




















## Resilience of the macroalgae *Gongolaria barbata* under ocean acidification: physiological responses and restoration perspective

Ilaria D'Aniello<sup>a,1,\*</sup> , Maria Nardiello<sup>b,1</sup> , Carmen Arena<sup>c,f</sup> , Denis Badocco<sup>d</sup> ,  
 Alberto Barausse<sup>a,f</sup> , Alberto Colletti<sup>c,f</sup> , Roberto Danovaro<sup>e,f</sup> , Erika Fabbrizzi<sup>c,f</sup> ,  
 Marta Formentin<sup>a</sup> , Simonetta Frascchetti<sup>c,f</sup> , Folco Giomi<sup>g</sup> , Valerio Matozzo<sup>a</sup> ,  
 Isabella Moro<sup>a,b,f</sup> , Katuscia Petrosillo<sup>f,h,i</sup> , Chiara Silvestrini<sup>c,f</sup> , Ermenegilda Vitale<sup>c,f</sup> ,  
 Marco Munari<sup>a,b</sup> 

<sup>a</sup> Department of Biology, Stazione Idrobiologica 'Umberto d'Ancona', University of Padova, Chioggia, (VE), Italy

<sup>b</sup> Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Naples, Italy

<sup>c</sup> Department of Biology, University of Naples Federico II, Naples, Italy

<sup>d</sup> Department of Chemical Sciences, University of Padova, Padova, Italy

<sup>e</sup> Department of Life and Environmental Sciences, Università Politecnica delle Marche, Ancona, Italy

<sup>f</sup> NBFC, National Biodiversity Future Center, Palermo, Italy

<sup>g</sup> Department of Biology, University of Rome Tor Vergata, Rome, Italy

<sup>h</sup> Department of Earth and Marine Science (DiStEM), University of Palermo, Palermo, Italy

<sup>i</sup> National Institute of Oceanography and Applied Geophysics - OGS, Trieste, Italy

### ARTICLE INFO

#### Keywords:

Habitat former  
 Seaweeds  
 Ecophysiology  
 Climate change  
 Mesocosms

### ABSTRACT

The increasing CO<sub>2</sub> concentration is a major cause of the climate change phenomenon. Concurrently, the same increase is leading to ocean acidification (OA), which is projected to decrease seawater pH by 0.4 units by 2100. Here we investigated the potential impacts of OA on the canopy-forming brown macroalga *Gongolaria barbata* from the Venice Lagoon. One-year-old individuals were maintained in mesocosms under two pH levels: 8.1 (current ambient value) and 7.7 (the end-of-the-century value predicted under the current scenario of anthropogenic CO<sub>2</sub> emissions). The physiological responses of the algae were assessed during the experiment in terms of oxygen production and consumption, and maximal PSII photochemical efficiency. At the end of the experiment, we analyzed the percentage of mature receptacles, algal growth rate and the total polyphenolic content and antioxidant capacity as indicators of the stress response. The significant decrease in polyphenolic content indicates the impairment of the defence mechanisms, which could make the algae more vulnerable to grazing under acidified conditions. Yet, conversely, our results suggest that changes in pH levels do not significantly affect the physiological processes, growth or fertility of the algae. These findings suggest that while OA may weaken defence mechanisms, the preservation of physiological and reproductive functions would still support the potential of *G. barbata* populations from the Venice Lagoon to act as donor sources for restoration efforts, highlighting their resistance to the acidified conditions expected in the future.

### 1. Introduction

The increase of CO<sub>2</sub> in the atmosphere, and thereby its uptake by the sea, is leading to an ongoing phenomenon of Ocean Acidification (OA). Currently, the average pH of global oceans ranges from 7.9 to 8.2, with geographic and seasonal variations (Takahashi et al., 2014). However, it

is estimated that the pH of surface seawater will decrease by up to 0.4 units by 2100 with potentially relevant ecological consequences (Caldeira and Wickett, 2003, 2005; Raven et al., 2005; Doney et al., 2009; Jiang et al., 2023). Compared to the open ocean, coastal zones and estuaries exhibit relatively wider fluctuations in seawater carbonate chemistry due to small-scale interactions among physical, biological,

\* Corresponding author. Sola San Domenico 3, Chioggia, (VE), 30015, Italy.  
 E-mail address: [ilaria.daniello@studenti.unipd.it](mailto:ilaria.daniello@studenti.unipd.it) (I. D'Aniello).

<sup>1</sup> These authors contributed equally to this work: Ilaria D'Aniello; Maria Nardiello.

and anthropogenic variables (Andersson and Mackenzie, 2011). Specifically, OA significantly modifies the availability of dissolved inorganic carbon, leading to changes in the physiological performance not only of marine animals with calcareous structures (Palombo et al., 2023; Signorini et al., 2024; Signorini et al., 2025a; Signorini et al., 2025b; Figuerola et al., 2025) but also of primary producers. The photosynthetic and growth rates of marine macro-autotrophs are expected to increase under elevated CO<sub>2</sub> concentrations due to the augmented supply of dissolved inorganic carbon (Connell et al., 2013; Koch et al., 2013; Celis-Plá et al., 2015; Young and Gobler, 2016). At the same time, the impact of OA can vary across species, with some species being negatively impacted, such as calcifying macroalgae (Porzio et al., 2011; Hofmann and Bischof, 2014), while others potentially favoured.

Ocean acidification is expected to profoundly alter benthic ecosystems by acting simultaneously on organismal physiology, species interactions, and community structure (Cornwall et al., 2024). Reduced seawater pH directly affects the calcification processes of many benthic organisms, including molluscs, echinoderms, bryozoans and crustose coralline algae, often resulting in weakened skeletal structures, increased energetic costs, and reduced fitness. Beyond calcification, OA can impair physiological performance by altering acid–base regulation, increasing energetic cost and ultimately reducing animal fitness (Wittmann and Pörtner, 2013). These organism-level effects can cascade to higher levels of biological organisation, reshaping competitive hierarchies, predator–prey interactions, and herbivory pressure within benthic communities (Connell et al., 2013; Legrand et al., 2017; Rodríguez et al., 2018). Such changes are particularly relevant in structurally complex habitats, as the ones dominated by canopy-forming brown macroalgae. They are widespread in both intertidal and subtidal zones of most temperate and subpolar regions and are considered habitat-forming species (Smale et al., 2013; Assis et al., 2020; Duarte et al., 2022). In addition to playing a crucial role in coastal primary production and nutrient cycling, these marine forests act as "ecosystem engineers" (Jones et al., 1994), increasing the three-dimensional complexity and spatial heterogeneity of rocky bottoms. They provide food, shelter, nurseries, and habitat for many other species, e.g., fish, invertebrates, and other algae (Cheminée et al., 2013; Piazzini et al., 2018; Fragkopoulou et al., 2022). In the Mediterranean Sea, brown algae of the genus *Cystoseira sensu lato* form forest-like assemblages that are considered important biodiversity hotspots (Bulleri et al., 2002). The regression of these habitats is now well documented and attributed to multiple factors, notably warming, urbanization and intense overgrazing (Sales and Ballesteros, 2009; Grech, 2017; Fabbrizzi et al., 2020; Verdura et al., 2021). Habitat loss is particularly concerning, as it is linked to massive biodiversity loss (Butchart et al., 2010; Bianchelli and Danovaro, 2020). In particular, the loss of these habitats often results in a regime shift and less biodiverse ecosystems, such as turf assemblages (Benedetti-Cecchi et al., 2001; Orlando-Bonaca and Rotter, 2018). Consequently, several studies have been focused on the restoration potential of these ecosystems (Cebrian et al., 2021 & references therein). However, ecological restoration requires a comprehensive knowledge of the species' ecology (Tamburello et al., 2019), including their tolerance to the ongoing environmental changes (Orfanidis et al., 2021; Tamburello et al., 2022; Fabbrizzi et al., 2023a). Studies investigating the effects of acidification on these brown algae show that elevated CO<sub>2</sub> often increases growth and photosynthesis in *Cystoseira* species, but the effects are highly context-dependent on environmental factors (Celis-Plá et al., 2015, 2017).

*Gongolaria barbata* (Stackhouse) Kuntze is a fucalean species widely distributed across the Mediterranean Sea and is especially common in the Northern Adriatic Sea and in transitional environments (Fabbrizzi et al., 2023b; Falace et al., 2024; Lenzi et al., 2024), where it forms dense forests at shallow depths (0.5–3m). This species exhibits a high degree of morphological plasticity which enable the colonization of areas subjected to wide climatic and ecological ranges (Falace and Bressan, 2006; Savonitto et al., 2019; Orlando-Bonaca et al., 2021; Sadogurska et al.,

2021; Marletta et al., 2024). Large populations of *G. barbata* are also found in the Venice Lagoon, which is a transitional environment exposed to multiple anthropogenic stressors and wide spatio-temporal environmental fluctuations in temperature, dissolved oxygen, salinity, and nutrient content (Solidoro et al., 2004; Fusi et al., 2024). These populations represent potentially an ideal donor for the restoration of these macroalgal habitats (Fabbrizzi et al., 2023b), given their tolerance to a wide array of stressors, including marine heatwaves (Bilajac et al., 2024; Cimini et al., 2024). However, the resilience of this species to acidified conditions has never been investigated.

In this study we tested the null hypothesis that a pH decrease from 8.1 to 7.7, as expected by ocean acidification in 2100 scenarios, would not significantly affect the physiological responses (oxygen consumption/production rates and maximal PSII photochemical efficiency) and fertility (percentage of mature receptacles) of *G. barbata* juveniles from the Venice Lagoon. The inclusion of juveniles is particularly pertinent due to the limited knowledge surrounding early life stages, as acknowledged in previous studies (Irving et al., 2009; Orlando-Bonaca et al., 2021). The overarching goal is to ascertain whether the *G. barbata* population in the southern Venice Lagoon could serve as a donor population for the restoration of deforested areas in the context of global change, with specific reference to ocean acidification.

## 2. Materials and methods

### 2.1. Study area and specimens' collection

The experiment was set up using one-year-old individuals of *Gongolaria barbata* from Ca' Roman, in the southern part of the Venice Lagoon (45° 14' 42.2" N 12° 17' 44.7" E). The Venice Lagoon is a coastal transitional environment characterized by a great variety of habitats, such as marshes, mudflats, seagrass meadows, tidal creeks, canals, and islands (Micheletti et al., 2011). It receives freshwater from watercourses in a densely inhabited and intensely cultivated drainage basin with an annual average flow rate of 34.5 m<sup>3</sup>/s, and it is subject to high anthropogenic pressure from harbors, tourism, fishing and aquaculture, as well as agriculture and industries in the watershed (Zuliani et al., 2005). The lagoon is connected to the Adriatic Sea through three mouths, which allow for water exchange primarily governed by the tidal regime (Solidoro et al., 2010).

Recruits of *G. barbata* were obtained from adult macroalgal forests already present at the Ca' Roman site (Sfriso and Curiel, 2007). In February/March 2022, natural stones coming from the same site (stones' surface area about 45 cm<sup>2</sup> and average depth of 1.5m) were placed underneath reproductively mature adults, as the species' reproductive period begins at the end of winter (Falace and Bressan, 2006). After the zygote implantation onto the stones, the juveniles' growth was monitored for one year until they were collected in December 2022 (35.16PSU, 10 °C, pH 8.15). The stones covered by juveniles were then transported (5 min by boat) in dark conditions at 10 °C to the facilities of the University of Padova, "Umberto D'Ancona" Hydrobiological Station in Chioggia (Venice, Italy), located in the Northern Adriatic, and part of affiliated with the University of Padova, where they remained for the entire experimental period. Thalli were never detached from their original substratum; instead, the stones bearing juveniles were carefully divided so that each stone fragment hosted a single attached thallus.

### 2.2. Experimental setup and maintenance

The outdoor experimental facility was established at the Hydrobiological Station of Chioggia. Individuals were acclimated to mesocosms' conditions for 50 days. After the acclimation time, the experiment lasted for six weeks from mid-January to February 2023.

A substantial tank with a capacity of 3.5 m<sup>3</sup> was utilized, supplied with water sourced directly from the southern basin of the lagoon of Venice in order to mirror temperature fluctuations occurring daily in the

Lagoon. Within this main tank, six smaller submerged tanks (each with a capacity of 50 L) were employed to host mesocosms, receiving a continuous flow of water directly from the lagoon at a rate of 1 L/min. Three of these tanks functioned as “controls” (named C1, C2 and C3), maintaining naturally fluctuating pH levels akin to those found in lagoon water, with an average pH of  $8.11 \pm 0.04$ . In contrast, the remaining three tanks were “treated” (named T1, T2 and T3) and consistently maintained at a pH of ambient pH  $-0.4$  units of pH (an average pH of  $7.71 \pm 0.1$ ) through the introduction of  $\text{CO}_2$  by an automated control system (Aquarium Controller Evolution, mod. ACQ110, Aquatronica, Italy) connected to pH electrodes (ACQ310N-PH, Aquatronica, Italy). This value of pH is close to the projected value under the RCP 8.5 scenario for the year 2100 (Kwiatkowski et al., 2020). Hence, the experimental design included one fixed factor, the pH, with two experimental levels: low ( $\sim 7.7$ ) and ambient ( $\sim 8.1$ ). Salinity was mirroring the lagoon's natural conditions, with mean values of  $35.41 \pm 1.07$  PSU in the low-pH treatment and  $35.39 \pm 1.06$  PSU in the ambient-pH treatment. Additional details on the temporal variability of environmental variables throughout the experimental period are provided in the Supplementary Material.

At the beginning of the experiment, 20 thalli of varying lengths were placed in each tank to ensure an even representation. The maintenance of mesocosms consisted of daily removal of sediments, by brushing the stones, the whole thalli, the tanks and all the devices. Additionally, daily monitoring of key environmental variables (temperature, pH, dissolved oxygen, and salinity) was conducted. Water samples from each mesocosm were collected twice a day (at 10:00 in the morning and at 15:00 in the afternoon) and analyzed with Basic 20 CRISON pHmeter (calibrated every morning with pH buffer 4.01, 7.01, and 10.01). Salinity (PSU), dissolved oxygen (%DO) and temperature ( $^{\circ}\text{C}$ ) were measured daily with a multiparameter probe (HANNA Instruments). All the environmental variables but the pH were not manipulated in the tanks and remained at their ambient levels. The measured response variables were respiration rate (RR), oxygen production rate (PR), gross primary production (GPP), maximal PSII photochemical efficiency, polyphenols content and total antioxidant capacity analysis, thalli growth, and fertility.

### 2.3. Carbonate system

Once a week, discrete water samples were filtered, collected in borosilicate glass bottles, and immediately fixed with saturated mercuric chloride using standard operating protocols during the whole experiment. Total alkalinity (TA) was measured via potentiometric titration using an automated titrator (836 Titrand, Metrohm) after equilibrating each seawater sample to  $25^{\circ}\text{C}$ . Calcium concentrations were subsequently determined with an Agilent 7700 ICP-MS operated under conditions described by Badocco et al. (2015). The mean calcium content across all measurements was  $10.2 \pm 0.2$  mmol  $\text{L}^{-1}$ , consistent with the theoretical estimate of  $10.1 \pm 0.2$  mmol  $\text{L}^{-1}$  derived from the empirical relationship proposed by Millero (1982). Based on TA data and pH on the total scale ( $\text{pH}_T$ ), the dissolved inorganic carbon (DIC), carbonate ion concentration, and partial pressure of carbon dioxide ( $\text{pCO}_2$ ) were computed at *in situ* sampling temperatures. Standard deviations for DIC,  $\text{CO}_2$ , and  $\text{pCO}_2$  were estimated through error-propagation analysis, yielding  $8.4$  mmol  $\text{kg}^{-1}$ ,  $3.4$  mmol  $\text{kg}^{-1}$ , and  $17$   $\mu\text{atm}$ , respectively. Thermodynamic constants for the carbonate system followed formulations from Millero (1979, 1995) and Millero et al. (2006). Solubility data for calcite and aragonite were taken from Mucci (1983) and Ingle (1975), and the corresponding saturation states ( $\Omega_{\text{Ca}}$ ,  $\Omega_{\text{Ar}}$ ) were calculated using the solubility products provided by Millero (1979).

### 2.4. Respiration/production rate

Oxygen consumption rate or respiration rate (RR) of randomly

selected algae was determined once a week during the 6 weeks experiment (sampling time named d7, d14, d21, d28, d35 and d42, refers respectively, to the 7th, 14th, 21st, 28th, 35th, 42nd day from the beginning of the experiment). Each tank contained 20 thalli; at each sampling time, respiration rate was measured on three thalli per tank, randomly selected from the individuals present. The same procedure used for respiration rate was applied also for computing oxygen production rate, in the same sampling time but only from d21 to d42, using other thalli than those used for respiration. Thalli were prepared by brushing the fronds and stones with a brush to eliminate accumulated sediments and potential epiphytes. Once cleaned, they were placed inside respirometric chambers, i.e. 850 mL glass jars, equipped with oxygen sensor spots (OXSP5, Pyro Science GmbH, Aachen, Germany). Each chamber was weighted empty, then with thalli (and stones) and finally filled with water, in order to compute the volume of water present inside each chamber. Filtered seawater at ambient pH was added into the chambers containing individuals coming from the control tanks (C1, C2 and C3), while filtered acidified water (using a ASKOOL pure  $\text{CO}_2$  tank) was added into the chambers with individuals coming from tanks T1, T2 and T3. The oxygen concentration inside the chambers was measured using a 4-channel fiber optic oxygen meter FireSting<sup>®</sup>-O2 (Pyro Science GmbH, Aachen, Germany), combined with the Pyro Workbench software under 1 h of trial, at intervals of 5 min. Each trial had an empty chamber as blank, used to correct the final results. A magnetic stirrer moving a magnetic stir-bar inside the chambers ensured the continuous mixing and so homogeneity of water during trials. During respiration trial, dark conditions were maintained throughout the experiment, while uniform light exposure was allowed during the production trial to allow photosynthetic activity. Temperature was kept stable, depending on the daily temperature of the mesocosms, by using a cold-water bath connected to an aquarium refrigerator (Teco TK 500).

Respiration rate (RR,  $\% \text{O}_2 \text{ h}^{-1}$  per chamber) was calculated as:

$$\text{RR} = - \left( \left( \text{O}_2^{\text{final}} - \text{O}_2^{\text{initial}} \right) \times \left( V_{\text{chamber}} - V_{\text{occupied}} \right) \times 60 \right) / \left( t_{\text{final}} - t_{\text{initial}} \right)$$

while Net Production Rate (NPR,  $\% \text{O}_2 \text{ h}^{-1}$  per chamber) was calculated as:

$$\text{NPR} = \left( \left( \text{O}_2^{\text{final}} - \text{O}_2^{\text{initial}} \right) \times \left( V_{\text{chamber}} - V_{\text{occupied}} \right) \times 60 \right) / \left( t_{\text{final}} - t_{\text{initial}} \right)$$

where  $\text{O}_2$  is the dissolved oxygen percentage,  $V_{\text{chamber}}$  is the total chamber volume (L), and  $V_{\text{occupied}}$  is the estimated volume of the thallus and attached substrate. The factor 60 converts the rate from  $\% \text{O}_2 \text{ min}^{-1}$  to  $\% \text{O}_2 \text{ h}^{-1}$ . Rates were expressed per chamber (i.e. per thallus) (Munari et al., 2020). Reliable estimates of thallus biomass (wet or dry weight, volume, or surface area) were not available for individual replicates. Each thallus was naturally attached to a piece of rock of irregular size and shape, which prevented accurate determination of the algal volume or mass without compromising the integrity of the specimen or the internal volume of the chamber. For this reason, respiration and production rates were expressed as oxygen flux per chamber (i.e., per thallus) rather than normalized to biomass. This approach ensured consistency across treatments and avoided uncertainties introduced by imprecise biomass corrections, while still providing meaningful comparisons of whole-organism metabolic performance.

### 2.5. Photosynthetic efficiency

The chlorophyll-a fluorescence emission measurements were performed using the Underwater Chlorophyll Fluorometer DIVING-PAM-II (Walz GmbH, Germany). Pulsed Amplitude Modulation (PAM) technology has been previously used on *G. barbata* individuals from the Adriatic Sea (Bilajac et al., 2024), and in other studies about the effect of ocean acidification of marine macroalgae (Porzio et al., 2020). Measurements were carried out in each tank every 5 days, using 6 replicates for each tank, i.e. 6 different thalli. Sampling time named d4, d9, d13,

d18, d23, d27, d32, d37, d42, refers respectively to the 4th, 9th, 13th, 18th, 23rd, 27th, 32nd, 37th and 42nd day from the beginning of the experiment. Thalli were darkened for 15 min, to ensure full oxidation of PSII reaction centres, through the use of specific "leaf clips". The ground basal fluorescence level ( $F_0$ ) was measured by a weak blue measuring signal ( $5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). The maximum fluorescence level ( $F_m$ ) in the dark-adapted state, was induced by a light saturation pulse of  $8000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . The maximum photochemical efficiency of photosystem II (PSII) (namely  $F_v/F_m$  ratio) was calculated as:  $F_v/F_m = (F_m - F_0)/F_m$ .  $F_v/F_m$  ratio is used to assess the stress/healthy status of the PSII including photoinhibition (Beer et al., 2014).

## 2.6. Total polyphenolic content and antioxidant capacity

At the end of the exposure period, ten thalli from each tank were selected (30 per condition). Thalli were washed and brushed to remove all attached sediments and epibionts. After determining the fresh weight and the relative size, they were pooled per condition and dried in an oven at  $37^\circ\text{C}$  for 24h. For each condition, ten dried samples were frozen with liquid nitrogen, powdered with mortar and pestle and subjected to the Folin-Ciocalteu colorimetric method, as reported in Fabbrizzi et al. (2023b) and Pica et al. (2024). This method was chosen since it provides consistent results without interfering with non-phenolic compounds with respect to other colorimetric methods (Singleton and Rossi, 1965; Waterman and Mole, 1994; Kamiya et al., 2010). Briefly, samples (0.200 g) were extracted in methanol and stored at  $4^\circ\text{C}$  for 24h. Extracts were centrifuged at 11.000 rpm per 10 min and the supernatants were mixed with 10 % Folin-Ciocalteu solution (1:1 v/v) and 700 mM  $\text{Na}_2\text{CO}_3$  solution (1:5, v/v). The mixtures were incubated in the darkness for 2 h and the absorbances were measured at 765 nm by a spectrophotometer. The total polyphenol content was calculated using a gallic acid standard curve and expressed as mg of gallic acid equivalents per gram of dry weight ( $\text{mg GAE g}^{-1} \text{DW}$ ). For each condition and analytical procedure, ten technical replicates were performed.

The total antioxidant capacity was assessed on the same powdered samples, according to George et al. (2004), using the FRAP method (ferric reducing antioxidant power). For each technical replicate, dried samples (0.250 g) grouped per condition were treated with a methanol: water solution (60:40 v/v) and stored at  $4^\circ\text{C}$  for 24 h. Extracts were centrifuged at 14.000 rpm for 15 min at  $4^\circ\text{C}$  and supernatants were mixed with FRAP reagents: 300 mM acetate buffer pH 3.6 (1:16 v/v), 10 mM tripyridyltriazine (TPTZ) in 40 mM HCl solution (1:1.6 v/v), 12 mM  $\text{FeCl}_3$  solution (1:1.6 v/v). After the mixture incubation in the darkness for 1 h at room temperature, the sample absorbance was read at 593 nm. The total antioxidant capacity was quantified by means of a Trolox standard curve and expressed as  $\mu\text{mol Trolox equivalents per gram of dry weight}$  ( $\mu\text{mol TE g}^{-1} \text{DW}$ ).

## 2.7. Thalli growth and fertility

All thalli used during the experiment were measured at the beginning and at the end of the experiment using a ruler. Fertility was assessed at the end of the experiment considering all thalli for each tank. It was evaluated according to Rindi et al. (2023) and expressed as the percentage of branches bearing receptacles relative to the total number of branches per thallus. Only terminal branches (i.e. last-order branches) were considered in the assessment. Fertility was subsequently classified into five categories according to the proposed criteria: 1) NF: non fertile, a thallus devoid of receptacles; 2) F1: <25 % of the branches bears receptacles; 3) F2: 25–50 % of the branches bears receptacles; 4) F3: 50–75 % of the branches bears receptacles; 5) F4: >75 % of the branches bear receptacles. The occurrence of receptacles was determined through direct visual observation of thalli.

## 2.8. Statistical analysis

Following the above-mentioned experimental design, to assess the impact of pH reduction on each physiological response variable of *G. barbata*, we employed PERMANOVA analysis using Primer 6 software. The factor pH was tested as a fixed factor with two levels. Tested response variables were: respiration rate, production rate, maximum photochemical efficiency (i.e.  $F_v/F_m$ ), total polyphenolic content, antioxidant capacity, thalli length and fertility. Given the non-independence of respiration, production and photosynthetic efficiency measurements over time, the analysis for these variables was conducted separately for each sampling time to account for temporal dependencies. The analyses were based on Euclidean distance matrices and performed using 9999 permutations. Statistical significance was set at  $p \leq 0.05$ .

## 3. Results

### 3.1. Respiration/production rate

Oxygen consumption in organisms under both pH conditions gradually increased until the 21st day of the experiment. However, results showed that there is not a statistically significant difference between the respiration of one-year-old thalli exposed to an acidified pH and those in the ambient pH condition (Fig. 1A). This trend likely stemmed from a rise in the lagoon's temperature, which also affected the mesocosms and caused a temperature variation of approximately  $+2.5^\circ\text{C}$  (see supplementary material). After day 21, as the temperature decreased drastically, a corresponding decline in oxygen consumption was observed in algae exposed to both pH levels. Net oxygen production also showed no statistically significant difference between individuals in the pH 8.1 (i.e., "ambient") vs pH 7.7 (i.e., "low") conditions throughout the experiment (Fig. 1B).

### 3.2. Maximal PSII photochemical efficiency

The maximal PSII photochemical efficiency ( $F_v/F_m$ ) showed distinct patterns across different sampling times and between organisms exposed to pH 8.1 (ambient) and those exposed to pH 7.7 (low) (Fig. 2). Specifically, up to the 13th day of exposure (d13), the photosynthetic efficiency increased more rapidly in organisms at low pH. After this time, the  $F_v/F_m$  ratio in organisms at low pH remained relatively stable for the duration of the experiment, while in those at the ambient pH (pH 8.1) declined until day 27, and subsequently it increased again.

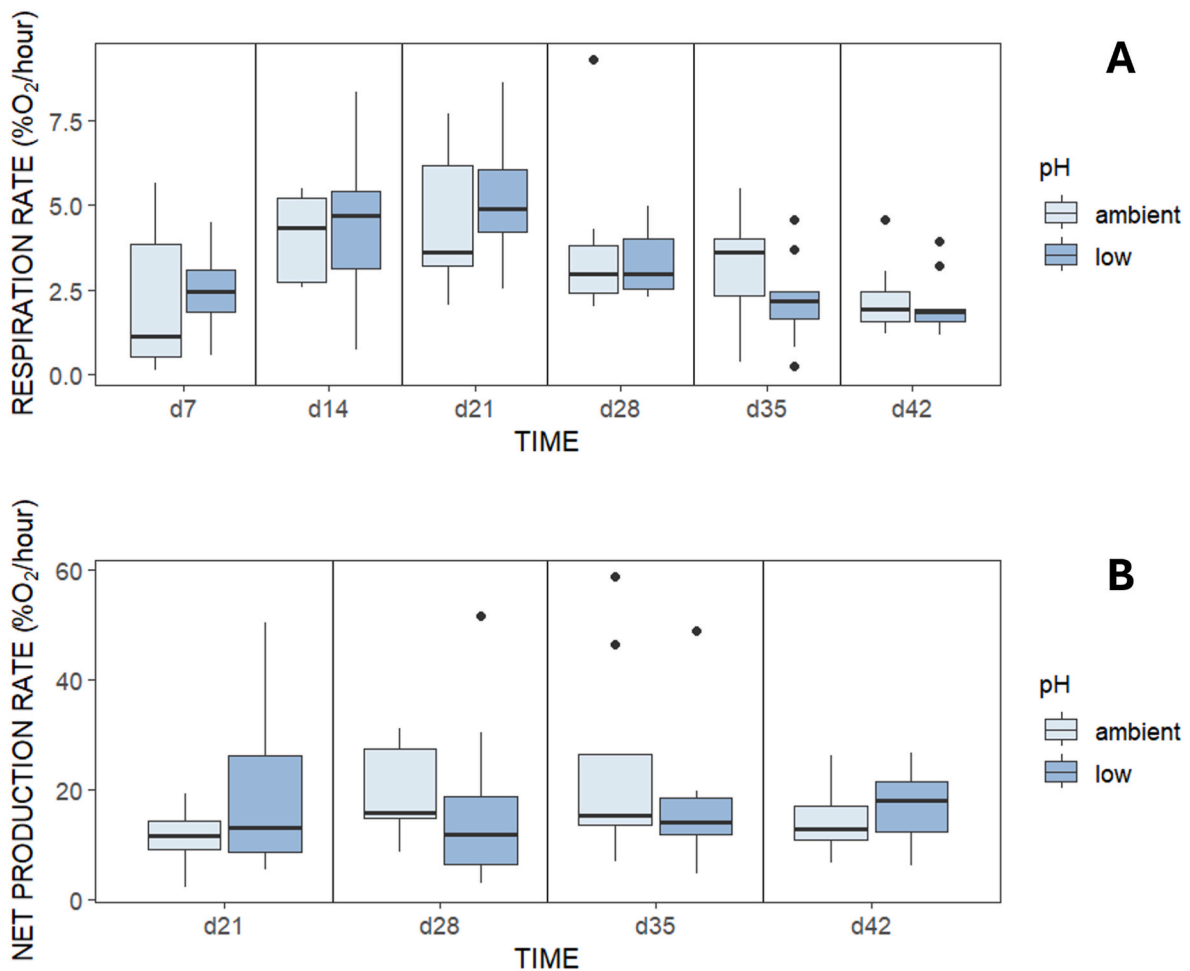
Significant differences in photosynthetic efficiency between the two treatments were found on the 13th, 23rd and 27th day of exposure, with higher values for the ambient pH at 13th day and lower in the other two sampling times, compared to the acidified condition. While the trend for individuals at pH 8.1 appears to follow natural temperature fluctuations, both rising and decreasing, the photosynthetic activity of organisms at pH 7.7, after initially rising with temperature increase, remains steady despite a sudden and rapid temperature decline (Supplementary material).

### 3.3. Total antioxidant capacity and polyphenolic content

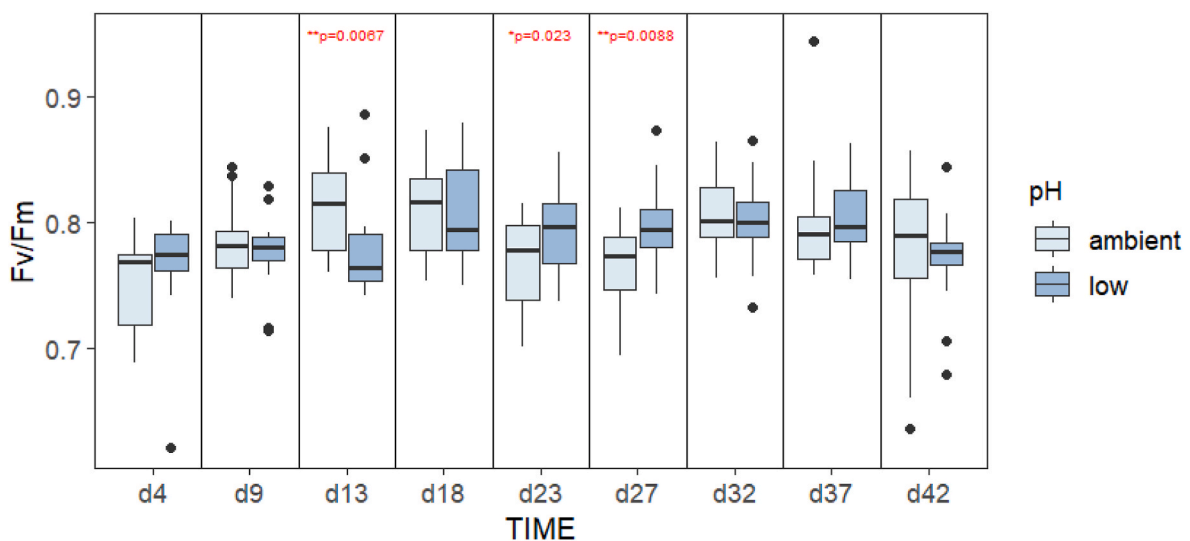
Statistical analysis showed no significant difference in total antioxidant capacity between thalli at ambient-pH ( $3.61 \pm 0.41 \mu\text{mol TE g}^{-1} \text{DW}$ ) and those exposed to low-pH ( $3.54 \pm 0.63 \mu\text{mol TE g}^{-1} \text{DW}$ ) (Fig. 3A). Conversely, significant differences in total polyphenolic content were found, with a higher concentration in thalli at pH 8.1 ( $2.13 \pm 0.09 \text{ mg GAE g}^{-1} \text{DW}$ ) compared to those at pH 7.7 ( $2.03 \pm 0.09 \text{ mg GAE g}^{-1} \text{DW}$ ) (Fig. 3B).

### 3.4. Thalli growth and fertility

At the beginning of the experiment (d0), the average length of thalli



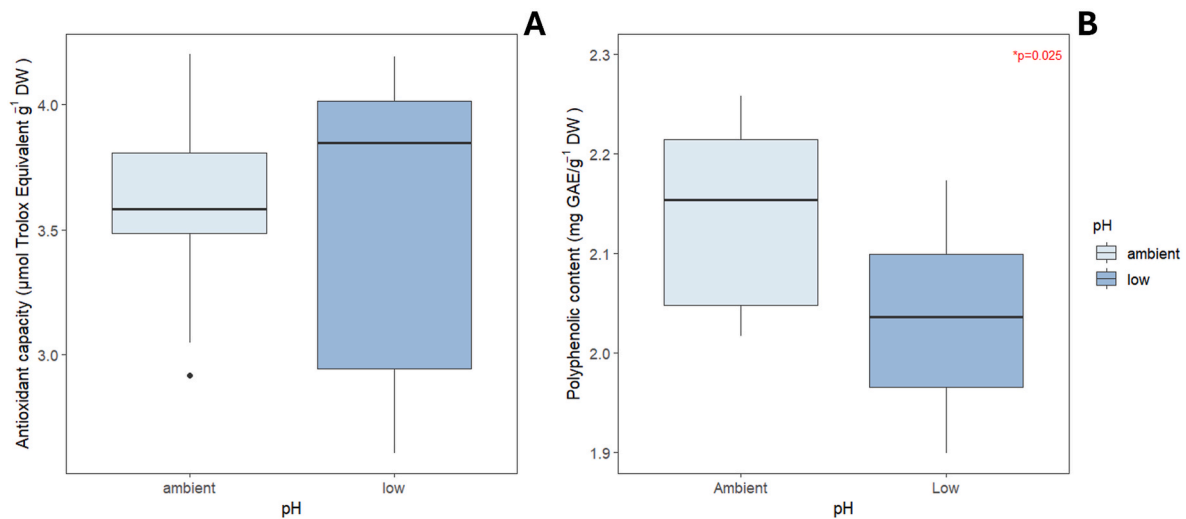
**Fig. 1.** (A) Respiration rate and (B) net production rate in *G. barbata* under the two experimental conditions across all sampling times. Boxplots show medians and quartiles for each experimental treatment ( $n = 3$ ). Light-blue plots represent individuals maintained at ambient pH, while blue ones indicate individuals kept under low-pH conditions. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



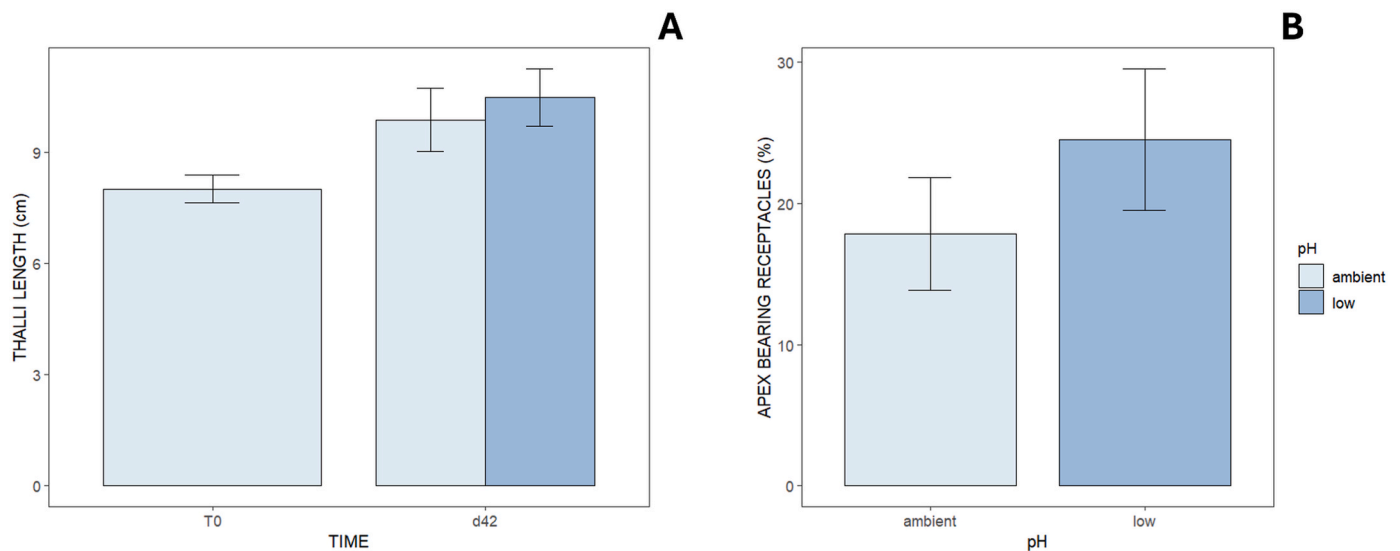
**Fig. 2.** Maximum PSII photochemical efficiency ( $F_v/F_m$ ) in *G. barbata* under the two experimental conditions across all sampling times. Boxplots show medians and quartiles for each experimental treatment ( $n = 6$ ). Light-blue boxes represent individuals maintained at ambient pH, while blue boxes indicate individuals exposed to low-pH conditions. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

was  $8.00 \pm 2.08$  cm. The measures taken at the end of the exposure period showed an average length of  $10.01 \pm 1.84$  cm for thalli at

ambient-pH and an average length of  $10.53 \pm 1.53$  cm for thalli at low-pH (Fig. 4A). No significant effects of pH levels on thalli length were



**Fig. 3.** (A) Total antioxidant capacity and (B) polyphenolic content in *G. barbata* under the two experimental conditions (n = 10). Boxplots show medians and quartiles for each experimental treatment. Light-blue boxes represent individuals maintained at ambient pH, while blue boxes indicate individuals exposed to low-pH conditions. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



**Fig. 4.** (A) Mean thallus length ( $\pm$ SE, cm) of *G. barbata* individuals at the beginning of the experiment (T0) and after 42 days (d42) of exposure to different pH conditions. Light-blue bars represent individuals maintained at ambient pH, while blue bars indicate individuals exposed to low-pH conditions. (B) Percentage (mean  $\pm$  SE) of apices bearing receptacles in *G. barbata* at the end of the exposure period. Light-blue bars refer to individuals at ambient pH, while blue bars represent individuals maintained under low-pH conditions. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

detected. Likewise, fertility was not affected by pH since the coverage of apex bearing receptacles present on each thallus did not differ significantly between the tested conditions (Fig. 4B).

#### 4. Discussion

Understanding the physiological responses of macroalgae such as *Gongolaria barbata* to environmental changes is crucially important to gather insights into their potential adaptation to ongoing global change. The knowledge of the response of habitat forming species such as the macroalgae of the *Cystoseira sensu lato* group, is useful also to identify the suitable donor populations enabling the ecological restoration of these habitats under future environmental scenarios affected by climate change and ocean acidification. The primary goal of this study was to test if the population of *G. barbata* inhabiting an extremely variable environment such as the Venice Lagoon are affected by ocean

acidification. Individuals were collected from this transitional system, an environment characterized by a wide range of environmental conditions (Solidoro et al., 2004; Bartolini et al., 2013; Fusi et al., 2024). Furthermore, the use of juveniles, obtained through either *in situ* (as in this case) or *ex situ* protocols, is essential for investigating the potential impact of acidification or other environmental challenges. This approach is fundamental for comprehensively evaluating the success of future restoration efforts and for ensuring that these strategies can proceed without harming populations by collecting adult specimens for experimental purposes (Verdura et al., 2018).

One of the earliest impacts of ocean acidification can be observed in the primary production of marine photosynthetic organisms. Previous studies on *Treptacantha abies-marina* (S.G. Gmelin) Kützting (another fucales species, formerly referred as *Cystoseira abies-marina* (S.G. Gmelin) C. Agardh) from the Canary Islands, reported the lack of effects of ocean acidification on the respiration rates (Hernández et al., 2018). However,

in that case, production rates were significantly enhanced at reduced pH, suggesting that increased CO<sub>2</sub> availability may boost primary production. Conversely, in our study, respiration rates and primary production showed no significant differences between controls and acidified conditions throughout the experimental period, suggesting that neither the increase of CO<sub>2</sub> nor reduced pH of the seawater influenced these metabolic processes. This is likely because the imposed changes in pH and the corresponding shifts in the carbonate system may reflect the natural variability of the Venice Lagoon, where pH fluctuations of even more 0.4 units can occur at scales of months (Sfriso et al., 1988, 1992). Also, studies conducted in other lagoonal systems have shown that pH fluctuations in lagoon environments can also exceed 0.4 units in some seasons over sub-daily temporal scales (López et al., 2022). From an evolutionary perspective, this is certainly significant for understanding how this population has adapted to cope with pH fluctuations. Such plasticity or tolerance likely reflects selective pressures exerted by naturally variable environments, shaping physiological and behavioral traits that confer resilience to ocean acidification (Munday et al., 2013).

The exploration of the maximum photochemical quantum yield of PSII was conducted to assess the photosynthetic efficiency of thalli at different pH levels (Schermer et al., 2016). Ocean acidification (OA) can alter protein metabolism in photosynthetic organisms (Edmunds and Wall, 2014; Li et al., 2017), which may consequently affect the proteins responsible for maintaining and functioning of photosystems, further affecting photo-physiological performance (Briggs and Carpenter, 2019). The negative effects of high pCO<sub>2</sub> levels on photosynthetic performances have been demonstrated for different macroalgal calcifying species, especially (Wei et al., 2020). Consistently with this, our analysis of maximal PSII photochemical efficiency revealed that, after 13 days of exposure, the F<sub>v</sub>/F<sub>m</sub> in thalli under low pH was lower than in those maintained under ambient condition. With prolonged exposure (23–27 days), F<sub>v</sub>/F<sub>m</sub> ratio became higher in acidified thalli, with increases occurring faster over time than in thalli at ambient pH. The results suggest that both duration and timing of pH variations may significantly influence the response of juveniles of *G. barbata* demonstrating the potential adaptation of this species to future climate scenarios. These results are in agreement with those reported for exposure to MHWs by Fabbrizzi et al. (2023b). In fact, when considering both short and long-term exposure to MHWs, F<sub>v</sub>/F<sub>m</sub> ratio of *G. barbata* decreased after short-term exposure (12 days) and return to control values in the long-term (25 days) indicating the occurrence of acclimation mechanisms to the new environmental conditions (Fabbrizzi et al., 2023b).

Our findings suggest also the absence of oxidative stress responses which in macroalgae involve the production of antioxidant defence molecules aiming to prevent organisms from cell damage (Lesser, 2006; Birben et al., 2012). This is particularly true for intertidal species, which are more exposed to UV radiation (Norton, 1991). Previous studies highlight that the oxidative responses in macroalgae are species-specific (Graiff and Karsten, 2021) and stress conditions can induce changes in this molecule's production, therefore representing an optimal proxy for macroalgal physiological conditions (Aguilera et al., 2002). In our study, the exposure to a lower pH did not significantly alter *G. barbata* antioxidant capacity, suggesting the absence of major effects on photo-protective mechanisms, even though a higher variance has been observed in the low-pH group. Among secondary metabolites produced by macroalgae, there are some of particular interest due to their role in defence against herbivores. In particular, polyphenols are photo-protective compounds that influence thallus palatability and their production often correlates with grazing pressure (Pavia and Brock, 2000; Mannino et al., 2015; Haavisto et al., 2017).

Previous studies on *Cystoseira compressa* (Esper) Gerloff & Nizammuddin, revealed that short-term exposure to acidic conditions increased the synthesis of polyphenolic compounds thereby enhancing phenolic-based photoprotection (Celis-Plá et al., 2015). Similarly, Kumar et al. (2017), demonstrated that in *Sargassum vulgare* C. Agardh,

physiological functions may improve following short-term OA exposure; however, long-term exposure appears to reduce phenolic compound content, suggesting that this species relies less on these compounds over time, likely due to the activity of other compounds that maintain cellular redox balance, resulting in no significant differences between control and acidified populations in antioxidant activity or photosynthetic rates. Nevertheless, since the effects of OA are species-specific, the reduction observed in our study may also reflect the energetic costs of coping with acidification, which could therefore impair natural defence mechanisms. As polyphenolic compounds act as defence tools against grazers, macroalgal vulnerability could be amplified under an acidification scenario, especially considering ongoing lagoon's oligotrophication, which will make them more exposed to UV radiation too by increasing water transparency (Cacciatore et al., 2025; Sfriso et al., 2025). Our study underscores the importance of examining the effects of future climate change on marine species and their restoration within a broader ecological framework to develop more reliable predictions. A species may appear resilient to the direct effects of environmental stressors such as ocean acidification, as macroalgal physiology remained largely unaffected in this study, yet still experience subtle biochemical or ecological shifts. In our case, the observed variation in polyphenolic content under lower pH conditions may indicate an indirect response with potential implications for trophic interactions and the macroalgae's ecological role within the food web.

Finally, low pH conditions did not affect macroalgal growth and fertility. This underscores the strong plasticity of this species to acidified conditions, making it a potential candidate as donor population for restoration efforts, at least considering near-future OA scenarios.

This experiment was specifically designed to test the effects of pH, by directly bubbling CO<sub>2</sub> to maintain the desired pH levels, while the other variables were maintained at the natural levels present in the lagoon. Although the physiological responses in macroalgae are typically very short (Celis-Plá et al., 2015) and the duration of this experiment may be considered perfectly adequate to evaluate the response to low pH exposure and potential physiological adaptations, our study presents some limitations. It lacks additional analyses on the combined effect of multiple stressors, particularly the potential synergistic impact of low pH and warming, including extreme forms such as heatwaves, on macroalgal physiology and photosynthetic performance.

Therefore, further research is required to assess *G. barbata* resilience to multiple interacting stressors. Recent evidence on the effects of marine heatwaves on *G. barbata* recruits (Fabbrizzi et al., 2023) showed no major physiological impairments under elevated temperature conditions, similarly to the absence of negative responses to acidification observed in the present study. Together, these findings support the hypothesis that *G. barbata* is a suitable candidate for restoration actions in changing coastal environments. Nonetheless, the observed variation in polyphenolic compounds, potentially involved in anti-grazing defences, may increase the species' vulnerability to herbivory under low-pH conditions. These results emphasize the importance of adopting a multiple-stressor approach in future studies to fully capture the complexity of macroalgal responses to climate change. A deeper understanding of these complex interactions is crucial for enhancing effective restoration strategies and securing the long-term conservation of these habitat-forming species under global change.

#### CRedit authorship contribution statement

**Ilaria D'Aniello:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Maria Nardiello:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Carmen Arena:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology. **Denis Badocco:** Writing – review & editing, Validation, Methodology, Formal analysis. **Alberto Barausse:** Writing – review & editing,

Validation, Supervision, Resources. **Alberto Colletti**: Writing – review & editing, Methodology, Formal analysis. **Roberto Danovaro**: Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Erika Fabbri**: Writing – review & editing, Methodology, Formal analysis. **Marta Formentin**: Writing – review & editing, Investigation, Formal analysis. **Simonetta Frascchetti**: Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Folco Giomi**: Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. **Valerio Matozzo**: Writing – review & editing, Validation, Supervision. **Isabella Moro**: Writing – review & editing, Validation, Supervision. **Katiuscia Petrosillo**: Writing – review & editing, Validation, Supervision, Formal analysis, Data curation. **Chiara Silvestrini**: Writing – review & editing, Methodology, Formal analysis. **Ermengilda Vitale**: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology. **Marco Munari**: Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

### Funding information

This work was funded by the EASME – EMFF (Sustainable Blue Economy) Project AFRIMED (<http://afrimed-project.eu/>, grant agreement N. 789059), supported by the European Community, and the Biodiversa+ FORESCUE (Biodiversa2021-134) project.

### Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Roberto Danovaro reports financial support was provided by European Biodiversity Partnership. Isabella Moro reports financial support was provided by European Biodiversity Partnership. Simonetta Frascchetti reports was provided by European Biodiversity Partnership. Roberto Danovaro reports financial support was provided by European Maritime and Fisheries Fund. Marco Munari reports financial support was provided by European Maritime and Fisheries Fund. 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.

### Acknowledgment

The authors wish to thank Andrea Sambo, Cristina Breggion and Mattia Panin at the Hydrobiological Station “Umberto D’Ancona” in Chioggia for their technical support.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2026.107887>.

### Data availability

Data will be made available on request.

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