

Response of *Cymodocea nodosa* to ocean acidification and warming in the Canary Islands: Direct and indirect effects

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ABSTRACT

As detected in warming and ocean acidification, global change can have profound impact on marine life. Its effects on seagrasses are becoming increasingly well-known, since several studies have focused on the responses of these species to global change conditions. However a few studies have assessed the combined effect of temperature and acidification on seagrasses. Overall in this study, the combined effects of increased ocean temperature and pH levels expected at the end of this century (+5 °C and pH 7.5) on *Cymodocea nodosa* from Canary Islands, were evaluated for one month through manipulative laboratory experiments. Growth, net production, respiration, gross primary production, chlorophyll-a concentration and its vulnerability to herbivory were quantified. Results showed a positive effect of decreased pH on growth and gross primary production, as well as greater vulnerability to consumption by the sea urchin *Paracentrotus lividus*. In contrast, increased temperature limited net and gross primary production. This study shows that in future scenarios, *C. nodosa* from the Canary Islands may be a losing species in the global change stakes.

1. Introduction

In recent decades, elevated levels of greenhouse gases in the atmosphere has led to an increase in temperature and CO₂. As consequence of heat absorption, ocean temperature has increased and the oceans absorbed over 30% of these emissions (Feely et al., 2004; Sabine et al., 2004). It has led to a global modification of the seawater systems, causing a decrease in ocean pH, known as ocean acidification, of up to 0.1 units between the years 1750 and 2000 (Gatusso and Hanson, 2011). In the last decades an increase of 4 °C in sea surface temperature per year has been observed (IPCC, 2019). These processes are known as global change and their effects on marine ecosystems have been widely studied in recent years. Diverse organisms have been affected by rising temperature and acidification conditions, such as calcareous invertebrates (Michaelidis et al., 2005; Shirayama and Thornton, 2005), corals (Langdon et al., 2003), calcified algae (Feely et al., 2004), algae and seagrasses (Koch et al., 2013; Rodríguez et al., 2018).

Seagrasses have been considered one of the most productive ecosystems worldwide; they host wide biodiversity and are ecosystem engineers (Jones et al., 1994; Wright and Jones, 2006) providing numerous ecological service to humans such as stabilize sediments and

produce large quantities of organic carbon, nutrient cycling alter water flow (Hemminga and Duarte, 2000). The effects of global change on seagrass have been studied in various species (Egea et al., 2019; Hernán et al., 2017; Traboni et al., 2018) and regions, such as, Mediterranean, Iberian (Collier et al., 2018; Egea et al., 2019; Tomas et al., 2015), and varied responses have been observed under different geographical regions (Tuya et al., 2017, 2019; Bennett et al., 2021). However the effects of global change on seagrasses of Canary Islands have been poorly studied.

The knowledge accumulated to date on the effects of individual stressors on seagrasses is impressive (see review Nguyen et al., 2021), however stressors rarely occur in isolation in the environment and when acting together, they can be synergistic, additive or antagonistic. From positive effects of rise temperature have been observed on seagrasses, increasing photosynthesis (Campbell et al., 2006; Winters et al., 2011) respiration rates (Schulze et al., 2005) to negative effects, such as limited growth (Traboni et al., 2018; Lee et al., 2007) deterioration of shoot status and eventually shoot mortality (Duarte, 2001). The responses of seagrasses to increased temperature are relatively well documented (Nguyen et al., 2021); however, little is known of the potential distortion of these responses caused by acidification (Koch et al.,

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2013; Egea et al., 2018b). Such effects on key ecosystems are very important to foresee the response to future scenarios of global change. Furthermore, several studies highlight the different intraspecific responses to global change according to their distribution (Tuya et al., 2019) and genetic diversity (Alberto et al., 2008; Marín-Guirao et al., 2016).

In the Canary Islands, the key seagrass is *Cymodocea nodosa* Ucria (Ascherson), with its geographical range encompassing several thermal regimes (Tuya et al., 2019). It extends from the entire Mediterranean Sea to adjacent eastern Atlantic coasts, including the Madeira and Canary archipelagos (Alberto et al., 2006, 2008; Chefaoui and Serrão, 2017; Mascaro et al., 2009; Tuya et al., 2014) and provides food and shelter for diverse organisms, mainly invertebrate and fish assemblages (Tuya et al., 2014; Espino et al., 2015; Casas et al., 2021). It can be forming extensive monospecific meadows, varying in density in sandy and bottoms in bays, harbours and sheltered areas along the eastern and southern coasts of the Islands (Reyes et al., 1995a,b). The populations in the Canaries differ genetically from both Atlantic and Mediterranean populations (Alberto et al., 2008).

The threats posed to *C. nodosa* are similar to those to seagrass in general. Faced with global change, seagrasses have sometimes been considered “winners” in this struggle because of their higher rates of photosynthesis, carbon fixation and growth when exposed to high levels of oceanic acidification (Palacios and Zimmerman, 2007; Jiang et al., 2010; Takahashi et al., 2016; Egea et al., 2018a). However, neutral effects have been showed on biomass, shoot density, growth rates under CO₂ enrichment (Campbell and Fourqurean, 2013; Cox et al., 2016; Palacios and Zimmerman, 2007). Conversely doubts remain whether this improvement in growth compromises their biomechanical properties, increasing their vulnerability to structural damage, leaf loss and an increased pressure of herbivores on them as suggested by several authors (De los Santos et al., 2017; Jiménez-Ramos et al., 2017; Tomas et al., 2015).

Changes in the growth or content of seagrass leaves due to global change can exert a significant impact on seagrass communities because these changes may influence leaf palatability, and thus make plants more or less vulnerable to herbivore (Cebrian and Duarte, 1998; Prado and Heck, 2011). Some studies have addressed the indirect effects of climate change on seagrass-herbivore interactions (Hernán et al., 2016, 2017; Tomas et al., 2015) and few have addressed the outcome of interactions between more than one stressor (Jiménez-Ramos et al., 2017; Tomas et al., 2015; Burnell et al., 2013; Ontoria et al., 2019b). Multiple stressors, such as warming temperatures may alter C/N ratios or the production of chemical defences (secondary metabolites) reducing the palatability and nutritional quality of leaves (Staeher and Wernberg, 2009; Sudatti et al., 2011; Beca-Carretero et al., 2018). The effect of ocean acidification has also been shown to down-regulate the production of phenolic compounds in marine plants and thus enhance herbivore consumption of seagrass tissues (Arnold et al., 2012).

Several studies highlight positive effects of high temperatures in the seagrass metabolism and in the maintenance of a positive carbon balance, since warmer temperatures favors photosynthesis and respiration through their effects on kinetic reactions and metabolism (Pérez and Romero, 1992; Zimmerman et al., 1989). However other studies have shown negative effects on seagrasses (Collier and Waycott, 2014; Jordà et al., 2012; Moreno-Marín et al., 2018).

Positive, neutral and negative effects of global change on seagrass have been registered and diverse responses have been observed from individuals of *C. nodosa* from Iberian or Mediterranean regions (Egea et al., 2018a, 2018b; Jiménez-Ramos et al., 2018; Hernán et al., 2017) as consequence of their geographical distribution, thermal tolerance and its optimal temperature, as has been observed in other seagrasses (Collier et al., 2018; Masini and Manning, 1997; Short and Neckles, 1999). Therefore, while the plant response to a single factor can be well described, the combination of multiple stressors can induce a complex response. Egea et al. (2018b) showed that the seagrasses may exhibit

non additive responses or additive responses and these responses can be antagonistic (combined effect is lower than expected additive effect) or synergistic (higher than expected additive effect) with stressors such as warming, acidification and eutrophication. Therefore future research with multiple-stressor studies in seagrass are needed to provide more information about resilience of seagrass in future global changes scenarios (Nguyen et al., 2021).

In this context, through laboratory experiments, this study examined for the first time the combined effects of ocean acidification and warming on growth, metabolic rates, chlorophyll-a content, and the relationship herbivory-seagrass of *C. nodosa* from the Canary Islands. We hypothesized that *C. nodosa* from the Canary Islands can be vulnerable to future global change conditions, evaluating which factors, high temperature or low pH, may have major negative repercussions on their populations in future global change scenarios.

2. Material and methods

2.1. Sampling site and experiment design

Seagrass *Cymodocea nodosa* was collected at random from a large submarine meadow in the locality of Abades (28°31.05' N, 16° 26' 12.49' W) in November 2018. Use of plant biomass from various genetically independent patches was ensured by scuba-diving over a large area, approximately 5000 m² (Monterroso et al., 2019). Plants were transported to the laboratory at the University of La Laguna in dark wet conditions within 2 h of collection. Upon arrival, experimental plant units of *C. nodosa* (consisting of one vertical shoot with its first rhizome segment) were carefully selected for healthy appearance. Epiphytes were removed by soaking in distilled water. Then, 4 such units were planted individually by hand in each of 60 transparent incubation beakers (2 L) containing natural sandy sediment, also from Abades. The sand was previously sieved (1 mm mesh). Each incubation beaker was a replicate. The plants were left to acclimate for 7 days before randomly assigning the experimental treatments, maintaining constant salinity, temperature 20 °C and pH 8.1 (values of seawater temperature and pH in the Canary Islands) under a 12 h light/12 h dark cycle using LED lighting (Leddy Tube, 6 W) in 300 L seawater tanks.

After acclimation to laboratory conditions, seawater parameters (temperature and CO₂ concentration) were modified. We implemented a full factorial experimental design, combining two of the main drivers currently acting together in coastal areas (warming and acidification), to determine how these affect the seagrass. A two-level experiment was conducted for a month, using a control pH treatment ca. 8.1 and another “low” pH ca. 7.5 equivalent to future conditions forecasted for the region in 2100 (Caldeira and Wickett, 2005). Two thermal treatments were also set, control temperature ca. 21 °C and high temperature with the seawater heated to +5 °C above ambient levels, ca. 26 °C, following the predictions for the region for the 2100 (IPCC, 2019). For the “low” pH treatment, filtered seawater was enriched by injecting CO₂, which was controlled by a computer system (AQUAMEDIC). The high temperature treatment was controlled using thermostatic coolers (ECHEIM AQUATIC, 50 W ± 0.5 °C) and heaters (Tetra HT200). The system consisted of two interconnected 300 L tanks for each treatment. In the header tank the seawater variables were adjusted (pH or temperature) and the seagrass beakers were placed in the other tank, see Fig. 1A. In each tank, temperature and light were continuously recorded using HOBO data loggers (Onset®), while salinity was measured daily with a salinometer (WTW COND 3110). In total, four treatments were established: control (21 °C and pH 8.1), low pH (21 °C and 7.5 pH), high temperature (26 °C and 8.1 pH) and future climate conditions (26 °C and 7.5 pH) (Fig. 1B).

Throughout the experiment period, the water was completely replaced every week with fresh filtered and sterilized seawater prepared at the appropriate pH and temperature conditions for each treatment. Water quality parameters for marine aquariums (NO₃, NO₂, NH₃/NH₄

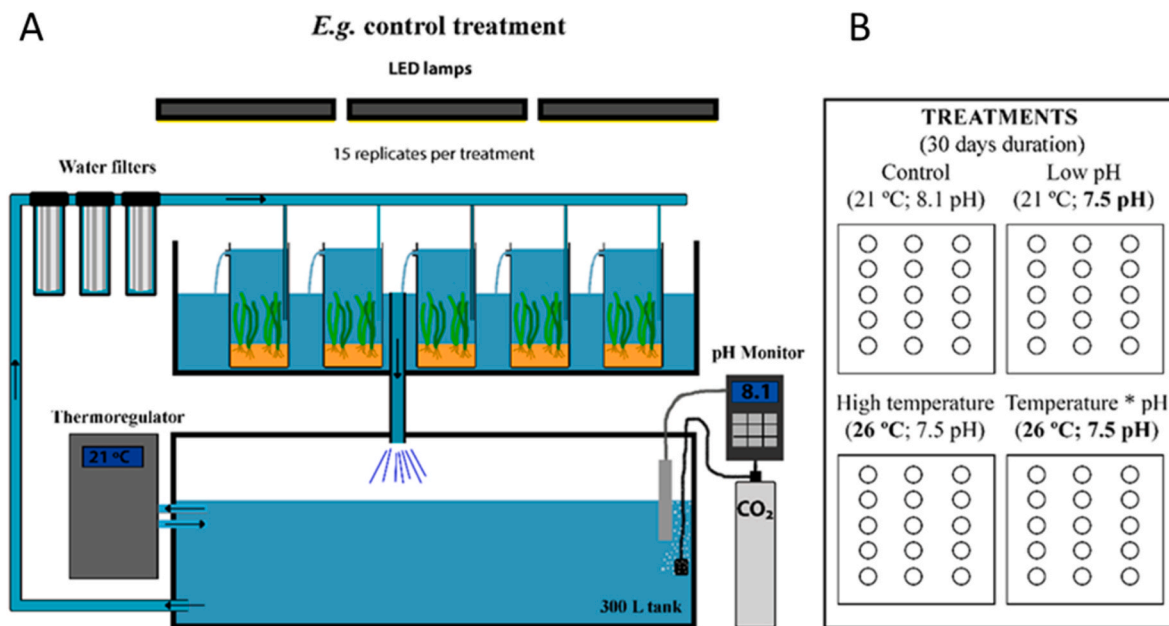


Fig. 1. A. Simplified diagram of one experimental treatment. B. Diagram of experimental design. See detailed description in the text.

and PO₄) were checked weekly using the Marine CareTest Kits (Red Sea). Alkalinity was monitored by titration twice a week for each treatment. The other chemical parameters of the seawater: pCO₂, and calcite (Ω_{Ca}) and aragonite (Ω_a) saturations, were calculated from total alkalinity (TA) and pH using the CO₂SYs software (Lewis and Wallace, 1998). Calculations were based on the K1 and K2 from Mehrbach et al. (1973), modified by Dickson and Millero (1987).

2.2. Growth

The growth was recorded through weight increase, and several morphometric variables: maximum leaf length, total root length and number of leaves of each experimental plant unit, modified from Hernán et al. (2017). The morphometric variables were registered on 15 replicates in each treatment with a meter rule and weight increase was recorded as the difference between final and initial weights on a precision balance (± 0.001), where all entire plants were weighed at the start and end of the experiment. Algae, epiphytes and dead leaves were removed before starting the experiment.

2.3. Metabolic rates

After one month in experimental conditions, metabolic measurements were performed on *C. nodosa* using incubations in beakers. Eight replicates were chosen of each treatment, each consisting of four individuals of *C. nodosa*, were incubated for 2 h in the same beakers with 2 L seawater in each treatment. In parallel five controls were blanks, without plants, to estimate the possible oxygen consumption of other microorganisms. Oxygen concentrations were measured at the beginning and end of each incubation, using an optical fiber system (FIBOX 3, PreSens, Regensburg, Germany). Reactive spots were calibrated with 0% and 100% buffer solutions. The 0% buffer was prepared by dissolving 1 g of sodium sulfite (Na₂SO₃) in 100 mL of seawater, while the 100% by bubbling air into 100 mL of seawater with an air-pump for 20 min to obtain air-saturated seawater. Net primary production and respiration rates were calculated from the following formula, modified from Legrand et al. (2019).

$$NPP \text{ or } R = \frac{\Delta O_2 \times V}{\Delta T \times WW}$$

where NPP is Net primary production ($\mu\text{mol O}_2 \text{ g WW}^{-1} \text{ h}^{-1}$), R is respiration ($\mu\text{mol O}_2 \text{ g WW}^{-1} \text{ h}^{-1}$), ΔO_2 is the difference between the initial and final oxygen concentrations ($\mu\text{mol O}_2 \text{ L}^{-1}$), V the volume of the beaker (L), ΔT the incubation time (h), and WW the wet weight of the incubated plants (g) obtained at the end of the incubations.

Gross primary production (GPP) was calculated following the equation

$$GPP = NPP + R$$

Control beakers containing only seawater were incubated to correct for oxygen fluxes due to any additional biological activity in seawater. Oxygen fluxes measured and calculated in control beakers were subtracted from those in beakers with seagrass.

2.4. Chlorophyll-a

At the end of the experiment, 3 replicates of each treatment were collected and immediately frozen at -20°C , pending analyses in dark conditions. The pigment content of leaves (chlorophyll-a) was measured on two segments (1 cm) cut out at 2 cm above the third leaf blade of every plant. Following the methodology used by Dennison (1990) the pigments were extracted from leaf segments in 10 mL of 96% acetone for 24 h in the dark at 4°C and the extracted solution was analyzed spectrophotometrically with a DU 800-Beckman Coulter apparatus. Pigment concentration was estimated using the Dennison (1990) formula:

$$Chl-a (\mu\text{g ml}^{-1}) = 12.7 \times A_{663} - 2.69 \times A_{645}$$

Where *Chl-a* is the concentration of *Chl-a* ($\mu\text{g ml}^{-1}$), A_{663} is the absorbance standardized with a turbulence value measured at 725 nm ($A_{663-A725}$) and A_{645} is the absorbance standardized with the turbulence value ($A_{645-A725}$).

2.5. Herbivory experiment

After 30 days under experimental conditions, 5 *C. nodosa* replicates from each treatment were used in an herbivory experiment. We evaluated potential indirect effects of such sea water conditions on *C. nodosa*'s susceptibility to herbivory by the sea urchin *Paracentrotus lividus*. Specimens of *P. lividus* were collected in the same locality as the plants, Abades, 5 days prior to starting the experiment. They were then

acclimated to laboratory conditions for 5 days, feeding them with *Dyctiota* sp. in the first 4 days of acclimation. All experimental units of *C. nodosa* were weighed on a precision balance (± 0.001 g). After 48 h, seagrasses from each replicate were weighed again and the horizontal test diameter of each echinoid was previously registered using a digital caliper (± 0.01 mm). For 2 days, sea urchins were left to feed on each replicate. In order to measure any possible changes in leaf tissue biomass not related to grazing, 3 controls without herbivores in each treatment were used to correct for such fluctuations. At the end, each experimental unit was weighed following the above procedure. To estimate the consumption rate of *C. nodosa* by sea urchin *P. lividus*, we used the following equation (see Rodríguez et al., 2018):

$$C_{tot} = Tt \times \left(\frac{C_f}{C_i} \right) - T_f$$

where C_{tot} is total consumption in grams (g), T_i is initial seagrass weight (g) and T_f its final weight after the urchin treatment, C_i is the initial seagrass weight and C_f the final seagrass weight in the control treatment without urchins.

2.6. Statistical analyses

Growth, chlorophyll-a content, and metabolic rates (gross primary production, respiration, net primary production) were analyzed through a two-way ANOVA by permutations (Anderson, 2004), with two fixed factors. Treatment pH with 2 levels: low pH (7.5) vs. control pH (8.1) and Treatment Temperature also with 2 levels: high temperature (26 °C) vs. control (21 °C).

To evaluate the differences in consumption rates of *C. nodosa* by *P. lividus*, changes in weight were analyzed with a two-way ANCOVA performed by permutations (Anderson, 2004), where the fixed factors were the same as above and the covariable was sea urchin size (horizontal test diameter, as above).

All permutational ANOVAs were calculated using PRIMER6 & PERMANOVA + software, using Euclidean distances. Significant terms in analyses of variance were further examined using *a posteriori* pairwise comparison by permutations (Anderson and Robinson, 2003). Whenever there were not enough possible permutations for a reasonable test, corrected *p*-values were obtained with random Monte Carlo draws from the asymptotic permutation distribution.

3. Results

3.1. Seawater parameters

Target temperatures and pH were recorded for each replicate and treatment (Table 1). The partial pressure of CO₂ (pCO₂) increased at low pH levels in all temperature treatments, and calcite (Ω_{Ca}) and aragonite (Ω_{Ar}) saturation levels decreased in low pH conditions (Table 1). Sea water only approached subsaturation values with respect to aragonite ($\Omega_{Ar} < 1$) under low pH conditions (Table 1).

Table 1

Seawater parameters measured during the experiment. Values shown are means (\pm SD) of Temperature (°C), Salinity (‰), Total pH (pH_T), Total alkalinity (TA). The other values: Partial pressure CO₂ (pCO₂), Calcite saturation (Ω_{Ca}), Aragonite saturation (Ω_{Ar}), were calculated using CO2SYS.

Treatment	T (°C)	Salinity	pH _T	pCO ₂ (µatm)	A _r (mmol·kg ⁻¹)	CO ₃ ⁻² (mmol·kg ⁻¹)	CO ₂ (mmol·kg ⁻¹)	Ω_{Ca}	Ω_{Ar}
High temperature – low pH	25.8 ± 1.5	38.47 ± 0.32	7.5 ± 0.1	1964.1 ± 87.25	2805.2 ± 81.91	100.92 ± 4.40	100.92 ± 4.40	2.36 ± 0.10	1.57 ± 0.06
High temperature - control pH	25.7 ± 1.4	38.47 ± 0.32	8.1 ± 0.05	401.3 ± 55.47	2864.8 ± 378.4	326.16 ± 45.09	326.16 ± 45.09	7.63 ± 1.05	5.06 ± 0.69
Control temperature- low pH	20.3 ± 0.1	38.07 ± 0.32	7.5 ± 0.1	2237.3 ± 259.4	3230.4 ± 370.7	97.04 ± 11.25	97.04 ± 11.25	2.27 ± 0.26	1.48 ± 0.17
Control temperature - control pH	20 ± 0.55	38.00 ± 0.26	8.1 ± 0.05	282.3 ± 2.74	2645.2 ± 24.46	307.55 ± 2.99	307.55 ± 2.99	7.20 ± 0.07	4.70 ± 0.04

3.2. Effects of ocean acidification and warming on *Cymodocea nodosa* growth

At the end of experiment the mortality rate was zero. The variables measured (maximum leaf length, number of leaves and total root length) were similar between treatments; no significant difference between them was observed (Fig. 2, Table 2). The maximum leaf length registered as increment varied between treatments, where in control temperature and pH showed highest lengths. The low leaf lengths were observed under high temperature and low pH, but these differences were not significant (Fig. 2A). A decrease of total root length was registered under high temperature and control pH treatment. In the rest of treatments a rise in total root length was observed (Fig. 2B). The number of new leaves registered at the end of experiment was around 1 in each treatment (Fig. 2C).

Only significant differences in weight increase were found between pH treatments (Fig. 2D, Table 2), where individuals reared under low pH showed a higher weight increase. In the other treatments, no significant differences were registered (Table 2).

3.3. Effects of ocean acidification and warming on metabolic rates of *Cymodocea nodosa*

Temperature and pH significantly affected Net primary production (NPP), in which the highest oxygen concentration was observed at low seawater pH (7.5) and at 21 °C (see Fig. 3A and B and Table 3A), while no effects attributable to interaction between them were found (Table 3A).

However, no significant differences in respiration processes were observed between treatments (Fig. 4, Table 3B). The lowest oxygen concentrations were recorded in the control treatment (pH = 8.1 units and 21 °C) and the highest oxygen concentrations in the high temperature-low pH treatment. Despite these differences, the results were not significant (Fig. 4, Table 3B).

Temperature negatively affected GPP. On average, biomass production in the high temperature treatment (26 °C) was, on average, 4 times lower than in the 21 °C treatment (Fig. 5; Table 3C). However, pH treatments showed no significant differences (see Table 3C, Fig. 5).

3.4. Chlorophyll-a

None of the factors significantly affected chlorophyll-a content. Although control temperature treatments show higher chlorophyll-a contents than the others, these differences were not significant (Fig. 6). The highest values were registered in seagrass reared under the 21 °C treatment. In control pH at 26 °C, similar chlorophyll-a contents to those in low pH were detected, but they were higher in control pH than low pH at 21 °C. Despite these results, no significant effects of temperature (Pseudo-F = 0.57316; *p* = 0.4765), pH (Pseudo-F = 2.4947, *p* = 0.1581) or the interaction between them (Pseudo-F = 0.42681, *p* = 0.5386) were found.

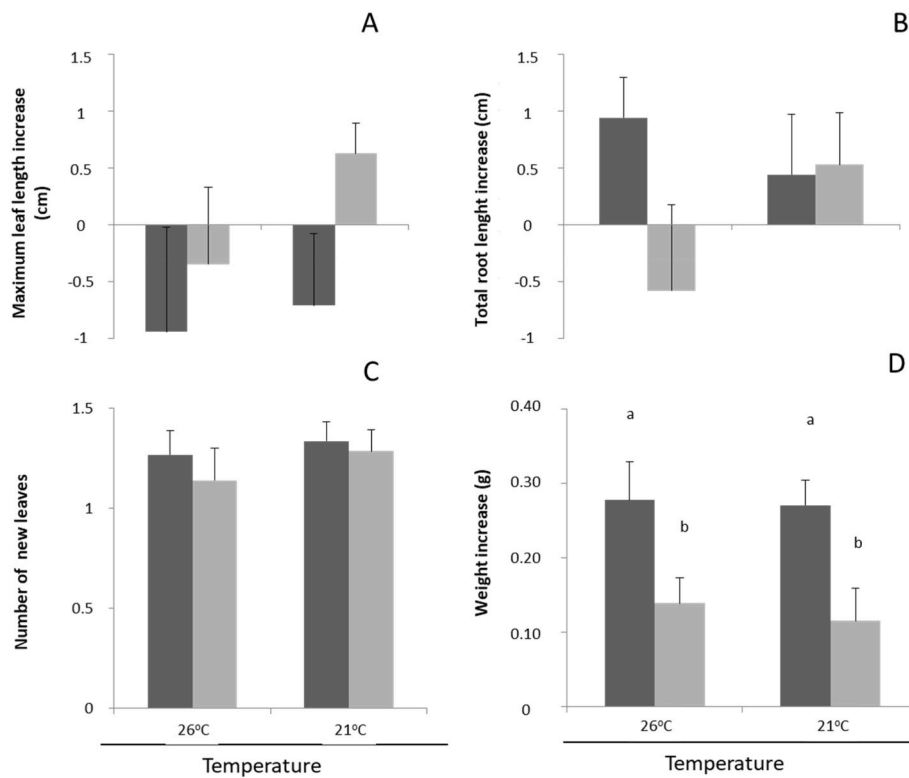


Fig. 2. Mean growth values \pm SE of *Cymodocea nodosa* under each pH and Temperature treatment (n = 15) measured as A) Maximum leaf length increase (cm), B) Total root length increase (cm), C) Number of new leaves and D) Weight increase (g). Dark grey show pH = 7.5 units and light grey pH = 8.1 units. Significant results are shown with different letters.

Table 2

Results of 2-way ANOVA by permutations on growth, in terms of morphometric variables (maximum leaf length, number of leaves and total root length) and as weight increase through growth of *Cymodocea nodosa* under each pH and temperature treatment (n = 15). Significant effects highlighted in bold.

Variable	Source of variation	df	SS	MS	Pseudo-F	p(perm)	Pairwise test
Maximum leaf length (cm)	pH treatment (pH)	1	17.092	17.092	2.4999	0.1132	
	Temperature treatment (T)	1	7.4366	7.4366	1.0877	0.3035	
	pH*T	1	3.33	3.33	0.48706	0.4942	
	Residual	56	382.86	6.8369			
	Total	59	410.72				
Number of leaves	pH treatment (pH)	1	0.11852	0.11852	0.52188	0.4698	
	Temperature treatment (T)	1	0.16713	0.16713	0.73593	0.3894	
	pH*T	1	3.33	3.33	0.48706	0.4942	
	Residual	56	12.718	0.2271			
	Total	59	13.026				
Total root length (cm)	pH treatment (pH)	1	7.6505	7.6505	1.7243	0.1895	
	Temperature treatment (T)	1	1.403	1.403	0.31621	0.5782	
	pH*T	1	9.7003	9.7003	2.1862	0.1472	
	Residual	56	248.47	4.437			
	Total	59	267.22				
Weight increase	pH treatment (pH)	1	0.31716	0.31716	12.684	0.0009	7.5 > 8.1
	Temperature treatment (T)	1	3.5394E-3	3.5394E-3	0.14155	0.7062	
	pH*T	1	1.0475E-3	1.0475E-3	4.1892E-2	0.8362	
	Residual	55	1.3753	2.5005E-2			
	Total	58	1.6989				

3.5. Herbivory experiments

Results of the permutational ANCOVA analyzing herbivory of *P. lividus* upon *C. nodosa* showed significant differences in the pH factor (Fig. 7, Table 5). The seagrass reared under low pH treatment was

consumed faster by *P. lividus* than that reared in control pH (Fig. 7). Temperature had no noticeable effect on feeding rates of *P. lividus*. The horizontal test diameter, recorded as sea urchin size (S), was not significant (Table 5).

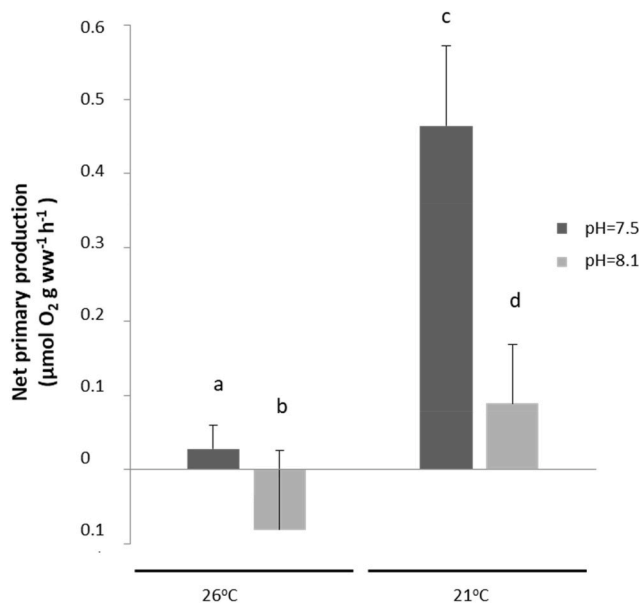


Fig. 3. Mean values ± SE of net primary production (NPP) by *Cymodocea nodosa* under each pH and temperature treatment (n = 8). Significant results are shown with different letters.

4. Discussion

In this study we highlighted negative, positive and neutral effects of global change on *C. nodosa* seagrass from Tenerife, Canary Islands, depending of factor analyzed, pH or temperature. The positive effects were observed under low pH, where higher growth rates were recorded as weight increase. However, negative effects of increase in temperature were registered on metabolic rates of this species independently pH effects, limiting its primary gross production at 26 °C. Neutral effects of pH and temperature were observed too on chlorophyll- a content. The ecological consequences of marine heatwaves can be catastrophic to the health of seagrass ecosystems in Canary Islands, where high temperatures, such as 26 °C, can limit their production. Additionally future acidification conditions, pH = 7.5, increased their vulnerability to grazing by *P. lividus*.

Neutral effects of temperature factor were observed on growth, weight increase or the morphometric variables measured. The effects of

ocean acidification or ocean warming on growth of many species of seagrass have been studied in numerous studies (Hernán et al., 2016; Borum et al., 2016; De los Santos et al., 2017; Ow et al., 2015; Palacios and Zimmerman, 2007), however few attention have been focused in multi stressor studies, incorporating multiple factors (Tomas et al., 2015; Egea et al., 2019; Ontoria et al., 2019b). In *C. nodosa*, diverse responses have been recorded about effects of warming on their growth (see review Nguyen et al., 2021), where decrease in growth rates and in biomass ratio have been observed (Savva et al., 2018; Marín-Guirao et al., 2018). In our study, combining temperature and pH, the temperature factor played no role on seagrass growth. Previous studies of *C. nodosa* growth in Tenerife Island under natural environmental conditions showed regular seasonal variations with their mean highest growth rates in spring-summer and their mean lowest ones in autumn-winter, where the sea surface is 23 °C and 21 °C respectively (Reyes et al., 1995a,b). An increase of 3 °C does not seem to favor the growth of *C. nodosa* from Canary Islands.

However low pH had a positive effect on weight increase, independently of temperature. The effects of low pH on seagrass growth are varied, Tomas et al. (2015) found no effect in the short term on leaf growth or rhizome elongation in *C. nodosa*, coinciding with the results obtained by Ow et al. (2015) with *C. serrulata* or in longer experiments

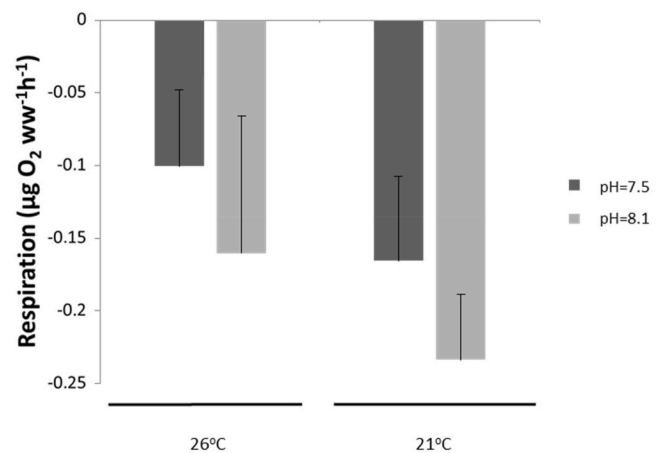


Fig. 4. Respiration rates (mean ± SE, n = 10) of *Cymodocea nodosa* in each treatment (n = 8): low pH- high temperature; low pH - control temperature, control pH - high temperature, control pH -control temperature.

Table 3

Results of 2-way ANOVA by permutations on oxygen concentration in: A) Net primary production (NPP), B) Respiration, and C) Gross primary production of *Cymodocea nodosa* under each pH and temperature treatment (n = 8). Significant effects highlighted in bold.

Variable	Source of variation	df	SS	MS	Pseudo-F	p(perm)	Pairwise test
A. Net primary Production (NPP)	pH treatment (pH)	1	0.50086	0.50086	11.842	0.0014	7.5 > 8.1
	Temperature treatment (T)	1	0.70036	0.70036	16.559	0.0001	21 °C > 26 °C
	pH*T	1	0.15794	0.15794	3.734	0.0664	
	Residual	28	1.1843	4.2295E-2			
	Total	31	2.5434				
B. Respiration (R)	pH treatment (pH)	1	0.11852	0.11852	0.52188	0.4698	
	Temperature treatment (T)	1	0.16713	0.16713	0.73593	0.3894	
	pH*T	1	3.33	3.33	0.48706	0.4942	
	Residual	56	12.718	0.2271			
	Total	59	13.026				
C. Gross Primary Production (GPP)	pH treatment (pH)	1	6.9371E-2	6.9371E-2	3.3726	0.0788	
	Temperature treatment (T)	1	0.26641	0.26641	12.952	0.0009	21 °C > 26 °C
	pH*T	1	3.722E-2	3.722E-2	1.8095	0.1939	
	Residual	28	0.57592	2.0569E-2			
	Total	31	0.94893				

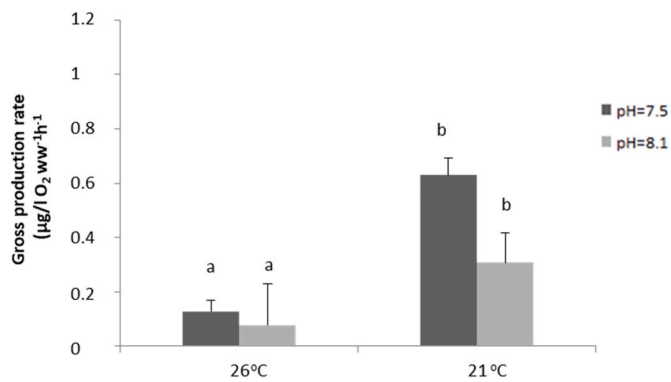


Fig. 5. Gross primary production rates (mean ± SE, n = 8) by *Cymodocea nodosa* under different treatments (n = 8): low pH- high temperature, low pH - control temperature, control pH -high temperature, control pH - control temperature. Significant results are shown with different letters.

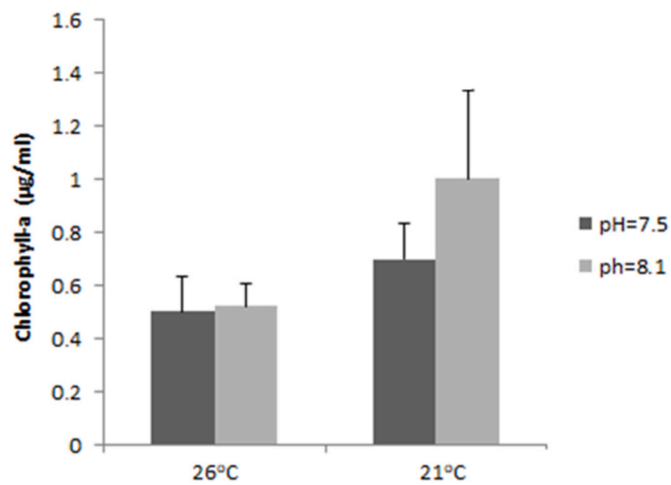


Fig. 6. Chlorophyll-a content (means ± SE) of *Cymodocea nodosa* under different treatments (n = 3): low pH- high temperature, low pH - control temperature, control pH - high temperature, control pH - control temperature.

(see Alexandre et al., 2012; Campbell and Fourqurean, 2013). However, a growth increment in leaf length of *C. nodosa* from Iberian region reared under 7.6 and 8.0 units of pH was reported in Egea et al. (2018b) and De los Santos et al. (2017) respectively. In our study the lack of effect on morphometric variables, except weight increase, may be explained by the short duration of the experiment or by low pH used (7.5 units), coinciding the neutral effect observed with De los Santos et al. (2017) when the seagrass were reared under 7.8 units of pH. On the other hand, the positive effects of low pH on weight could be attributed to having more resources stored to resist or recover from stressful conditions.

In the present study, higher oxygen production within the Net primary production (NPP) in plants subjected to low pH treatments was detected, coinciding with the results of other work with the same species (De los Santos et al., 2017). However in our study, gross primary production (GPP) was conditioned by temperature, noting a limitation in those treatments where the temperature was 26 °C, unlike the results obtained by Egea et al. (2018b) where the maximum values of NPP and GPP in *C. nodosa* were registered when reared at 26 °C. The differing responses obtained in the same species may be mainly due to *C. nodosa* from the Mediterranean and Iberian regions maintaining their production range between 10 and 32 °C (Drew, 1978; Pérez and Romero, 1992), where high temperature favors the enzymatic action in photosynthesis (Pérez and Romero, 1992; Terrados and Ros, 1995). In contrast, our case of *C. nodosa* from the Canaries does not have the same

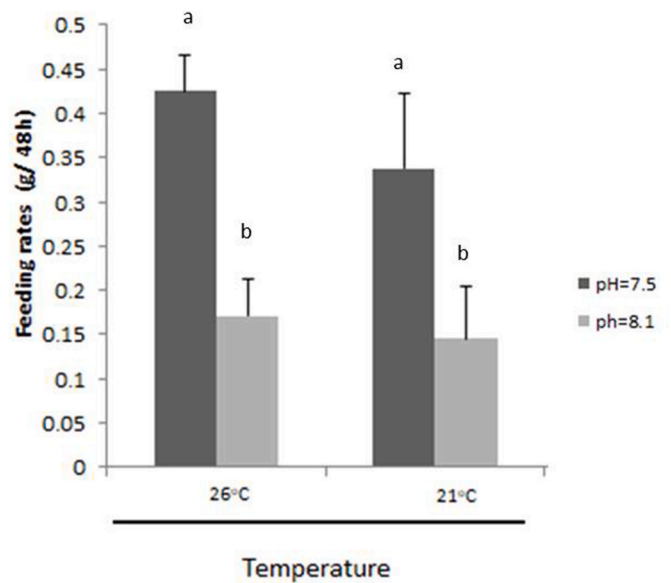


Fig. 7. Mean (±SE) feeding rates (g/48 h) of *Paracentrotus lividus* upon *Cymodocea nodosa* under each pH and temperature treatment in the laboratory experiment (n = 5). Significant results are shown with different letters.

Table 5

Results of two-way permutational ANCOVA comparing feeding rates of juvenile *Paracentrotus lividus* on *Cymodocea nodosa* between different temperature and pH treatments (n = 5). Sea-urchin size (S) measured as horizontal test diameter was chosen as covariable. Significant values in bold.

Herbivory						
Factor	df	SS	MS	Pseudo-F	p(perm)	Pairwise test
S	1	1.5091E-2	1.5091E-2	0.79126	0.3865	
pH	1	0.23638	0.23638	12.394	0.0023	7.5 > 8.1
T	1	1.5059E-2	1.5059E-2	0.78955	0.3799	
pHxT	1	3.8352E-3	3.8352E-3	0.20109	0.6561	
Res	15	0.28609	1.9072E-2			
Total	19	0.55645				

optimal thermal production range, as demonstrated by the results of this study, 26 °C being the limiting temperature for GPP, despite the fact that NPP is favored by lower pH. Other studies showed that under oceanic acidification conditions (7.6; 6.6 pH units) *P. oceanica* reaches higher levels of photosynthesis (Guilini et al., 2017). Net photosynthetic rates of *Thalassia testudinum*, *Zostera marina*, *Halodule wrightii* and *Syringodium filiforme* also responded positively to short-term decreases in pH (Campbell and Fourqurean, 2013; Thom, 1996; Zimmerman et al., 1997). Therefore, photosynthetic CO₂ responses appear to be species-specific and it is also possible that short-term laboratory experiments fail to show the potential effects of long-term exposure and adaptation. In other work with three species of seagrass (*Cymodocea serrulata*, *Halodule uninervis* and *Zostera muelleride*) the results show that, under conditions of global change, ocean acidification increases net primary production, while high temperature lowers it (Collier et al., 2018). The results obtained in this work show us that plants subjected to high temperature treatments have a lower gross primary production than those at control temperatures. This contrasts with Egea et al. (2018b), where GPP was favored by high temperature, again explained by the different responses of *C. nodosa* in the Canaries compared to the Mediterranean and Iberian regions. Under long-term global change in the Canary Islands, this study indicates that high temperature would be the factor that most harms the plants, thus counteracting the minor improvements caused by acidification. These results once again highlight the different responses of seagrasses to global change conditions

according to species, thermal tolerance, distribution range, environment, and genetic variability (Tuya et al., 2021; Alberto et al., 2008).

Differences in metabolic activity after the pH and temperature treatments were not reflected in chlorophyll-a content because it did not increase significantly, although photosynthetic activity increased at low pH. However, a trend was observed where the highest concentration of chlorophyll-a occurred at the control temperature, independent of pH. These results coincide with those of Hernán et al. (2017), in which *P. oceanica* was exposed to three temperature treatments (25, 27 and 29 °C) and also with those obtained in *C. nodosa* by Jiménez-Ramos et al. (2017). These authors also found no significant differences in chlorophyll-a content after undergoing different pH, temperature and nutrient treatments during a month of experimentation. The reasonable conclusion from these cases would be that if the NPP of plants subjected to global change was higher, it should be because they have more chlorophyll-a, possibly through their growth providing a larger surface area. However, this did not happen in any of the above research, or in our experiment. This may be because under high temperature treatments these plants increase their metabolic demands, causing stress and tissue necrosis (Hernán et al., 2017). Studies with other species of seagrass show similar responses, such as in *Thalassia hemprichii*, where lower pH led to a significant improvement in photosynthetic performance despite no concurrent increase in chlorophyll-a content (Jiang et al., 2010). In other trials in the natural environment, an increase in chlorophyll-a content was detected according to the higher photosynthetic rates under CO₂ enrichment (see Apostolaki et al., 2014 for *C. nodosa*), allowing plants to invest in biochemical pathways for C and electron fixation. All of this suggests that experimentation time is a limiting factor, longer exposure being needed for these changes to be appreciable.

Our study show the vulnerability of plants reared at low pH to being consumed by the sea urchin *P. lividus*. Low pH played a role more important than increase a temperature. The results suggest than these higher consumptions by sea urchins could be explained by the lower nutritional energy value of these plants; they would have to graze more to become satiated. On the other hand, structurally they are weaker and easier to consume. This situation seems to indicate that nutritional content is essential to interpret these data, as well as chemistry and fiber content, as suggested by various studies (De los Santos et al., 2012, 2017; Hernán et al., 2016; Jiménez-Ramos et al., 2017). In other studies with *C. nodosa* was also reflected higher consumption by herbivores on submitting plants to low pH (7.87 and 7.69 units) and high nutrient concentrations, in Tomas et al. (2015) and Jiménez-Ramos et al. (2017) respectively. In this study the temperature play not role on herbivore however in others studies such as Hernán et al. (2017), using *P. oceanica* with only temperature treatments, also showed higher consumption by herbivores under high temperature treatments. Our results suggest that low pH may trigger an increase in the grazing of seagrass tissues, with important implication for the resilience of seagrass ecosystems.

Seagrass species act differently in the face of environmental changes, even within the same species depending on the region (Tuya et al., 2019). Diverse studies have shown that populations of the same species may differ in their thermal tolerance across its distribution range (Bennett et al., 2019; 2021; King et al., 2018; Marín-Guirao et al., 2018). Other authors have obtained similar results by comparing the negative effects of marine heat waves (MHW) on various seagrasses (Fraser et al., 2014; Marba and Duarte, 2010; Reusch et al., 2005; Thomson et al., 2015). Genetic differences are also important, leading to varied resistance mechanisms and adaptations to change, such as *C. nodosa* in the Mediterranean and Atlantic, showing how a high range of genetic variability gives rise to different responses to change. This is the case of Mediterranean *C. nodosa* meadows, which are more resistant to high temperatures due to their greater genetic variability (Alberto et al., 2008). In this study we highlight the different responses of *C. nodosa* from the Canaries against future conditions of global change and we contrast them with those obtained on *C. nodosa* from Mediterranean and

Iberian region. The high plasticity and high genetic variability of *C. nodosa* from the Iberian region aid in its resistance to warming (Egea et al., 2018b; Marín-Guirao et al., 2018; Savva et al., 2018). Contrastingly, *C. nodosa* from the Canaries has a low genetic variability (Alberto et al., 2008) and its tolerance range term is narrower than the same species from the Mediterranean and Iberian regions. These factors highlight the negative effect on GPP, limiting its metabolic rates at 26 °C and above.

In conclusion, we results show a negative response of *C. nodosa* from Canary Islands to the forecast future conditions of warmer temperature and CO₂ increase as its productivity was aggravated by rise in temperature in low and control pH conditions. Marine heatwaves can be catastrophic to the health of seagrass ecosystems in Canary Islands as suggested by various studies on other seagrasses (Fraser et al., 2014; Marba and Duarte, 2010; Reusch et al., 2005; Thomson et al., 2015). Recent studies already show a clear decline in the *C. nodosa* meadows in the Canary Islands (Tuya et al., 2013a, b), where natural (storms, diseases) and anthropogenic factors (eutrophication, temperature) often interact with each other, which are recognized as major threats seagrass survival (Tuya et al., 2013b, Grech et al., 2012). In future years is possible that the temperature plays a role fundamental in decline of Canary Islands meadows. In addition, low pH led to an overall increase in plant palatability and, therefore, enhanced their consumption by a generalist herbivore *Paracentrotus lividus*, which could strengthen top-down effects in seagrass habitats (Tomas et al., 2015; Jiménez-Ramos et al., 2017) and modify the fluxes of matter and energy in coastal areas (Lavery et al., 2013). Even though we found negative effects, it is important to keep in mind that extrapolating these results to *in situ* conditions must be done with caution, since complex relationships in the ecosystem and other indirect effects may should these effects. Overall future research is required to address longer exposure times to global change conditions, so as to more precisely evaluate the plasticity of this key species in response to global change and the need of actions to evaluate the future conservation status of *C. nodosa* meadows in Canary Islands. Furthermore is necessary promoting a management actions to conserve *C. nodosa* meadow of Canary Islands under a global change vision.

Author statement

AR and AB planned and designed the study. AR and SM conducted the experiments. AR analyzed the data. AR led the writing, with contributions from the other authors. All authors reviewed the manuscript.

Declaration of competing interest

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

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