficiency. In this experiment, the Medium and High treatments exhibited the highest mass values, despite the absence of statistically significant differences compared to the Control. This suggests that the surplus alkalinity may have been utilized by serpulids to increase tube size, an interpretation further supported by a similar trend observed in tube length. Accordingly, larger tube size may indicate increased biologically mediated carbonate production, although the magnitude of this contribution could not be quantified in the present study. From this perspective, the results indicate that below a certain threshold, represented here by the Oversaturated treatment, an increase in treatment concentration may lead to enhanced biogenic CO_2 production, thereby reducing the overall effectiveness of the treatment in coastal areas characterized by abundant calcifying benthic communities. However, the magnitude and direction of these effects are strongly context-dependent, highlighting the need for site-specific evaluations. Moreover, the relative contribution of biological calcification and abiotic carbonate precipitation to the overall carbon budget was not directly quantified. While abiotic precipitation was inferred from alkalinity loss and visible carbonate deposition, biological calcification was assessed only indirectly through organism growth and biomass-related metrics. Future studies combining carbonate chemistry mass balances with direct measurements of calcification rates will be necessary to quantify the relative importance of these processes and their implications for carbon removal efficiency.

Taken together, the results indicate that $pH_{eq}OA$ method does not negatively affect benthic settlement except under conditions of oversaturation. Moderate to high alkalinity enhancement promoted recruitment and growth of *Serpula* sp., while oversaturation produced the opposite effect. These findings stress the importance of optimizing alkalinity enhancement strategies to balance carbon sequestration efficiency with ecological sustainability. They also underline the need to avoid excessive supersaturation, which triggers abiotic carbonate precipitation, reduces the effectiveness of alkalinity addition, and may negatively affect benthic communities. Rather than identifying a universal $\Omega_{aragonite}$ threshold, the present results highlight the importance of defining site- and system-specific operational limits that minimize carbonate precipitation while maintaining ecological compatibility.

5. Conclusions

This study provides the first comprehensive ecological evaluation of the $pH_{eq}OA$ approach under controlled yet environmentally realistic port-mesocosm conditions, offering new insights into how alkalinity enhancement interacts with coastal carbonate chemistry and benthic communities. The results demonstrate that, within a system-specific saturation range, alkalinity enhancement does not affect the settlement and the growth of MBCs such as *Serpula* sp., likely by increasing bicarbonate availability for biomineralization. These findings indicate that $pH_{eq}OA$ can be ecologically compatible with biologically active coastal systems, where MBCs represent an important component of ecosystem functioning, provided that alkalinization remains below locally defined thresholds. Moreover, these findings should not be interpreted as defining a universal $\Omega_{aragonite}$ threshold, but rather as demonstrating that precipitation risk and ecological responses depend on local environmental conditions and saturation state.

In contrast, oversaturation led to runaway abiotic carbonate precipitation, rapid alkalinity consumption, CO_2 release, and the formation of unsuitable settlement substrates, ultimately suppressing MBCs recruitment. This shift from biologically mediated calcification to chemically driven precipitation highlights a critical threshold beyond which alkalinity enhancement becomes counterproductive, both by reducing the efficiency of carbon storage and by negatively affecting benthic ecosystem processes. Together, these results emphasize the importance of defining site-specific saturation thresholds that balance carbon removal efficiency with ecological integrity when implementing OAE technologies.

This experiment represents an important first step toward demonstrating the ecological feasibility of $pH_{eq}OA$ deployment in port environments; however, further research is required to refine operational thresholds across diverse coastal settings. Future studies extending over longer time scales will be necessary to evaluate chronic responses, community succession and potential delayed effects of alkalinity enhancement on benthic assemblages. In addition, the variability observed in recruitment within the Medium treatment indicates that settlement success may be influenced by additional biological interactions or microhabitat-scale factors that were not explicitly addressed in this study, highlighting the need for future investigations that integrate community-level processes, biofilm dynamics, and fine-scale habitat heterogeneity when assessing the ecological outcomes of ocean alkalinization. Addressing these knowledge gaps will be essential to optimize pH_{eq}OA strategies and to ensure that large-scale deployment maximizes carbon removal potential while safeguarding coastal benthic ecosystems.

CRediT authorship contribution statement

Davide Calvi: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Sara Groppelli:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Federico Comazzi:** Writing – review & editing, Investigation. **Samira Jamali Alamooti:** Writing – review & editing, Investigation. **Arianna Azzellino:** Writing – review & editing, Formal analysis, Data curation. **Piero Macchi:** Validation, Supervision, Resources. **Daniela Basso:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Davide Calvi reports financial support, administrative support, equipment, drugs, or supplies, and travel were provided by Limenet s.r.l. Sara Groppelli reports financial support, administrative support, equipment, drugs, or supplies, and travel were provided by Limenet s.r.l. Samira Jamali Alamooti reports financial support, administrative support, and equipment, drugs, or supplies were provided by Limenet s.r.l. Federico Comazzi reports financial support, administrative support, equipment, drugs, or supplies, and travel were provided by Limenet s.r.l. Davide Calvi reports a relationship with Limenet s.r.l. that includes: funding grants, non-financial support, and travel reimbursement. Sara Groppelli reports a relationship with Limenet s.r.l. that includes: funding grants, non-financial support, and travel reimbursement. Samira Jamali Alamooti reports a relationship with Limenet s.r.l. that includes: funding grants, non-financial support, and travel reimbursement. Federico Comazzi reports a relationship with Limenet s.r.l. that includes: employment, non-financial support, and travel reimbursement. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2026.120198.

Data availability

Data will be made available on request.

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Glossary

MBCs: Macro-Benthic Calcifiers
AWL: Accelerated Weathering of Limestone
BAWL: Buffered Accelerated Weathering of Limestone
CDR: Carbon Dioxide Removal
DIC: Dissolved Inorganic Carbon
DO: Dissolved Oxygen
GLM: General Linear Model
LDPE: Low-Density PolyEthylene
OAE: Ocean Alkalinity Enhancement
$pH_{eq}OA$: pH-equilibrated Ocean Alkalinization
TA: Total Alkalinity
TAC: Total Alkalinity Control
TAT: Total Alkalinity Treatment
$\Omega_{aragonite}$: Aragonite saturation state