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Effect of ocean acidification on the oxygen consumption of the sea urchins *Paracentrotus lividus* (Lamarck, 1816) and *Arbacia lixula* (Linnaeus, 1758) living in CO₂ natural gradients

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Ocean acidification (OA) stands out as one of the main threats to marine ecosystems. OA leads to a reduction in the availability of carbonate ions, which are essential for marine calcifiers such as echinoderms. We aim to understand the physiological responses of two sea urchin species, *Paracentrotus lividus* and *Arbacia lixula* to low pH conditions and determine whether their responses result from phenotypic plasticity or local adaptation. The study is divided into two parts: plasticity response over time, measuring respiration rates of individuals from the Mediterranean Sea exposed to low pH over seven days, and adaptation and plasticity under changing pH, analyzing individuals inhabiting a pH gradient in a natural CO₂ vent system located in La Palma Island, Spain. Over the seven days of low pH exposure, distinct patterns in respiration rates were revealed, with both species demonstrating potential for acclimatization. Notably, *P. lividus* and *A. lixula* displayed unsynchronized acidosis/alkalosis cycles, suggesting different physiological mechanisms. Additionally, environmental history seemed to influence adaptive capacity, as specimens from fluctuating pH environments exhibited respiration rates similar to those from stable environments with heightened phenotypic plasticity. Overall, our results suggest that both species possess the capacity for metabolic plasticity, which may enhance their resilience to future OA scenarios but likely involve energetic costs. Moreover, CO₂ vent systems may serve as OA refugia, facilitating long-term survival. Understanding the plastic responses versus adaptations is crucial for predicting the effects of OA on species distribution and abundance of marine organisms in response to ongoing climate change.

Introduction

The anthropogenic rise in atmospheric CO_2 leads to its absorption by the oceans resulting in the decreasing of seawater pH, known as ocean acidification (OA). Under current emissions, a drop in oceanic pH by 0.4 units is projected by the end of the 21st century compared to the current values (Allan et al., 2021). OA severely threatens marine ecosystems (Dupont and Pörtner, 2013), due to the reduction in the availability of carbonate ions, which affects marine calcifiers who use these minerals to synthesize carbonate biostructures. This vulnerability encompasses a broad range of taxa, including echinoderms (Byrne and Hernández, 2020). The consequences of vulnerable systems are disruptions to food webs, alteration of biodiversity on marine invertebrates, and impacts on the overall health of the oceans (Stumpp et al., 2013).

The sea urchins *Paracentrotus lividus* (Lamarck, 1816) and *Arbacia lixula* (Linnaeus, 1758) are calcifying species and key components in benthic communities of the Mediterranean Sea and northeast Atlantic due to their role in algae population regulation (Palacín et al., 1998). Within the Mediterranean, the combined grazing action at high densities promotes the formation of barren ground areas (Bonaviri et al., 2011; Agnetta et al., 2015), making changes in their abundance a concern for coastal conservation (Vergés et al., 2014). Studying their adaptability to overcome OA is essential to predict potential impacts on shallow Atlantic-Mediterranean waters.

Seawater pH affects the metabolism of the organisms by altering biochemical pathways (Dupont et al., 2010). External rising CO_2 concentration can diffuse into body tissues causing hypercapnia and acidosis (a decreased internal pH) (Collard et al., 2013) which disrupt physiological processes such as calcification, nutrition, and metabolism (Melzner et al., 2009; Byrne and Fitzer, 2019; Pörtner, 2008). Understanding metabolic responses is necessary for assessing the species survival, health, and fitness under environmental challenges (Brown et al., 2004; Calosi et al., 2013). Stabilized metabolic rates can occur via adaptation (selecting genotypes), or acclimatization (phenotypic plasticity) (Norin and Metcalfe, 2019). However, physiological plasticity and adaptation to low pH in marine organisms remain poorly understood (Calosi et al., 2013; Di Giglio et al., 2020). Both *P. lividus* and *A. lixula* have a likely efficient pH compensation system, essential for survival in acidified areas. However, *P. lividus* is less effective in regulating extracellular acid-base balance than *A. lixula* (Calosi et al., 2013). Nevertheless, this buffering ability may involve an energy reallocation (Catarino et al., 2012), making it essential to study these hidden physiological responses for predicting their long-term viability and ecosystem dynamics.

Much of our knowledge of OA effects mainly comes from short to medium-term laboratory experiments (e.g. Stumpp et al., 2012; Spicer et al., 2011; Wood et al., 2008), but long-term exposure often reveals more complex responses. Long-term studies on tropical sea urchins, particularly those in the *Echinometra* genus, showed varied OA responses, depending on life stage and environmental context (Uthicke et al., 2020, 2021; Karelitz et al., 2019). Moreover, studies on natural CO_2 emission points, also known as CO_2 vents, such as those at Ischia, Papua New Guinea and Canary Island, show that some populations of sea urchins exhibit adaptations to high CO_2 environments, with adults demonstrating resilience (Foo et al., 2022; Di Giglio et al., 2020; Uthicke et al., 2016, 2019; González-Delgado et al., 2024). In this sense, marine CO_2 vents, which create natural pH gradients, offer valuable scenarios to study the long-term effects of OA on marine ecosystems (González-Delgado and Hernández, 2018; González-Delgado et al., 2023), allowing scientists to investigate the biological responses of organisms, as well as their adaptive potential and resilience (Hofmann et al., 2014).

The Canary Islands, situated in the northeastern Atlantic Ocean, are home to the only subtropical marine CO_2 vent under investigation (Hernández et al., 2024). La Palma Island presents a continuous degassing of CO_2 in the south, in Fuencaliente (Figure 1) (Carracedo et al., 2001; Padrón et al., 2015). The features of the natural CO_2 vent include a pH gradient that decreases from 8.1 at open sea (150m from the vent center) to less than 7.4 at the source during low tide (González-Delgado et al., 2021), allowing us to test the effect of decreasing pH on marine organisms in a situation analogous to future oceans.

This study investigates how the metabolic rates vary in two calcifying echinoderms, the sea urchins *P. lividus* and *A. lixula*, under different pH conditions during experiments in a laboratory and along a naturally acidified area. The main goal is to better understand their physiological responses to OA conditions predicted for the near future and whether those responses are a result of large phenotypic plasticity or local adaptation to long-term exposure to low pH. Specifically, we measured metabolic responses by analyzing respiration rates (VO_2 consumption) of organisms under experimental pH changes to assess their innate plasticity to pH shifts. Additionally, we compared organisms from natural CO_2 vent systems (pH ~7.5) and ambient sites (pH ~8.1) to identify potential adaptive differences between them. We expected organisms from more stable environments to be more affected by environmental changes, while those accustomed to greater environmental variability may respond more effectively, having adapted to wider fluctuations in abiotic factors (Asnicar et al., 2021). It is critical to have a clear understanding of when plastic responses occur instead of genetic adaptations and vice versa, as this knowledge is essential for predicting how OA affects the distribution and abundance of species and thus predicting the responses of marine organisms to ongoing climate change.

Materials and methods

Our study is divided into two different parts. For investigating the plasticity response over time experiments were performed with individuals collected from ambient sites at pH 8.1 to measure their plasticity responses to low pH conditions over seven–days. Adaptation and plasticity under changing pH was analyzed using individuals living along a pH gradient from a natural CO_2 vent

system. Each part of the study was performed in a different laboratory and locality due to infrastructure and field requirements.

Plasticity response over time

To measure metabolic responses to experimental changes of pH, we collected individuals by freediving at 0.5–5 m depth at Cala Sant Francesc in Blanes (NW Mediterranean) (see Supplementary Table S1). All specimens collected were adults with volumes between 16.72–38.6 mL in *P. lividus* and 8.55–27.76 mL in *A. lixula*. Twelve individuals of *P. lividus* were collected in February 2024, six specimens were used for control conditions (individuals maintained at the same collection chemical parameters) and six specimens for experimental conditions (individuals collected and exposed to low pH)(Supplementary Table S2 for chemical parameters). *For A.lixula* six specimens were collected in December 2023 for control conditions and six in May 2024 for experimental conditions (Supplementary Table S3 for chemical parameters). The individuals maintained at the collected parameters (control condition) were used to control the housing effect. All experiments were conducted at the University of Barcelona. Seawater pH at the sampling point was measured with the multiparametric sensor AAQ-RINKO by JFE Advantech Co., Ltd. Experiments were conducted separately for both species, following the same protocol.

Once in the laboratory, animals were acclimatized in a 25L settling tank. After 48 hours, they were transferred to experimental tanks (125L), containing individual 10L tanks for each replicate. Then, they were exposed to either control or experimental low-pH conditions for seven days as explained above. Oxygen consumption (VO_2 consumption) of each specimen was measured before placing it in the experimental tank (Day 0) and after 24h (Day 1), 48h (Day 2), 72h (Day 3), and 168h (Day 7) of the experiment. Animals were allowed to feed over the rocks placed at the bottom of the tank throughout the experiments. However, before each VO_2 measurement of the corresponding days, animals were starved for 12h to ensure a similar physiological status for all of them (Grosjean, 1998; Shpigel et al., 2004). Values of pH were achieved using a pH-controlling setup (Milwaukee PRO Digital controller) connected to a pH electrode which regulated pH by bubbling CO_2 gas into the seawater to maintain or reach the desired pH 7.5. Dissolved oxygen (DO) levels were maintained by the simultaneously bubbling air. Alkalinity was also checked using a SERA KH test to keep it constant at 11–14 dKH by adding Reef Carbonate (4,000 meq/L) from Seachem when necessary. Temperature was experimentally maintained at the temperature of the collection day (16°C±1°C) using a cooler (HAILEA HS28A).

Respiration rate (VO_2; mg/kg/h) was independently measured per specimen using an intermittent-flow respirometry system (Q-box mini-AQUA aquatic respirometer, Qubit Systems) with the Logger Pro 3.16.1 software from Vernier Software Technology. A respirometer chamber was placed in a 20L aquarium equipped with a pH-controlling setup connected to a pH electrode to control the desired pH and a cooler to maintain a constant temperature. The total volume of the respirometry setup was 465ml. Once introduced

into the chamber, the specimens were allowed 5 minutes to acclimate as performed by Christensen et al. (2011). The decreasing DO levels were measured with three cycles of oxygen consumption consisting of a 5-minute flush phase, 7-minute rest phase, and 10-minutes of closed circulation. A run without specimen (blank run) was performed before each condition to control the background VO_2 consumption from microorganisms. Each specimen was weighed (g), and the height (mm) and the width (mm) were measured to calculate its volume (mL) using the Elliott et al. (2012) formula:

$$\frac{4}{3}\pi \times \left(\frac{1}{2}Diameter\right)^2 \times \frac{1}{2}Height \qquad .$$

The respiration rate for each specimen was calculated with the difference between final VO_2 and background VO_2 (blank run).

Adaptation and rapid response under natural pH gradients

To determine potential differences in metabolic rates for individuals that naturally live under different pH values, we collected adult individuals of both species in La Palma (Canary Islands, Spain) in four different places in January 2024 (see Supplementary Table S1). We sampled at two "Ambient" sites (pH 8.1) at Muelle del Faro (A1) for *P. lividus* and La Bajita (A2) for both species, and two CO_2 "Vent" sites at Playa del Faro (V1) for both species with a pH fluctuation between 8.01–7.4, and Las Cabras (V2) for *A. lixula*, with a pH fluctuation of 8.0–7.7 (See Figure 1, Supplementary Table S1) (González-Delgado et al., 2021; Hernández et al., 2016). Specimens were collected following the same criteria applied before. There were some differences in the sampling scheme and experimental design between species due to their different abundances at the sampling points. The analyses were conducted at the Observatorio Marino de Cambio Climático (OMACC) in Fuencaliente, La Palma Island.

The experiments were conducted using freshly collected seawater from the sampling points keeping the variables salinity and temperature constant (see Supplementary Tables S2, S3). The temperature, salinity, and pH were measured at each sampling point using a multiparametric sensor (AAQ-RINKO by JFE Advantech Co., Ltd.) and specimens were acclimatized at the same seawater parameters as the collection site. Unfortunately, due to infrastructure limitations, experiments at the OMACC had to be limited to a 24-hour acclimatization. After acclimatization in the settling tank (25L), the specimens were transferred to an experimental tank (125L), with individual tanks (10L) inside for each replicate. Following a 24-hour exposure period to the conditions described below, the VO_2 consumption was measured for each specimen after 12h of starvation and following the same protocol previously detailed.

To investigate if metabolic rates vary due to local adaptation to pH, we tested individuals from the sampling sites under their natural conditions, hereafter basal conditions. For *P. lividus* (N=24), we collected eight samples from each of the three locations: Ambient 1 (A1A, pH = 8.1), Ambient 2 (A2A, pH = 8.1),
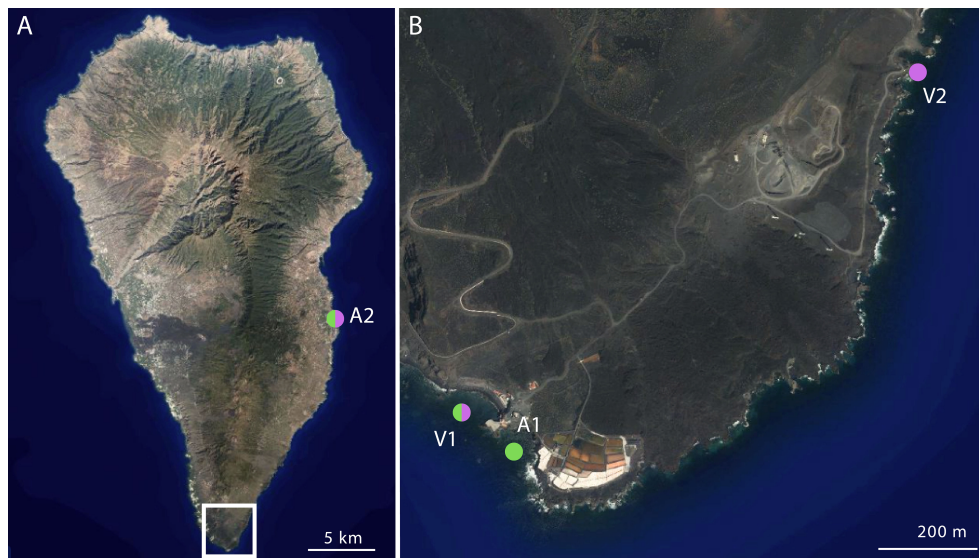
and Vent 1 (V1V, pH = 7.5). For *A. lixula* (N=24), we took eight samples from three locations as well: Ambient 2 (A2A, pH = 8.1), Vent 1 (V1V, pH = 7.5), and Vent 2 (V2V, pH = 7.8). *A. lixula* was not present in the Ambient 1 site, thus we collected only ambient specimens from La Bajita (A2). Therefore, achieving equivalent sampling points for both species was not feasible, although the design was maintained as initially planned.

Finally, to determine whether specimens which are geographically closely related but living under different pH conditions do display different immediate responses to pH changes, we experimentally varied pH values in the laboratory, hereafter experimental conditions. In *P. lividus*, eight specimens from the Vent 1 were exposed to ambient pH (V1A: pH=8.1), and eight specimens from Ambient 2 were exposed to low pH (A2V: pH=7.5). In *A. lixula*, eight specimens from Ambient 2 were exposed to low pH (A2V: pH= 7.5). The abundance of individuals of *A. lixula* in both vent sites was low, which prevented us from conducting the exposure of specimens from the vent to ambient pH.

Statistical analysis

For the plasticity response over time experiments, we fitted mixed-effects models (Harrison et al., 2018) to evaluate the interaction between TREAT (Control *vs* pH 7.5) and DAY (Day 0, Day 1, Day 2, Day 3 and Day 7) on VO_2 consumption, incorporating random effects for individuals, thereby accounting for repeated measures. The analyses were conducted in RStudio version 4.2.2 (2022-10-31; R Core Team, 2022) and using *lme4 package* (Bates et al., 2015). Different distributions (Normal

and Gamma) and log transformations were tested. Models were selected based on the lowest AIC and convergence (Akaike, 1998). To further explore the interaction effects, *post-hoc* and pairwise comparisons were performed using *emmeans* package (Lenth, 2024). To illustrate the results, box plots were created using the *ggplot2* package (Wickham, 2016). The normality of the residuals of the models was tested with the Shapiro-Wilk test.

For investigating the adaptation and rapid response under natural pH gradients, we compared the VO_2 consumption among individuals in their basal and experimental conditions. We normalized the VO_2 values of *A. lixula* using a log transformation whereas the transformation was not needed for *P. lividus* data. Initially, normality of the residuals was checked using the Shapiro-Wilk test, while the homogeneity of variances was evaluated for the variables 'meanVO_2' (dependent variable) and 'pH' (independent variable) using the Levene test through *rstatix* package (Kassambara, 2023). An analysis of variance (ANOVA) was performed in RStudio followed by the *post-hoc* Tukey test through the function TukeyHSD (R Core Team, 2022) to determine differences between the respiration rates from vent and ambient specimens and at different pH conditions. Boxplots were created for data visualization using the *ggplot2* package.

Results

Plasticity response over time

The VO_2 consumption of both species was log-transformed to normalize the data for subsequent analyses. A linear mixed model was then employed for *P. lividus*, as its residuals were normally

distributed, whereas a generalized mixed model with gamma distribution was applied for *A. lixula*, to account for its non-normal distribution. The best model in both species followed the formula: $VO_2 \sim DAY*TREAT+(1|IND)$. The Shapiro-Wilk test confirmed the normal distribution of residuals for both species after applying the respective models (*P. lividus*, p = 0.053; *A. lixula*, p = 0.07).

We assessed mean VO_2 across days under control conditions for each species to account for potential housing effects, we assessed mean VO_2 across days under control conditions for each species. In *P. lividus*, the VO_2 consumption under control conditions ranged from 7.70 to 24.65 mg/kg/h over the seven days, showing minor variations in respiration rates over time (Figure 2, Supplementary Table S4). Although comparisons among some days were significantly different (Supplementary Tables S5), the overall pattern remained stable, indicating no major housing effect. For *A. lixula*, there was no evidence of housing effect, as VO_2 consumption range of 8.00 to 18.13 mg/kg/h and showed no significant differences among days under control conditions (Figure 2, Supplementary Tables S4, S5).

In *P. lividus*, VO_2 consumption during the experimental condition was consistent on Day 0, Day 2, and Day 7 (11.00–31.90 mg/kg/h) but spiked on Day 1 and Day 3 (98.50– 79.80 mg/kg/h). In *A. lixula* the VO_2 consumption was stable on Day 0, Day 2, and Day 7 (42.60–57.20 mg/kg/h), but dropped significantly on Day 1, and Day 3 (17.40–16.31 mg/kg/h) (Figure 2, Supplementary Material Tables S4, S5). Interestingly, for each species the VO_2 consumption before low pH exposure (Day 0) and the last day of experiment at low pH 7.5 (Day 7) showed similar values. However, only *A. lixula* showed no significant difference among those values (Supplementary Table S5).

Adaptation and rapid response under natural pH gradients

The Shapiro-Wilk test confirmed normal distribution of the residuals for both, *P. lividus* (p = 0.68), and *A. lixula* (p = 0.73). The Levene test proved homogeneity of variances for both, *P. lividus* (p = 0.29), and *A. lixula* (p = 0.13).

The ANOVA analysis indicated significant differences among the measured respiration rates in both species (*P. lividus*: F= 20.94, p <0.05; *A. lixula*: F= 25.17, p < 0.05) across all conditions, indicating differences in the VO_2 consumption after the 24 hours of the experiment. Pairwise comparisons (Tukey tests) found no significant differences in basal VO_2 consumption between specimens from the Vent and Ambient sites in both species (Figure 3, Supplementary Tables S6–8). Basal conditions in *P. lividus*, both ambient (A1 the closest to the Vent site and A2 the furthest to the Vent site) and the Vent showed a similar VO_2 consumption (29.00 mg/kg/h). Moreover, in *A. lixula*, both Vents and the Ambient ranged from 27.50 to 53.20 mg/kg/h. Significant differences were found between the experimental and the basal conditions in both species (Figure 3, Supplementary Tables S6–8). *P. lividus* from ambient sites exposed to pH 7.5 showed a mean VO_2 consumption (A2V = 150.90 mg/kg/h) twice as high as the

individuals from the Vent exposed to ambient pH (V1A = 74.20 mg/kg/h) (Figure 3). *A. lixula* from the Ambient exposed to pH 7.5 (A2V = 276.41 mg/kg/h) increased 10 times the VO_2 consumption compared to the basal levels (27.50–53.20 mg/kg/h).

Discussion

Echinoderms, as marine calcifiers, are among the most sensitive organisms to future OA conditions (Byrne and Hernández, 2020; Byrne and Fitzer, 2019). Studies that combine laboratory experiments and OA natural analogues are useful for assessing the stress response and long-term effects of OA (Hernández et al., 2024). In the present study, we combined the study of short, medium, and long-term metabolic responses to OA conditions in two keystone species of sea urchins.

The ability of sea urchins such as *P. lividus* and *A. lixula* to cope with pH fluctuations stems from their coelomic fluid's buffering capacity (Calosi et al., 2013; Di Giglio et al., 2020). Moreover, other physiological processes such as osmoregulation, and excretion, are also interrelated for maintaining metabolic activities (Catarino et al., 2012). After seven days of experiments at acidified conditions (pH=7.5), respiration rates of both species reached similar VO_2 levels to Day 0 (pH=8.1) before the low pH exposure, indicating a potential recovery of the basal respiration rates. However, *A. lixula* showed significant differences when compared to the respiration rates found in the controls, possibly related to the sampling season. The specimens for the control conditions were collected at the end of December of 2023 while the specimens used for the experimental conditions were collected at the beginning of May of 2024. Although similar seawater temperature (16°C ± 1°C), other internal physiological (such as reproductive season) and external ecological (such as food supply) processes might be influencing the respiration rate of collected specimens (Burton et al., 2011).

During the seven days of low pH exposure, VO_2 consumption alternate between increases and decreases in both species, following opposite patterns. In *P. lividus*, elevated respiration rates may be explained through metabolic upregulation to compensate for tissue acidosis (Stumpp et al., 2012). This finding aligns with Calosi et al. (2013), who reported that *P. lividus* adjusts to low pH by accumulating bicarbonates, a buffering mechanism likely requiring increased metabolic activity. In contrast, *A. lixula* exhibited an initial decrease in VO_2 consumption, likely due to its non-bicarbonate buffering mechanisms requiring less metabolic adjustment (Calosi et al., 2013). These distinct physiological responses underline the species-specific strategies for coping with low pH.

Both species show potential for adaptation and/or long-term acclimatization in physiological responses since the basal respiration rates showed no differences between populations from the ambient sites (pH=8.1) and the CO_2 vent sites (pH=7.5 and 7.8) at La Palma. Migliaccio et al. (2019) also found a lack of differences in *P. lividus* respiration rates between specimens from a CO_2 vent and ambient sites, suggesting physiological mechanisms to survive under OA. Acclimatization to OA over time is not rare in sea
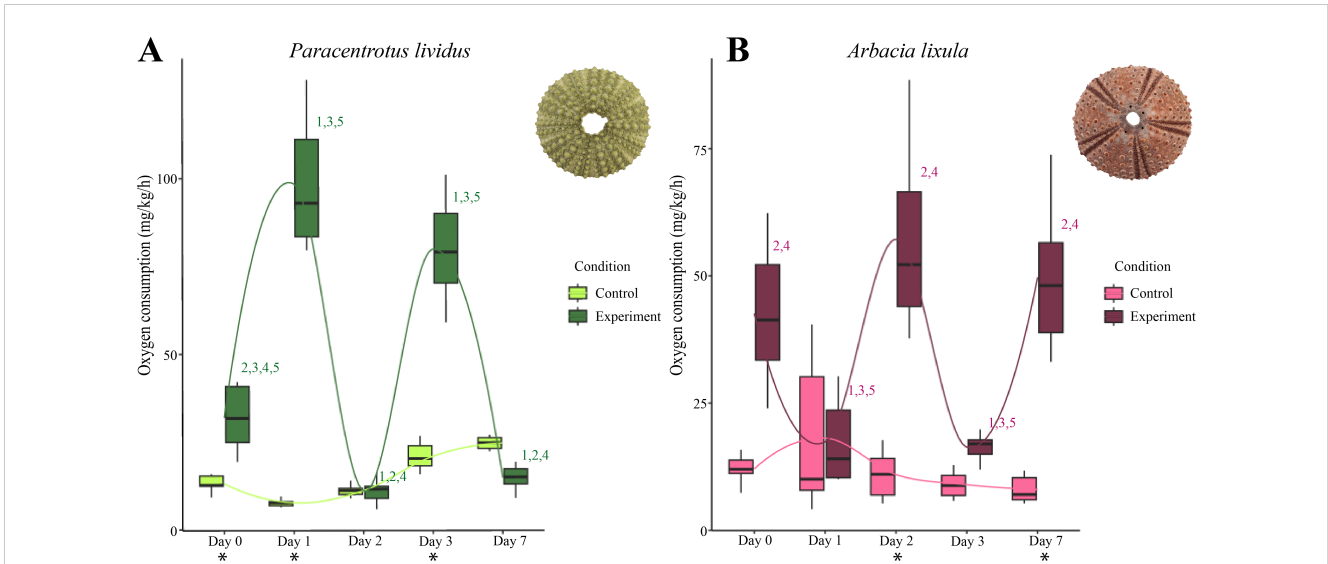
FIGURE 2
Plasticity responses over time. Means of VO_2 consumption from all the specimens on different experimental conditions of pH in *Paracentrotus lividus* **(A)** and *Arbacia lixula* **(B)**. For control conditions the pH was 8.1 and for experiment conditions the pH was 7.5 in both species. The pH during the Day 0 was 8.1 in both species and conditions. The numbers above the bars indicate the significance of pairwise comparisons (padj<0.05) among days in experimental conditions. Asterisks below pH treatment of each day indicate (padj<0.05) between control and experimental conditions.

urchins since several studies have already found no differences in respiration rates between pH treatments in the long-term, for instance, *P. lividus* (Catarino et al., 2012; Cohen-Rengifo et al., 2019), *Echinometra* sp (Asnicar et al., 2021; Moulin et al., 2014; Uthicke et al., 2020, 2021), *Hemicentrotus pulcherrimus* (Kurihara et al., 2013), *Strongylocentrotus droebachiensis* (Stumpp et al., 2012), and *Sterechinus neumayeri* (Suckling et al., 2015). However, studies such as Holtmann (2013) and Marčeta et al. (2020), indicate that maintaining coelomic fluid pH may involve metabolic trade-offs

impacting growth, reproduction and immune functions. In this study, the sex of the individuals was not recorded to avoid sacrificing them. Marčeta et al. (2020) found that females exposed to acidified conditions showed reduced gonad quality and impaired oocyte development. These findings suggests that sex-specific differences could exist in the responses of *P. lividus* and *A. lixula* to OA, with females potentially being more sensitive due to the energetic demands of reproduction. While maintaining acid-base homeostasis is energetically demanding and can reduce the energy
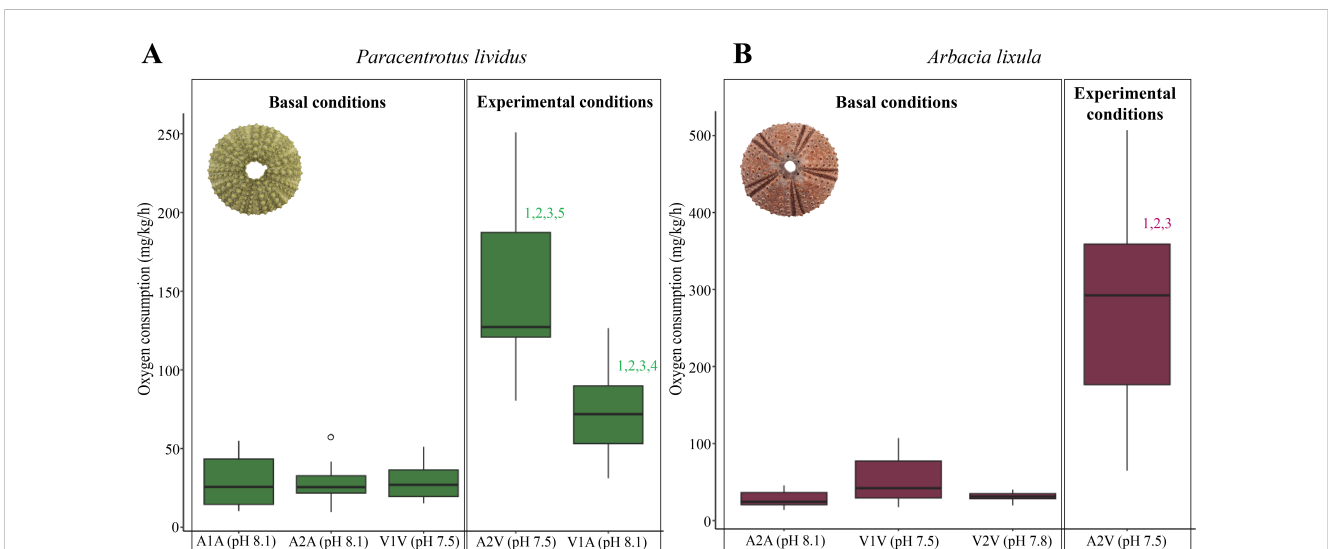

FIGURE 3
Adaptation and rapid responses to pH changes. Means of VO_2 consumption from all the specimens on different experimental conditions of pH in *Paracentrotus lividus* **(A)** and *Arbacia lixula* **(B)**. The exposure to each condition lasted 24 hours in both species. The numbers above the bars indicate the significance of pairwise comparisons (padj<0.05) among days in experimental conditions.

available for other essential processes (Catarino et al., 2012; Collard et al., 2013) it may not fully explain the shifts in energy budgets observed in OA environments. Indeed, other cofounding factors could contribute to a shift in energy budgets and VO_2 consumption rates, such as biomineralization (Kurihara et al., 2012). These trade-offs further highlight the metabolic challenges associated with ocean acidification (Dupont and Thorndyke, 2012; Stumpp et al., 2012; Hernroth et al., 2011).

To assess the phenotypic plasticity, individuals from ambient sites in La Palma of both species were placed under short-term exposure to low pH conditions (pH=7.5). VO_2 consumption increased significantly, especially in *A. lixula*, indicating a stress response to the sudden change. Interestingly, *P. lividus* showed a higher increase in VO_2 consumption when ambient individuals were exposed to low pH conditions (A-V) compared to vent specimens exposed to ambient pH conditions (V-A). This might be related to the natural exposure to fluctuating pH of individuals living at vent sites, ranging from pH=8.1 to pH=7.4 during tides (González-Delgado et al., 2021) while organisms from ambient sites live under constant pH values (pH=8.1). Differences in food consumption at varying pH levels may have influenced the observed VO_2 patterns, as sea urchins might adjust feeding to cope with acidification stress, aligning with other studies (e.g., Dupont et al., 2010). However, food consumption was not directly measured in this study. Populations exposed to high environmental variability may develop tolerance to environmental stressors (Asnicar et al., 2021; Teixidó et al., 2020) as well as maternal anticipatory effects (Palombo et al., 2023; Karelitz et al., 2019; Thor and Dupont, 2015). Studies have shown that echinoderms exhibit reduced sensitivity to OA when exposed to a highly variable environmental history (Asnicar et al., 2022; Kapsenberg and Cyronak, 2019) indicating higher phenotypic plasticity in specimens from more fluctuating environments, such as the CO_2 vent sites studied here. In those lines, high pH variability areas have been proposed as OA refugia, offering adaptive advantages for marine organisms (Bednaršek et al., 2022; García et al., 2018; Kapsenberg and Cyronak, 2019). Thus, CO_2 vent systems in particular, may serve as significant OA refugia to consider.

While *P. lividus* and *A. lixula* may acclimate their respiration rates to low pH over time, understanding their long-term physiological responses is key to predicting viability and ecosystem dynamics. This study suggests that adult sea urchins of both species can potentially acclimate to low pH conditions over the long-term, supporting previous findings (Calosi et al., 2013; Collard et al., 2013; Di Giglio et al., 2020; and Migliaccio et al., 2019). Incorporating sex identification and measuring feeding rates could reveal sex-specific differences and clarify dietary roles in metabolic homeostasis. Future research combining physiological data with population genomics and transcriptomics could uncover adaptive mechanisms and their inheritance.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Author contributions

RF: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing, Visualization. VA: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing, Data curation, Funding acquisition, Visualization. MM: Data curation, Formal analysis, Methodology, Writing – review & editing, Investigation, Visualization. JH: Conceptualization, Methodology, Resources, Supervision, Writing – review & editing, Funding acquisition. SG: Writing – review & editing, Investigation, Methodology. RP: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2025.1500646/full#supplementary-material

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