


Short-term interactive effects of ultraviolet radiation, carbon dioxide and nutrient enrichment on phytoplankton in a shallow coastal lagoon

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Abstract The main goal of this study was to evaluate short-term interactions between increased CO₂, UVR and inorganic macronutrients (N, P and Si) on summer phytoplankton assemblages in the Ria Formosa coastal lagoon (SW Iberia), subjected to intense anthropogenic pressures and highly vulnerable to climate change. A multifactorial experiment using 20 different nutrient-enriched microcosms exposed to different spectral and CO₂ conditions was designed. Before and after a 24-h in situ incubation, phytoplankton abundance and composition were analysed. Impacts and interactive effects of high CO₂, UVR and nutrients varied among different functional groups. Increased UVR had negative effects on diatoms and cyanobacteria and positive effects on cryptophytes, whereas increased CO₂ inhibited cyanobacteria but increased cryptophyte growth. A positive synergistic interaction between CO₂ and UVR was observed for

diatoms; high CO₂ counteracted the negative effects of UVR under ambient nutrient concentrations. Nutrient enrichments suppressed the negative effects of high CO₂ and UVR on cyanobacteria and diatoms, respectively. Beneficial effects of CO₂ were observed for diatoms and cryptophytes under combined additions of nitrate and ammonium, suggesting that growth may be limited by DIC availability when the primary limitation by nitrogen is alleviated. Beneficial effects of high CO₂ and UVR in diatoms were also induced or intensified by ammonium additions.

Keywords Nutrient limitation · Stress responses · Ultraviolet radiation · Acidification · Coastal lagoons

Introduction

Predicting how phytoplankton communities will reorganize in the future in response to multifaceted simultaneous changes to their environment is currently a major scientific challenge (Litchman et al. 2012; Reusch and Boyd 2013), vital for conservation and predicting ecosystem function and services (Collins 2011). Several research directions have been recently suggested to advance this field and increase its predictive power, including the collection of experimental data on phytoplankton major functional traits, such as growth and production responses to nutrient enrichment, light, ultraviolet radiation,

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warming and ocean acidification, and its combination with distribution patterns along environmental gradients (Litchman et al. 2012).

Although closely associated with climate change, these phytoplankton stressors also act on shorter timescales, affecting phytoplankton growth and mortality. For instance, ultraviolet radiation reaching the sea surface depends on cloud cover, which can vary on very short timescales, leading to intermittent exposure to either high UVR levels or limiting PAR intensities (Gao et al. 2007). Large diurnal fluctuations in pH are observed in coastal waters, unrelated to ocean acidification (Nielsen et al. 2012). Sea surface temperature may also vary significantly on a daily basis (Stuart-Menteth et al. 2003), with day–night differences up to 3.5 °C (Stramma et al. 1986).

Therefore, short-term perturbation experiments provide important insights on phytoplankton ecology and physiology and have been extensively used by ecologists to understand the (short-term) effects of environmental stressors on phytoplankton (e.g., Varona-Cordero et al. 2014; Reul et al. 2014; Sala et al. 2015). A caveat though, short-term incubations are not the most adequate to address phytoplankton responses over longer, climate-related timescales (Reusch and Boyd 2013), given that environmental change in natural ecosystems will not happen abruptly, but rather gradually, over much longer temporal scales (Collins 2011; Reusch and Boyd 2013). In nature, phytoplankton are progressively exposed to these changes and respond not only through phenotypic plasticity (short-term responses), but also through species sorting, genetic adaptation or a combination of these (Litchman et al. 2012). Evolution experiments, with longer incubation times, are the most adequate to address adaptive responses to climate change (Reusch and Boyd 2013) and other environmental drivers over longer timescales. However, experimental approaches with longer incubation times are laborious and require a thorough planning, including previous indications on phytoplankton responses to individual and multiple drivers, as well as their synergistic and antagonistic effects. These effects can be effectively addressed with short-term manipulation experiments, representing an initial step to comprehend phytoplankton acclimation and adaptation to short- and long-term environmental changes.

Ocean biota is indeed subjected to a complex matrix of multiple environmental change variables

that act on short- and long-term scales, and it is increasingly evident that synergistic and antagonistic interactions among drivers are essential to provide more realistic predictions of future ecosystems changes (Doyle et al. 2005; Boyd and Hutchins 2012). However, these complex interactions also present large uncertainties when predicting ecological change (Piggott et al. 2015). In addition, experimentally derived knowledge on combined impacts of multiple environmental drivers on natural phytoplankton communities, either short- or long-term, is limited (Doyle et al. 2005; Bouvy et al. 2011). For instance, and to the best of our knowledge, simultaneous manipulation of CO₂, UVR and nutrient enrichment was only undertaken in two aquatic ecosystems, the Ross Sea (Feng et al. 2010) and Mediterranean coastal waters (Neale et al. 2014 and references therein).

In this context, the main goal of this study is to evaluate the immediate isolated and interactive effects of increased CO₂, UVR and inorganic macronutrients (N, P and Si) on summer phytoplankton assemblages in the Ria Formosa coastal lagoon (southern Portugal), using a short-term multifactorial experimental design with nutrient-enriched microcosms subjected to different spectral and CO₂ conditions. This lagoon is one of the most important aquatic ecosystems in Portugal, from a biological and social–economical perspective. It is located in an extremely vulnerable region to climate change (IPCC 2014), and it is subjected to strong anthropogenic pressures. Previous experiments in the Ria Formosa have included the simultaneous manipulation of CO₂ and UVR (Domingues et al. 2014), and nutrients and light (Domingues et al. 2015). We hypothesize that UVR will have negative effects on phytoplankton growth, which will be compensated by increased CO₂ and by the alleviation of nutrient limitation.

Materials and methods

Study site

The Ria Formosa coastal lagoon is a shallow multi-inlet euryhaline system, located in the southern coast of Portugal and subjected to mediterranean climate with hot, dry summers and moderate winters, in a region classified as very vulnerable to climate change (IPCC 2014) (Fig. 1). The lagoon has a total humid area of 105 km² and a mean depth of 2 m and extends 55 km

(E–W) and 6 km (N–S) at its widest point (Andrade et al. 2004). Tides are semidiurnal and mesotidal, with tidal amplitudes ranging between 1.3 m during neap tides and 3 m during spring tides (Newton et al. 2003). The water column is well mixed, with no persistent or widespread haline or thermal stratification (Newton and Mudge 2003). The lagoon is a breeding and feeding ground for fish and birds and is subjected to multiple anthropogenic stressors, supporting a wide range of human activities, including tourism, fisheries and shellfish farming (Barbosa 2010); the Ria Formosa and its hinterland are also protected by national and international legislation. The coastal region adjoining the Ria Formosa, inserted in the Gulf of Cadiz, is affected by regular upwelling events that also impact the outer section of the lagoon, extending to at least 6 km upstream from the lagoon inlets (Barbosa 2010; Cravo et al. 2014). To minimize the influence of adjacent coastal waters, sampling was conducted at an inner lagoon location during low tide (Fig. 1).

Experimental set-up

The experiment was carried out in early September 2012 under typical summer conditions (see Barbosa

2010 and “Results” section). Sub-surface (approximately 0.5 m) water samples were collected into translucent 4.5 L UVR-transparent LDPE cubitainers (Nalgene I-Chem Certified Series LDPE cubitainers) with a reduced CO₂ diffusive loss (2.9 Pa day⁻¹; Sobrino et al. 2009). Water samples were not pre-screened, given that this procedure may significantly alter the structure of the initial phytoplankton community, thus increasing the problems associated with the extrapolation of experimental outcomes to natural communities (Nogueira et al. 2014). Two different spectral treatments were prepared: (a) exposure to photosynthetically active radiation (PAR) and UVR (i.e., total solar radiation), obtained by covering the cubitainers with a net that allowed transmission of 86 % of incident light, and (b) exposure to PAR only, obtained by covering the cubitainers with UV-absorbing film Llumar SHE ER PS7 that allowed transmission of 87 % of incident PAR and eliminated >99 % of incident UVR. In both spectral treatments, incident irradiance was higher than mean PAR and UVR intensities in the mixed layer. In addition, UV-B radiation attenuates rapidly in the water column in the Ria Formosa, approximately 91 % in the first 0.5 m (Machado 2010).

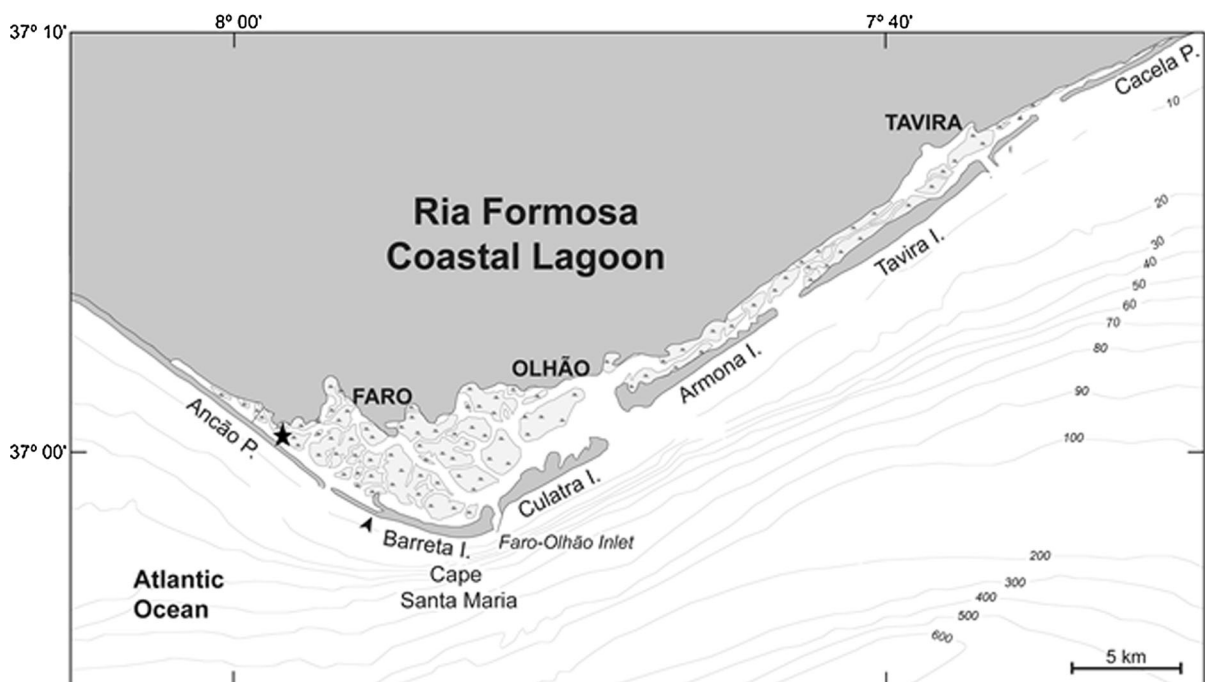


Fig. 1 Location of the Ria Formosa coastal lagoon and sampling station (black star)

For each spectral treatment, experimental treatments with ambient CO₂ and high CO₂ concentrations were prepared. CO₃²⁻ (as Na₂CO₃), HCO₃⁻ (as NaHCO₃) and HCl 0.01 N were added to each cubitainer to increase pCO₂ and DIC to the levels expected for the year 2100, according to Gattuso et al. (2010). Partial pressure of CO₂ (pCO₂) in each cubitainer at the beginning and end of the incubation period was estimated with Seacarb package for R. Alkalinity, determined by titration (Parsons et al. 1984), and pH were used as input variables. CO₂ partial pressure (pCO₂) in the beginning of incubation ranged between 418 and 712 µatm in the low- and high-CO₂ treatments, respectively, corresponding to pH values of 8.03 and 7.88. After 1-day incubation, pCO₂ and pH were 400 ± 218 µatm and 8.14 ± 0.14, respectively, in the low CO₂ treatments and 651 ± 236 µatm and 7.93 ± 0.13 in the high CO₂ treatments.

Four different experimental treatments were thus used to test the effects of increased CO₂ and UVR: “PAR” (PAR only, ambient CO₂ levels), “high CO₂” (PAR only, high CO₂), “UVR” (PAR + UVR, ambient CO₂) and “UVR + high CO₂” (PAR + UVR, high CO₂). For each of these four experimental treatments, five different nutrient-enriched treatments were prepared by adding saturating concentrations of N, P and Si in a single pulse, as follows: control (no nutrient additions); +NIT (100 µM of nitrate as potassium nitrate), +AMM (100 µM of ammonium as ammonium chloride), +NN (50 µM of nitrate and 50 µM of ammonium) and +NPSi (100 µM of nitrate, 100 µM of silicate as sodium hexafluorosilicate and 6.25 µM of phosphate as potassium dihydrogen phosphate). These concentrations were based on previous knowledge on nutrient availability and limitation in the Ria Formosa lagoon (Barbosa 2010) and were intended to be higher than nutrient concentrations in situ, to avoid the potential occurrence of nutrient limitation that would mask the effects of other variables on phytoplankton growth. Hence, a total of 20 experimental treatments, prepared in duplicate ($n = 40$), were used to test the isolated and combined effects of increased CO₂, increased UVR and increased dissolved inorganic macronutrients.

The cubitainers were incubated in situ for 24 h at the water surface, fixed to a buoy and exposed to in situ temperature, natural light–dark cycle, and PAR and

Fig. 2 Abundance (left column) and biomass (right column) of the main phytoplankton groups (diatoms, cryptophytes and cyanobacteria) at the beginning and end of the experiments. **a**, **b** Treatment PAR; **c**, **d** treatment high CO₂; **e**, **f** treatment UVR; **g**, **h** treatment UVR + high CO₂

UVR intensities higher than the mean light intensity in the mixed layer, to simulate the effects of increased radiation. This short incubation time was chosen due to the high metabolic rates of summer phytoplankton in the Ria Formosa (see Barbosa 2006) and to minimize the occurrence of “bottle effects” (see Nogueira et al. 2014). At the beginning and end of incubation, aliquots were taken from each experimental treatment for the determination of pH and alkalinity, nutrient concentration, chlorophyll *a* concentration, phytoplankton composition and abundance, and photosynthetic parameters. All materials used in the experiments and laboratorial analyses were previously washed with HCl 10 % and thoroughly rinsed with deionized water.

Physical–chemical variables

Photosynthetically active radiation (PAR) intensity was measured with a LI-COR radiometer, and mean light intensity in the mixed layer (I_m) was calculated as:

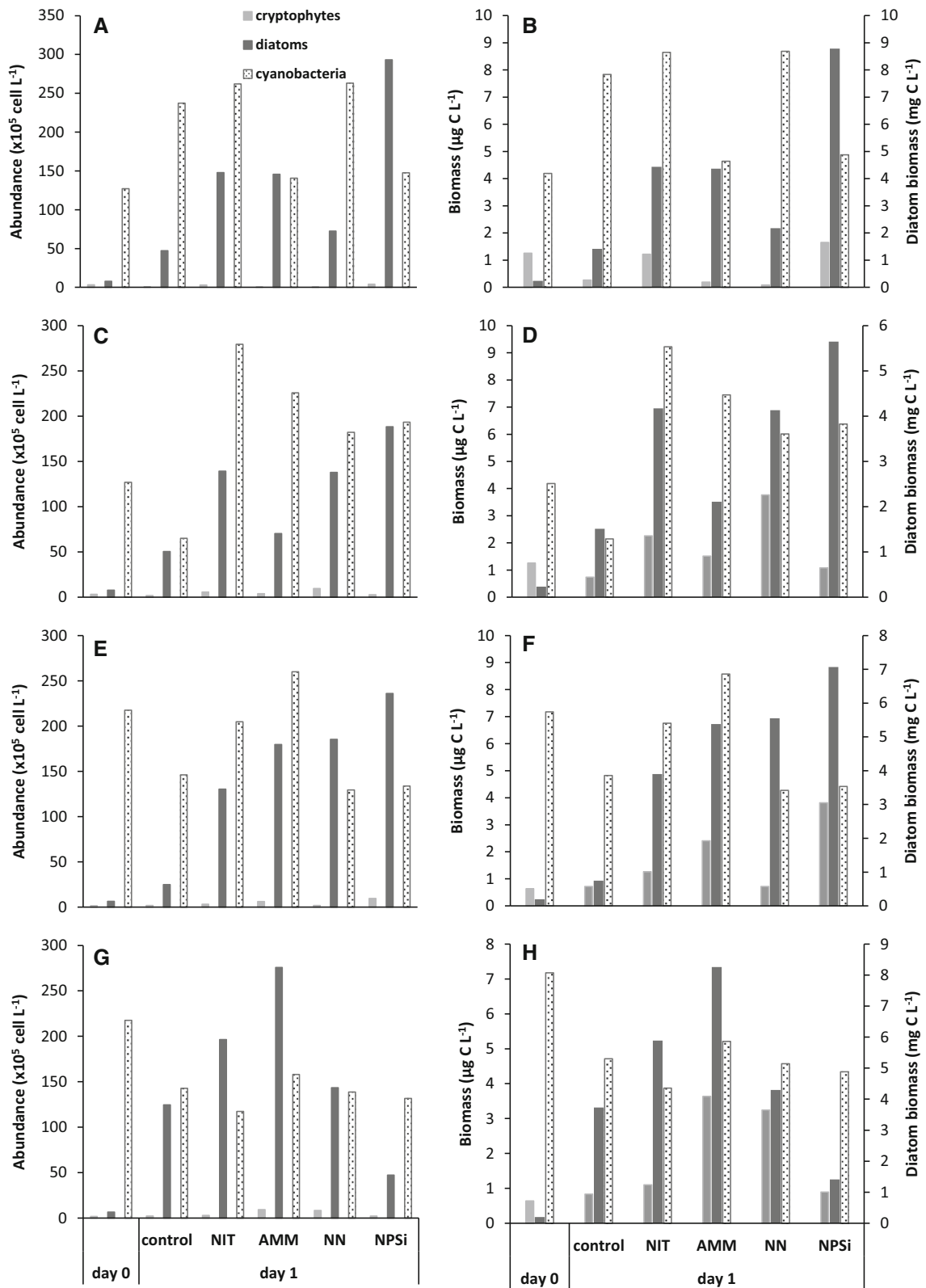
$$I_m = I_0 \left(1 - e^{(-K_e Z_m)} \right) (K_e Z_m)^{-1} \quad (1)$$

where Z_m (m) represents the mean depth of the mixed layer (2 m) and K_e (m⁻¹) is the light attenuation coefficient, estimated from the vertical profiles of PAR intensity versus depth, as:

$$I_z = I_0 e^{-K_d Z} \quad (2)$$

where I_z represents the light intensity at depth Z (m) and I_0 is the light intensity at the surface. Water temperature was measured with an YSI probe.

Samples for determination of dissolved inorganic macronutrient concentrations were filtered through 0.2-µm cellulose acetate filters. Soluble reactive phosphorus, dissolved silicate and ammonium were analysed immediately after sampling using spectrophotometric methods described by Grasshoff et al. (1983) on a spectrophotometer Hitachi U-2000. Samples for nitrate and nitrite were frozen (−20 °C) until analysis on a Skalar autoanalyser.



Phytoplankton biomass, abundance and composition

Chlorophyll *a* concentration was determined spectrophotometrically using glass fibre filters (GF/F, Whatman, nominal pore diameter = 0.7 µm), after extraction with acetone 90 % (Parsons et al. 1984).

Phytoplankton composition and abundance were determined using both epifluorescence (Haas 1982) and inverted microscopy (Utermöhl 1958). Samples for enumeration of cyanobacteria and autotrophic nanoflagellates were preserved with glutardialdehyde (final concentration 2 %) immediately after collection, stained with proflavine and filtered (<100 mm Hg) onto black polycarbonate membrane filters (Whatman, nominal pore diameter = 0.45 µm). Preparations were made within 24 h of sampling using glass slides and Cargille type A immersion oil and then frozen (−20 °C) in dark conditions, to minimize loss of autofluorescence. Enumeration was made at 787.5× magnification using an epifluorescence microscope (Leica DM LB). Samples for enumeration of diatoms and other microphytoplankton were preserved with acid Lugol's solution (final concentration c. 0.003 %) immediately after collection, settled in sedimentation chambers and observed at 400× magnification using an inverted microscope (Zeiss Axio Observer A1). For both methods, a minimum of 50 random visual fields, at least 400 cells in total and 50 cells of the most common genus were enumerated. Assuming that phytoplankton cells were randomly distributed, counting precision was ±10 % (Venrick 1978).

Phytoplankton abundance of specific phytoplankton groups was converted to biomass using carbon content mean values for specific phytoplankton groups, obtained within a 12-month monitoring programme in the Ria Formosa coastal lagoon. Values used for the three main phytoplankton groups were 299.61 pg C/cell for diatoms, 3.91 pg C/cell for cryptophytes and 0.33 pg C/cell for cyanobacteria (Domingues et al., unpublished data).

Phytoplankton net growth rates

Chlorophyll *a* was used as a proxy to calculate phytoplankton community net growth rates, whilst abundances were used to estimate net growth rates of specific phytoplankton functional groups. Net growth rates (day^{−1}) for each experimental treatment were

calculated assuming exponential growth as $(\ln N_t - \ln N_0)/t$, where N_0 and N_t represent Chl *a* concentration or cell abundance at the beginning and end of the experiment, respectively, and t represents incubation time.

Data analyses

Data analyses were performed using IBM SPSS Statistics 22 software. Data normality and homogeneity of variances were tested with Shapiro–Wilk and Levene's tests, respectively. For most dependent variables, normality assumptions were not met. Data transformation (reflect and square-root transformation for moderately, negatively skewed data) did not improve data normality. The nonparametric Mann–Whitney test was used to evaluate individual effects of nutrients (control vs. nutrient addition), CO₂ (ambient vs. high) and UVR (PAR vs. UVR) on phytoplankton net growth rates. For the evaluation of interactive effects between the three independent variables, a three-way ANOVA with replication was used. This parametric test was used because it is considered robust to violations of normality and no nonparametric alternatives exist. Nevertheless, results were regarded with caution (Dytham 2003). A Bonferroni post hoc test was used to assess significant differences between each experimental treatment and the control. All statistical analyses were considered at a 0.05 significance level.

Results

Environmental background

At time of sample collection, mean water temperature, mean incident PAR (I_0) and mean PAR intensity in the mixed layer (I_m) were 23.5 °C, 1115 µmol photons m^{−2} s^{−1} and 536 µmol photons m^{−2} s^{−1}, respectively, with approximately 13 h of daily solar insolation. Dissolved inorganic nitrogen (DIN = nitrate + nitrite + ammonium) concentration at the beginning of the experiments was 3.5 ± 2.8 µM. Dissolved reactive phosphorus and dissolved silica concentrations were 0.49 ± 0.12 and 8.41 ± 1.20 µM, respectively. Mean nutrient ratios at the beginning of the experiments were: NP = 7.1; SiN = 2.4; and SiP = 17.2.

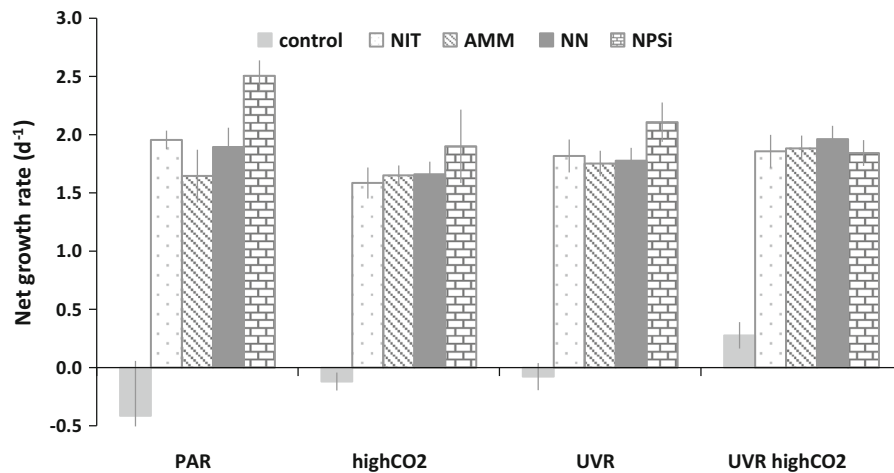


Fig. 3 Net growth rates (day^{-1}) of the phytoplankton community (based on chlorophyll *a* concentration), in the different nutrient, CO_2 and spectral treatments. Vertical lines represent ± 1 SE

Table 1 *p* values for the effects and interactions between CO_2 , UVR and nutrient enrichment for net growth rates of community and specific phytoplankton groups, obtained with three-way ANOVA, assuming a significance level of 0.05

	Community	Diatoms	Cryptophytes	Cyanobacteria
UVR	ns	0.000	0.000	0.000
CO_2	ns	ns	0.000	ns
NUTS	0.000	0.000	0.005	ns
$\text{UVR} \times \text{CO}_2$	ns	0.028	0.000	ns
$\text{UVR} \times \text{NUTS}$	ns	0.000	0.002	ns
$\text{CO}_2 \times \text{NUTS}$	ns	0.000	0.000	ns
$\text{UVR} \times \text{CO}_2 \times \text{NUTS}$	ns	0.017	ns	0.023

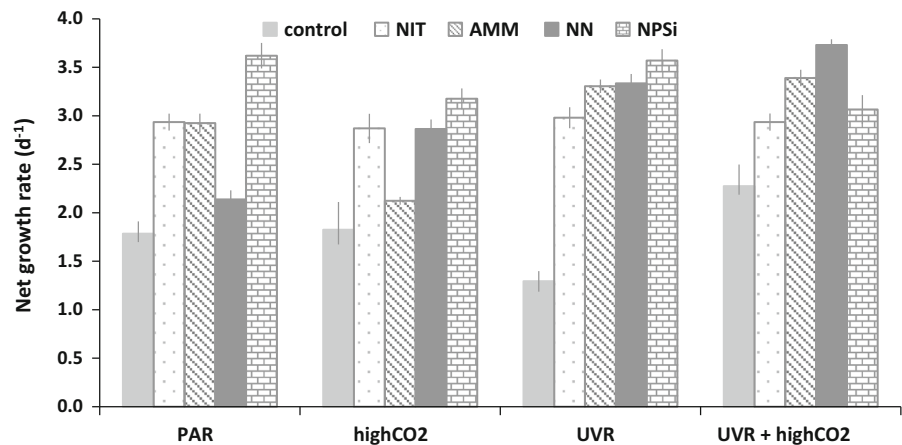
ns non-significant

Chlorophyll *a* concentration and phytoplankton abundance at the beginning of the experiments were $2.22 \pm 0.76 \mu\text{g C L}^{-1}$ and $1.82 \times 10^7 \pm 0.51 \times 10^7 \text{ cell L}^{-1}$, respectively. *Synechococcus*-like coccoid picocyanobacteria dominated the community, contributing 94 % ($1.72 \times 10^7 \pm 0.52 \times 10^7 \text{ cell L}^{-1}$) to total abundance. Diatoms ($7.21 \times 10^5 \pm 0.70 \times 10^5$), dominated by the solitary genera *Rhizosolenia* and *Thalassiosira*, and cryptophytes ($2.42 \times 10^5 \pm 0.82 \times 10^5 \text{ cell L}^{-1}$), dominated by nano-sized forms, accounted for 4 and 1.5 % of phytoplankton abundance, respectively (Fig. 2). In terms of biomass, diatoms dominated the community at the beginning of the experiments ($216.0 \pm 20.9 \mu\text{g C L}^{-1}$), followed by cyanobacteria ($5.7 \pm 1.6 \mu\text{g C L}^{-1}$) and cryptophytes ($0.9 \pm 0.3 \mu\text{g C L}^{-1}$), corresponding to 97, 2.6 and 0.4 %, respectively (Fig. 2).

Effects of increased nutrients, CO_2 and UVR on phytoplankton composition and growth

Phytoplankton community net growth rates (based on chlorophyll *a*) for all nutrient-enriched treatments ($1.65 \pm 0.23 \text{ day}^{-1} - 2.51 \pm 0.13 \text{ day}^{-1}$) were significantly higher ($p < 0.001$) in relation to the control ($-0.42 \pm 0.47 \text{ day}^{-1}$), and nitrogen source (nitrate, ammonium or both) had no significant effects on phytoplankton growth rate (Fig. 3). Bonferroni's post hoc tests showed no significant differences in net growth rates among the different nutrient additions. Overall, summer phytoplankton community in the Ria Formosa coastal lagoon was potentially limited by nitrogen. No interactive effects of nutrients, CO_2 and UVR on phytoplankton community net growth rates were revealed by three-way ANOVA (Table 1).

Fig. 4 Net growth rates (day^{-1}) of diatoms, in the different nutrient, CO_2 and spectral treatments. Vertical lines represent ± 1 SE



Diatom responses to nutrient enrichment mirrored those of the community, responding positively to N-additions in all experimental treatments (Fig. 4). Diatom net growth rates were significantly affected by nutrients and UVR exposure alone, but high CO_2 levels had no significant effect on growth rates, in the non-amended nutrient treatments. Two-way interactions between UVR and CO_2 ($p = 0.028$), UVR and nutrients ($p < 0.001$), and CO_2 and nutrients ($p < 0.001$) and also three-way interactions among the three independent variables ($p = 0.017$) were observed (Table 1). Specifically, in the nutrient-limited treatments (control), UVR exposure led to a decrease in diatom growth rates, whereas the combination of UVR and high CO_2 alleviated the negative effect of UVR on growth. Ammonium enrichments combined with high CO_2 and UVR significantly increased diatom net growth rates. Diatom abundance

and biomass (Fig. 2) also increased in all experimental treatments, and in some treatments exposed to UVR, diatom abundance even surpassed cyanobacteria (Fig. 2e, f). Overall, phytoplankton community structure in all experimental treatments changed towards diatom dominance.

Significant and positive responses of cryptophytes to nutrient addition were observed in treatments NIT ($p = 0.032$) and NPSi ($p = 0.005$), under PAR and ambient CO_2 levels (Fig. 5). Net growth rates of cryptophytes were significantly higher ($p = 0.029$) in the UVR treatments in relation to the control (PAR). Increases in CO_2 and UVR, either alone or in combination, were associated with significantly higher net growth rates for most nutrient additions, and significant two-way interactions between nutrients and CO_2 ($p < 0.001$) and CO_2 and UVR ($p < 0.001$) were observed (Table 1). Stimulation of cryptophyte net

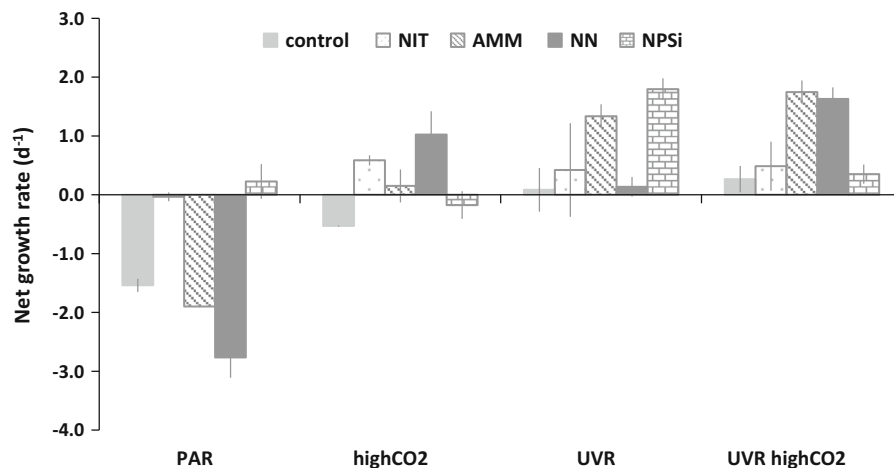


Fig. 5 Net growth rates (day^{-1}) of cryptophytes in the different nutrient, CO_2 and spectral treatments. Vertical lines represent ± 1 SE

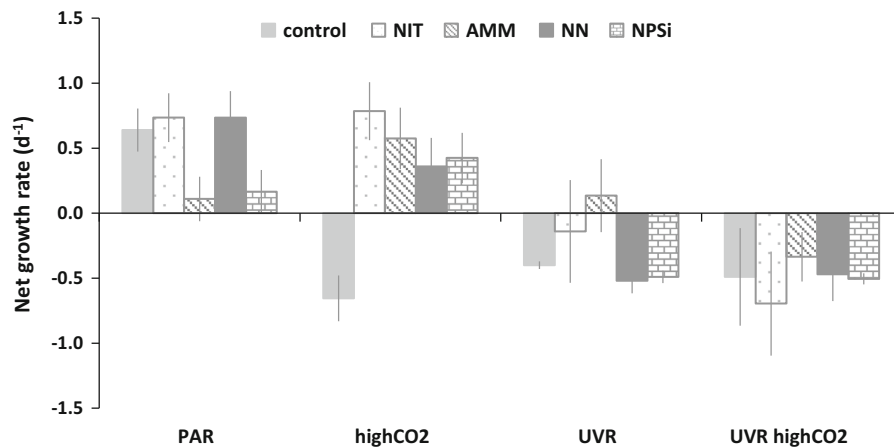


Fig. 6 Net growth rates (day^{-1}) of cyanobacteria in the different nutrient, CO_2 and spectral treatments. Vertical lines represent ± 1 SE

growth rates by increased CO_2 and/or UVR was more pronounced after ammonium additions (AMM and NN) (Fig. 5). In terms of abundance and biomass, cryptophytes remained the least important group in all experimental treatments (Fig. 2).

Cyanobacteria net growth rate was not affected by nutrients in the experimental treatments (Fig. 6). Conversely, net growth rates of cyanobacteria were negative in all CO_2 and UVR treatments in relation to the control (PAR), but these differences were not statistically significant, probably due to a high variability between replicates. Significant three-way interactions among nutrients, CO_2 and UVR ($p = 0.023$) on net growth rates were observed (Table 1). Growth rates were significantly lower under exposure to UVR, particularly after nitrate + ammonium (NN) and NPSi additions. An inhibitory effect of increased CO_2 , in isolation, was observed on cyanobacteria growth rates, but this negative effect was eliminated after nutrient additions (Fig. 6). Cyanobacteria importance in terms of abundance was surpassed by diatoms in some of the UVR treatments. Overall, cyanobacteria abundance and biomass decreased in the UVR treatments and increased in the high CO_2 treatments (Fig. 2).

Discussion

We hypothesized that UVR would have negative effects on phytoplankton growth, which would be compensated by increased CO_2 and by the alleviation of nutrient limitation. Indeed, nutrient limitation was

clear in our experiments, regardless of CO_2 levels and UVR exposure; community and diatom growth were limited by nitrogen (N), and cryptophytes seemed to be limited by phosphorus (P) or co-limited by N and P. UVR had positive effects on cryptophytes growth, under ambient nutrient levels, but negative effects on cyanobacteria and diatoms. Nutrient addition alleviated the negative effects of high CO_2 on cyanobacteria, and the combination of nutrients, particularly ammonium, and high CO_2 increased diatom growth rates under UVR exposure.

Regarding potential nutrient limitation, community response to nutrient enrichment was strongly associated with the response of diatoms, which dominated in terms of biomass (97 %). Nutrient limitation, particularly by nitrogen, is a common occurrence in the Ria Formosa coastal lagoon during summer (Domingues et al. 2015), when nutrient concentrations attain their lowest values (Barbosa 2010), and it has been experimentally demonstrated for the phytoplankton community and, specifically, diatoms (Loureiro et al. 2005; Domingues et al. 2015). However, a decoupling between community biomass (measured as chlorophyll *a* concentration) and diatom abundance was observed in the non-enriched treatments, where the former decreased over time, whilst the latter increased. This may be an indication of cell acclimation to higher light intensities (PAR exposure during the experiments was higher than I_m) by decreasing the cellular content of Chl*a*. Indeed, chlorophyll *a* concentration may not always represent the ideal proxy for phytoplankton biomass (see Domingues et al. 2008).

Unlike diatoms, cryptophytes were stimulated only under nitrate enrichments (NIT and NPSi), denoting a preferential use of nitrate and/or an inhibitory effect of ammonium on growth. In addition, the more pronounced growth response in treatment NPSi indicates co-limitation by nitrogen and phosphorus. Nutrient enrichment experiments conducted a few weeks earlier at the same location in the Ria Formosa coastal lagoon showed no nutrient limitation of cryptophytes growth (Domingues et al. 2015), suggesting a significant short-term variability in nutritional conditions and/or requirements. In contrast to diatoms and cryptophytes, *Synechococcus*-like picocyanobacteria were not nutrient limited, probably due to low half-saturation constants for nutrient uptake (Timmermans et al. 2005; Domingues et al. 2015).

Increased UVR was associated with negative effects on diatoms and cyanobacteria growth and positive effects on cryptophytes, whereas increased CO₂ inhibited cyanobacteria and increased cryptophytes net growth rates. Overall, short-term exposure to increased UVR and/or CO₂ under ambient nutrient concentrations led to changes in summer phytoplankton community structure in the Ria Formosa, from a dominance by *Synechococcus*-like picocyanobacteria towards dominance by centric diatoms. The same trend was observed in the Ria Formosa for winter phytoplankton assemblages (Domingues et al. 2014). These changes in phytoplankton community structure were mostly due to deleterious effects of UVR and CO₂ on cyanobacteria. *Synechococcus* net growth rates were negative in all UVR treatments, independently of nutrient enrichment, as previously referred for the Ria Formosa during winter (Domingues et al. 2014). *Synechococcus* mortality under UV exposure is well established, even at natural UV intensities (Llabrés et al. 2010), because, unlike larger phytoplankton, this dominant cyanobacteria and other picoplankters have low levels of photoprotective compounds (Garcia-Pichel 1994; but see Rastogi et al. 2014). Additionally, the high susceptibility of *Synechococcus* to UVR is probably related to its small size and high surface-to-volume ratio, leading to a shorter path length for UV absorption (Beardall et al. 2009a). Increased CO₂, under ambient nutrient concentration, also induced a significant decline in cyanobacteria net growth rates, as previously reported for the Ria Formosa and other ecosystems (Paulino et al. 2008; Domingues et al. 2014). However,

contrasting results, including no CO₂ effects (Fu et al. 2007) or increased growth rates (Sobrinho et al. 2009), have been observed for cyanobacteria. Downregulation of the metabolically costly carbon concentrating mechanisms (CCMs) of most cyanobacteria, including *Synechococcus*, under increased CO₂ concentrations, may increase the allocation of energy and nutrients available to other cellular processes, thus leading to increased growth rates (Badger et al. 2006; see Domingues et al. 2014). However, under ambient nutrient conditions, *Synechococcus* in the Ria Formosa during both winter (Domingues et al. 2014) and summer (this study) were apparently not able to explore this pathway.

Diatom net growth rates also decreased after short-term exposure to increased UVR under ambient (limiting) nutrient concentrations. Effects of UVR on diatoms are highly variable in the literature (Gao et al. 2012a; Domingues et al. 2014), but diatoms are usually considered highly resistant to UVR, due to the UV-absorbing capacities of their silica frustules (van de Poll et al. 2005; Ingalls et al. 2010), an effective xanthophyll cycle (Zudaire and Roy 2001) and the production of UV-absorbing compounds. Stimulation of diatom net growth rates under UVR exposure has also been observed in the Ria Formosa during winter and interpreted as a possible indirect response mediated through detrimental effects of UVR on diatoms grazers (Domingues et al. 2014). Negative effects of UVR on summer diatoms can also be explained by a lower capacity to repair UVR-induced damages under nitrogen limiting conditions (Shelly et al. 2002; Beardall et al. 2009b). Indeed, the negative effect of UVR on diatom net growth was counteracted by nutrient enrichment (see below).

Contrasting with the response of winter diatoms in the Ria Formosa coastal lagoon, which significantly increased their net growth rates following CO₂ additions probably due to the physiological benefits of the downregulation of CCMs (Domingues et al. 2014), diatom net growth rates were not affected by CO₂ enrichment in our summer experiments. Indeed, reported effects of increased CO₂ on diatoms are contradictory across the literature and depend on environmental levels of nutrients, light and temperature, with reports of neutral, stimulatory and inhibitory effects on diatom photosynthesis and growth (Gao et al. 2012a; Li and Campbell 2013; Gao and Campbell 2014). However, a relatively reduced

stimulation of diatom growth rate by high CO₂ is usually referred under nutrient limiting conditions (Sun et al. 2011; Tatters et al. 2012) and high PAR intensities (Wu et al. 2010; Gao et al. 2012b; Gao and Campbell 2014), conditions effectively observed in the Ria Formosa during summer (Barbosa 2010; Domingues et al. 2015).

Significant and positive synergistic effects (*sensu* Piggott et al. 2015) of increased CO₂ and UVR on diatom net growth rates were observed; high CO₂ levels suppressed the negative effects of increased UVR under ambient nutrient concentrations. Reports on interactive effects between light intensity, UVR and CO₂ on phytoplankton are divergent, depending on the phytoplankton species considered and other environmental drivers, including the relative contributions and intensities of UV-A and UV-B (Gao et al. 2007; Chen and Gao 2011). Some studies refer that increased CO₂ and UVR synergistically reduce the activity of phytoplankton natural assemblages (Sobrino et al. 2009, 2014), diatoms (Sobrino et al. 2008; Wu et al. 2012) and other taxa (Gao et al. 2009; Chen and Gao 2011). However, decreased sensitivity to UVR under high CO₂ was also reported for three phytoplankton species, including diatoms, in unialgal culture-based experiments (Sobrino et al. 2005; Li et al. 2012; García-Gómez et al. 2014; Wu et al. 2014). Regarding natural diatom assemblages, our study is the first to report an alleviation of the detrimental effects of UVR due to positive synergistic interactions between UVR and CO₂ (the sum of individual effects is greater than any individual effect in the same direction: Piggott et al. 2015). This interactive effect suggests that UVR damages specific processes related to HCO₃[−] transport in diatoms, being minimized at high CO₂ levels (Sobrino et al. 2005). Moreover, increased availability of energy due to downregulation of CCMs at high CO₂ may have actively stimulated non-photochemical quenching of cells or other photoprotective and recovery pathways after UVR damage (Li et al. 2012; Wu et al. 2014), partially counteracting the harmful UVR effects.

In contrast to cyanobacteria and diatoms, net growth rates of cryptophytes increased significantly after exposure to high CO₂ and UVR, alone and in combination, under both ambient and increased nutrient concentrations. Regarding UVR, our results contrast with previous studies that suggest that cryptophytes are highly sensitive to UVR (Gerber

and Häder 1995; Banaszak and Neale 2001). In the Ria Formosa coastal lagoon, cryptophytes seem to be less sensitive to UVR; winter cryptophytes were not affected (Domingues et al. 2014), whilst summer cryptophytes were stimulated by UVR exposure. The Ria Formosa is a shallow, well-mixed system, located at 37°N and thus subjected to high radiation intensities. Given that sensitivity to UVR increases under low light levels, cryptophytes in the Ria Formosa are probably not only acclimated to high irradiances (summer I_{opt} in the Ria Formosa is >500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$; Domingues et al. unpublished data), including high UVR, but are also less sensitive to UVR than communities from deeper ecosystems (Neale et al. 1998). High CO₂ levels also benefited cryptophytes growth in our experiments, contrasting with previous results in the Ria Formosa (and other coastal ecosystems, e.g., Nielsen et al. 2010), where no effects of CO₂ enrichment on winter cryptophytes were observed (Domingues et al. 2014). Divergent results regarding the effects of high CO₂ levels on phytoplankton may be explained by the lower sensitivity of coastal phytoplankton to pH and CO₂ changes due to the natural fluctuations of these drivers in coastal ecosystems, in relation to the more stable oceanic environment (Berge et al. 2010).

Beneficial effects of high CO₂ are evident and/or intensified under nutrient-replete conditions, in relation to nutrient-limited conditions, for different dissolved inorganic micro- and macronutrients and different phytoplankton species, including filamentous and coccoid cyanobacteria (Hutchins et al. 2007; Fu et al. 2008; Endo et al. 2013), diatoms (Sun et al. 2011; Tatters et al. 2012) and dinoflagellates (Fu et al. 2010). In our study, the negative effects of elevated CO₂ on cyanobacteria net growth rates under ambient nutrient concentrations were suppressed under all nutrient enrichments, indicating an additive or positive synergistic interaction between CO₂ and nitrogen. Regarding diatoms and cryptophytes, positive synergistic effects of inorganic nutrients and CO₂ on net growth rates were also detected, and responses to high CO₂ depended on nutrient concentrations and nitrogen source. In relation to ambient nutrient conditions, which were clearly limiting for diatoms, high CO₂ and combined additions of nitrate and ammonium stimulated net growth rates of diatoms and cryptophytes. These results suggest that diatoms and cryptophytes growth may be limited by the availability of dissolved

inorganic carbon only when the primary limitation by nitrogen is relieved (see Fu et al. 2010).

Nutrient concentrations were also a relevant modulator of high UVR impacts on net growth rates of all phytoplankton groups considered in this study. The suppression of negative effects of UVR on diatoms was associated with additions of ammonium combined with high CO₂. Ammonium additions also stimulated cryptophytes growth under UVR exposure. To our knowledge, there is only one study that observed an alleviation of UVR-induced growth suppression by ammonium enrichment in the red macroalgae *Gracilaria* (Xu and Gao 2012). Overall, nutrient limitation is reported to exacerbate the detrimental effects of UVR on growth rates and photosynthetic performance of phytoplankton assemblages (Xenopoulos and Frost 2003; Medina-Sánchez et al. 2006) or specific groups including diatoms (van de Poll et al. 2005; Li and Gao 2014), dinoflagellates (Litchman et al. 2002) and other autotrophic flagellates (Shelly et al. 2002; Heraud et al. 2005). Alleviation of UVR detrimental effects under nutrient-replete conditions can be explained by a higher capacity of phytoplankton to constrain defences against UVR and actively repair UVR-induced damages. Indeed, phytoplankton repair mechanisms involve the production of UVR screening compounds, such as mycosporine-like amino acids (MAAs), the xanthophyll cycle and the activity of specific enzymes, involved in scavenging reactive oxygen species or DNA repair (Litchman et al. 2002; Beardall et al. 2009a, Sobrino et al. 2014 and references therein). These processes depend on ATP supply, nitrogen and phosphorus and are usually promoted after nutrient additions.

Final remarks

Inconsistencies in phytoplankton responses to CO₂, UVR and nutrient enrichments can be found across the literature and may be related to factors such as a high degree of inter- and even intra-specific variability, different initial algal assemblages, different environmental/experimental conditions, different levels of pre-acclimation to experimental conditions and even adaptation to the experimental conditions. The occurrence of transient responses to the new environmental conditions, the possibility of cell acclimation (Sobrino et al. 2008; García-Gómez et al. 2014) and adaptation

after several generations (Lohbeck et al. 2012; Collins et al. 2014; Schlüter et al. 2014) should be considered in short-term perturbation experiments (Domingues et al. 2014). Indeed, short- and long-term effects of environmental drivers on phytoplankton may be quite different. For instance, phosphorus enrichment and UVR may have antagonistic effects over short-term (hours) exposures, but over long-term incubations (>2 months), they act synergistically (sensu Crain et al. 2008), leading to more severe UVR-induced damage to phytoplankton cells (Carrillo et al. 2008). Although long-term incubations are critical to evaluate potential phytoplankton adaptation to future environmental change scenarios, many environmental stressors typically associated with climate change also act on short timescales. Therefore, the study of short-term effects of environmental stressors is critical to understand basic physiological and ecological mechanisms that may further be conditioning phytoplankton acclimation and adaptation to environmental change.

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References

- Andrade C, Freitas MC, Moreno J, Craveiro SC (2004) Stratigraphical evidence of Late Holocene barrier breaching and extreme storms in lagoonal sediments of Ria Formosa. *Mar Geol* 210:339–362
- Badger MR, Price GD, Long BM, Woodger FJ (2006) The environmental plasticity and ecological genomics of the cyanobacterial CO₂ concentrating mechanism. *J Exp Bot* 57:249–265. doi:10.1093/jxb/eri286
- Banaszak AT, Neale PJ (2001) UV sensitivity of photosynthesis in phytoplankton from an estuarine environment. *Limnol Oceanogr* 46:592–600
- Barbosa AB (2006) Estrutura e dinâmica da teia alimentar microbiana na Ria Formosa (Structure and dynamics of the microbial food web in the Ria Formosa). Ph.D. Thesis. University of Algarve, 518 p
- Barbosa AB (2010) Seasonal and interannual variability of planktonic microbes in a mesotidal coastal lagoon (Ria Formosa, SE Portugal). Impact of climatic changes and local human influences. In: Kennish MJ, Paerl HW (eds) *Coastal lagoons: critical habitats of environmental change*. CRC Press, Boca Raton, pp 334–366
- Beardall J, Sobrino C, Stojkovic S (2009a) Interactions between the impacts of ultraviolet radiation, elevated CO₂, and

- nutrient limitation on marine primary producers. *Photochem Photobiol Sci* 8:1257–1265. doi:[10.1039/b9pp00034h](https://doi.org/10.1039/b9pp00034h)
- Beardall J, Stojkovic S, Larsen S (2009b) Living in a high CO₂ world: impacts of global climate change on marine phytoplankton. *Plant Ecol Divers* 2:191–205. doi:[10.1080/17550870903271363](https://doi.org/10.1080/17550870903271363)
- Berge T, Daugbjerg N, Andersen B, Hansen P (2010) Effect of lowered pH on marine phytoplankton growth rates. *Mar Ecol Prog Ser* 416:79–91
- Bouvy M, Bettarel Y, Bouvier C et al (2011) Trophic interactions between viruses, bacteria and nanoflagellates under various nutrient conditions and simulated climate change. *Environ Microbiol* 13:1842–1857. doi:[10.1111/j.1462-2920.2011.02498.x](https://doi.org/10.1111/j.1462-2920.2011.02498.x)
- Boyd P, Hutchins D (2012) Understanding the responses of ocean biota to a complex matrix of cumulative anthropogenic change. *Mar Ecol Prog Ser* 470:125–135. doi:[10.3354/meps10121](https://doi.org/10.3354/meps10121)
- Carrillo P, Delgado-Molina JA, Medina-Sánchez JM et al (2008) Phosphorus inputs unmask negative effects of ultraviolet radiation on algae in a high mountain lake. *Glob Chang Biol* 14:423–439. doi:[10.1111/j.1365-2486.2007.01496.x](https://doi.org/10.1111/j.1365-2486.2007.01496.x)
- Chen S, Gao K (2011) Solar ultraviolet radiation and CO₂-induced ocean acidification interacts to influence the photosynthetic performance of the red tide alga *Phaeocystis globosa* (Prymnesiophyceae). *Hydrobiologia* 675:105–117. doi:[10.1007/s10750-011-0807-0](https://doi.org/10.1007/s10750-011-0807-0)
- Collins S (2011) Many possible worlds: expanding the ecological scenarios in experimental evolution. *Evol Biol* 38:3–14. doi:[10.1007/s11692-010-9106-3](https://doi.org/10.1007/s11692-010-9106-3)
- Collins S, Rost B, Rynearson TA (2014) Evolutionary potential of marine phytoplankton under ocean acidification. *Evol Appl* 7:140–155. doi:[10.1111/eva.12120](https://doi.org/10.1111/eva.12120)
- Crain CM, Kroeker K, Halpern BS (2008) Interactive and cumulative effects of multiple human stressors in marine systems. *Ecol Lett* 11:1304–1315. doi:[10.1111/j.1461-0248.2008.01253.x](https://doi.org/10.1111/j.1461-0248.2008.01253.x)
- Cravo A, Cardeira S, Pereira C et al (2014) Exchanges of nutrients and chlorophyll a through two inlets of Ria Formosa, South of Portugal, during coastal upwelling events. *J Sea Res* 93:63–74. doi:[10.1016/j.seares.2014.04.004](https://doi.org/10.1016/j.seares.2014.04.004)
- Domingues RB, Barbosa A, Galvão H (2008) Constraints on the use of phytoplankton as a biological quality element within the Water Framework Directive in Portuguese waters. *Mar Pollut Bull* 56:1389–1395. doi:[10.1016/j.marpolbul.2008.05.006](https://doi.org/10.1016/j.marpolbul.2008.05.006)
- Domingues RB, Guerra CC, Barbosa AB et al (2014) Effects of ultraviolet radiation and CO₂ increase on winter phytoplankton assemblages in a temperate coastal lagoon. *J Plankton Res* 36:672–684. doi:[10.1093/plankt/ftb135](https://doi.org/10.1093/plankt/ftb135)
- Domingues RB, Guerra CC, Barbosa AB, Galvão HM (2015) Are nutrients and light limiting summer phytoplankton in a temperate coastal lagoon? *Aquat Ecol* 49:127–146. doi:[10.1007/s10452-015-9512-9](https://doi.org/10.1007/s10452-015-9512-9)
- Doyle SA, Saros JE, Williamson CE (2005) Interactive effects of temperature and nutrient limitation on the response of alpine phytoplankton growth to ultraviolet radiation. *Limnol Oceanogr* 50:1362–1367. doi:[10.4319/lo.2005.50.5.1362](https://doi.org/10.4319/lo.2005.50.5.1362)
- Dytham C (2003) Choosing and using statistics: a biologist's guide, 2nd edn. Blackwell, Oxford
- Endo H, Yoshimura T, Kataoka T, Suzuki K (2013) Effects of CO₂ and iron availability on phytoplankton and eubacterial community compositions in the northwest subarctic Pacific. *J Exp Mar Bio Ecol* 439:160–175. doi:[10.1016/j.jembe.2012.11.003](https://doi.org/10.1016/j.jembe.2012.11.003)
- Feng Y, Hare CE, Rose JM et al (2010) Interactive effects of iron, irradiance and CO₂ on Ross Sea phytoplankton. *Deep Res Part I Oceanogr Res Pap* 57:368–383. doi:[10.1016/j.dsr.2009.10.013](https://doi.org/10.1016/j.dsr.2009.10.013)
- Fu F-X, Warner ME, Zhang Y et al (2007) Effects of increased temperature and CO₂ on photosynthesis, growth, and elemental ratios in marine *Synechococcus* and *Prochlorococcus* (cyanobacteria). *J Phycol* 43:485–496. doi:[10.1111/j.1529-8817.2007.00355.x](https://doi.org/10.1111/j.1529-8817.2007.00355.x)
- Fu F, Mulholland MR, Garcia NS et al (2008) Interactions between changing pCO₂, N₂ fixation, and Fe limitation in the marine unicellular cyanobacterium *Crocospheara*. *Limnol Oceanogr* 53:2472–2484
- Fu FX, Place AR, Garcia NS, Hutchins DA (2010) CO₂ and phosphate availability control the toxicity of the harmful bloom dinoflagellate *Karlodinium veneficum*. *Aquat Microb Ecol* 59:55–65. doi:[10.3354/ame01396](https://doi.org/10.3354/ame01396)
- Gao K, Campbell DA (2014) Photophysiological responses of marine diatoms to elevated CO₂ and decreased pH: a review. *Funct Plant Biol* 41:449–459. doi:[10.1071/FP13247](https://doi.org/10.1071/FP13247)
- Gao K, Wu Y, Li G et al (2007) Solar UV radiation drives CO₂ fixation in marine phytoplankton: a double-edged sword. *Plant Physiol* 144:54–59. doi:[10.1104/pp.107.098491](https://doi.org/10.1104/pp.107.098491)
- Gao K, Ruan Z, Villafañe VE et al (2009) Ocean acidification exacerbates the effect of UV radiation on the calcifying phytoplankton *Emiliania huxleyi*. *Limnol Oceanogr* 54:1855–1862
- Gao K, Helbling E, Häder D-P, Hutchins D (2012a) Responses of marine primary producers to interactions between ocean acidification, solar radiation, and warming. *Mar Ecol Prog Ser* 470:167–189. doi:[10.3354/meps10043](https://doi.org/10.3354/meps10043)
- Gao K, Xu J, Gao G et al (2012b) Rising CO₂ and increased light exposure synergistically reduce marine primary productivity. *Nat Clim Chang* 2:1–5. doi:[10.1038/nclimate1507](https://doi.org/10.1038/nclimate1507)
- García-Gómez C, Gordillo FJL, Palma A et al (2014) Elevated CO₂ alleviates high PAR and UV stress in the unicellular chlorophyte *Dunaliella tertiolecta*. *Photochem Photobiol Sci* 13:1347. doi:[10.1039/C4PP00044G](https://doi.org/10.1039/C4PP00044G)
- García-Pichel F (1994) A model for internal self-shading in planktonic organisms and its implications for the usefulness of ultraviolet sunscreens. *Limnol Oceanogr* 39:1704–1717. doi:[10.4319/lo.1994.39.7.1704](https://doi.org/10.4319/lo.1994.39.7.1704)
- Gattuso J-P, Gao K, Lee K, et al (2010) Approaches and tools to manipulate the carbonate chemistry. In: Riebesell U, Fabry VJ, Hansson L, Gattuso J-P (eds) Guide to best practices ocean acidification research. Data Report. Publications Office of the European Union, pp 41–52
- Gerber S, Häder DP (1995) Effects of enhanced solar irradiation on chlorophyll fluorescence and photosynthetic oxygen production of 5 species of phytoplankton. *FEMS Microbiol Ecol* 16:33–41
- Grasshoff K, Ehrhardt M, Kremling K (1983) Methods of seawater analysis. Verlag Chemie, Weinheim

- Haas LW (1982) Improved epifluorescence microscopy for observing planktonic micro-organisms. *Ann l'Institut Oceanogr* 58:261–266
- Heraud P, Roberts S, Shelly K, Beardall J (2005) Interactions between UV-B exposure and phosphorus nutrition. II. Effects on rates of damage and repair. *J Phycol* 41:1212–1218. doi:[10.1111/j.1529-8817.2005.00149.x](https://doi.org/10.1111/j.1529-8817.2005.00149.x)
- Hutchins DA, Fu F-X, Zhang Y et al (2007) CO₂ control of trichodesmium N₂ fixation, photosynthesis, growth rates, and elemental ratios: implications for past, present, and future ocean biogeochemistry. *Limnol Oceanogr* 52:1293–1304. doi:[10.4319/lo.2007.52.4.1293](https://doi.org/10.4319/lo.2007.52.4.1293)
- Ingalls AE, Whitehead K, Bridoux MC (2010) Tinted windows: the presence of the UV absorbing compounds called mycosporine-like amino acids embedded in the frustules of marine diatoms. *Geochem Cosmochim Acta* 74:104–115
- IPCC (2014) Climate change 2014: synthesis report. Contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change. IPCC, Geneva
- Li G, Campbell DA (2013) Rising CO₂ interacts with growth light and growth rate to alter photosystem II photoinactivation of the coastal diatom *Thalassiosira pseudonana*. *PLoS ONE* 8:e55562. doi:[10.1371/journal.pone.0055562](https://doi.org/10.1371/journal.pone.0055562)
- Li G, Gao K (2014) Effects of solar UV radiation on photosynthetic performance of the diatom *Skeletonema costatum* grown under nitrate limited condition. *Algae* 29:27–34
- Li Y, Gao K, Villafañe VE, Helbling EW (2012) Ocean acidification mediates photosynthetic response to UV radiation and temperature increase in the diatom *Phaeodactylum tricornutum*. *Biogeosci Discuss* 9:7197–7226. doi:[10.5194/bgd-9-7197-2012](https://doi.org/10.5194/bgd-9-7197-2012)
- Litchman E, Neale PJ, Banaszak AT (2002) Increased sensitivity to ultraviolet radiation in nitrogen-limited dinoflagellates: photoprotection and repair. *Limnol Oceanogr* 47:86–94. doi:[10.4319/lo.2002.47.1.0086](https://doi.org/10.4319/lo.2002.47.1.0086)
- Litchman E, Edwards K, Klausmeier C, Thomas M (2012) Phytoplankton niches, traits and eco-evolutionary responses to global environmental change. *Mar Ecol Prog Ser* 470:235–248. doi:[10.3354/meps09912](https://doi.org/10.3354/meps09912)
- Llabrés M, Agustí S, Alonso-Laita P, Herndl G (2010) *Synechococcus* and *Prochlorococcus* cell death induced by UV radiation and the penetration of lethal UVR in the Mediterranean Sea. *Mar Ecol Prog Ser* 399:27–37. doi:[10.3354/meps08332](https://doi.org/10.3354/meps08332)
- Lohbeck KT, Riebesell U, Reusch TBH (2012) Adaptive evolution of a key phytoplankton species to ocean acidification. *Nat Geosci* 5:346–351. doi:[10.1038/ngeo1441](https://doi.org/10.1038/ngeo1441)
- Loureiro S, Newton A, Icely J (2005) Effects of nutrient enrichments on primary production in the Ria Formosa coastal lagoon (Southern Portugal). *Hydrobiologia* 550:29–45. doi:[10.1007/s10750-005-4357-1](https://doi.org/10.1007/s10750-005-4357-1)
- Machado LM (2010) A radiação UV-B na Ria Formosa: incidência e impactos biológicos. University of Algarve, Faro
- Medina-Sánchez JM, Villar-Argaiz M, Carrillo P (2006) Solar radiation-nutrient interaction enhances the resource and predation algal control on bacterioplankton: a short-term experimental study. *Limnol Oceanogr* 51:913–924. doi:[10.4319/lo.2006.51.2.0913](https://doi.org/10.4319/lo.2006.51.2.0913)
- Neale PJ, Cullen JJ, Davis RF (1998) Inhibition of marine photosynthesis by ultraviolet radiation: variable sensitivity of phytoplankton in the Wedell-Scotia Confluence during the austral spring. *Limnol Oceanogr* 43:433–448
- Neale P, Sobrino C, Segovia M et al (2014) Effect of CO₂, nutrients and light on coastal plankton. I. Abiotic conditions and biological responses. *Aquat Biol* 22:25–41. doi:[10.3354/ab00587](https://doi.org/10.3354/ab00587)
- Newton A, Mudge SM (2003) Temperature and salinity regimes in a shallow, mesotidal lagoon, the Ria Formosa, Portugal. *Estuar Coast Shelf Sci* 57:73–85. doi:[10.1016/S0272-7714\(02\)00332-3](https://doi.org/10.1016/S0272-7714(02)00332-3)
- Newton A, Icely JD, Falcao M et al (2003) Evaluation of eutrophication in the Ria Formosa coastal lagoon, Portugal. *Estuar Coast Shelf Res* 23:1945–1961. doi:[10.1016/j.csr.2003.06.008](https://doi.org/10.1016/j.csr.2003.06.008)
- Nielsen LT, Jakobsen HH, Hansen PJ (2010) High resilience of two coastal plankton communities to twenty-first century seawater acidification: evidence from microcosm studies. *Mar Biol Res* 6:542–555. doi:[10.1080/17451000903476941](https://doi.org/10.1080/17451000903476941)
- Nielsen L, Hallegraeff G, Wright S, Hansen P (2012) Effects of experimental seawater acidification on an estuarine plankton community. *Aquat Microb Ecol* 65:271–286. doi:[10.3354/ame01554](https://doi.org/10.3354/ame01554)
- Nogueira P, Domingues RB, Barbosa AB (2014) Are microcosm volume and sample pre-filtration relevant to evaluate phytoplankton growth? *J Exp Mar Bio Ecol* 461:323–330. doi:[10.1016/j.jembe.2014.09.006](https://doi.org/10.1016/j.jembe.2014.09.006)
- Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, New York
- Paulino AI, Egge JK, Larsen A (2008) Effects of increased atmospheric CO₂ on small and intermediate sized osmotrophs during a nutrient induced phytoplankton bloom. *Biogeosciences* 5:739–748. doi:[10.5194/bg-5-739-2008](https://doi.org/10.5194/bg-5-739-2008)
- Piggott JJ, Townsend CR, Matthaei CD (2015) Reconceptualizing synergism and antagonism among multiple stressors. *Ecol Evol* 5:1538–1547. doi:[10.1002/ece3.1465](https://doi.org/10.1002/ece3.1465)
- Rastogi RP, Sinha RP, Moh SH et al (2014) Ultraviolet radiation and cyanobacteria. *J Photochem Photobiol B Biol* 141:154–169. doi:[10.1016/j.jphotobiol.2014.09.020](https://doi.org/10.1016/j.jphotobiol.2014.09.020)
- Reul A, Muñoz M, Bautista B et al (2014) Effect of CO₂, nutrients and light on coastal plankton. III. Trophic cascade, size structure and composition. *Aquat Biol* 22:59–76. doi:[10.3354/ab00585](https://doi.org/10.3354/ab00585)
- Reusch TBH, Boyd PW (2013) Experimental evolution meets marine phytoplankton. *Evolution (NY)* 67:1849–1859. doi:[10.1111/evo.12035](https://doi.org/10.1111/evo.12035)
- Sala MM, Aparicio FL, Balagué V et al (2015) Contrasting effects of ocean acidification on the microbial food web under different trophic conditions. *ICES J. Mar. Sci*
- Schlüter L, Lohbeck KT, Gutowska MA et al (2014) Adaptation of a globally important coccolithophore to ocean warming and acidification. *Nat Clim Chang* 4:1024–1030. doi:[10.1038/nclimate2379](https://doi.org/10.1038/nclimate2379)
- Shelly K, Heraud P, Beardall J (2002) Nitrogen limitation in *Dunaliella tertiolecta* Butcher (Chlorophyceae) leads to increased susceptibility to damage by ultraviolet-B radiation but also increased repair capacity. *J Phycol* 38:1–8
- Sobrino C, Montero O, Lubián L (2005) Effect of UV-A and UV-B on diel patterns of growth and metabolic activity in *Nannochloris atomus* cultures assessed by flow cytometry. *Mar Ecol Prog Ser* 293:29–35. doi:[10.3354/meps293029](https://doi.org/10.3354/meps293029)

- Sobrinho C, Ward ML, Neale PJ (2008) Acclimation to elevated carbon dioxide and ultraviolet radiation in the diatom *Thalassiosira pseudonana*: effects on growth, photosynthesis, and spectral sensitivity of photoinhibition. *Limnol Oceanogr* 53:494–505
- Sobrinho C, Neale PJ, Phillips-Kress JD et al (2009) Elevated CO₂ increases sensitivity to ultraviolet radiation in lacustrine phytoplankton assemblages. *Limnol Oceanogr* 54:2448–2459
- Sobrinho C, Segovia M, Neale P et al (2014) Effect of CO₂, nutrients and light on coastal plankton. IV. Physiological responses. *Aquat Biol* 22:77–93. doi:[10.3354/ab00590](https://doi.org/10.3354/ab00590)
- Stramma L, Cornillon P, Weller RA et al (1986) Large diurnal sea surface temperature variability: satellite and in situ measurements. *J Phys Oceanogr* 56:345–358. doi:[10.1175/1520-0485\(1986\)016<0827:LDSSTV>2.0.CO;2](https://doi.org/10.1175/1520-0485(1986)016<0827:LDSSTV>2.0.CO;2)
- Stuart-Menteth AC, Robinson IS, Challenor PG (2003) A global study of diurnal warming using satellite-derived sea surface temperature. *J Geophys Res* 108:3155. doi:[10.1029/2002JC001534](https://doi.org/10.1029/2002JC001534)
- Sun J, Hutchins DA, Feng Y et al (2011) Effects of changing pCO₂ and phosphate availability on domoic acid production and physiology of the marine harmful bloom diatom *Pseudo-nitzschia multiseries*. *Limnol Oceanogr* 56:829–840. doi:[10.4319/lo.2011.56.3.0829](https://doi.org/10.4319/lo.2011.56.3.0829)
- Tatters AO, Fu FX, Hutchins DA (2012) High CO₂ and silicate limitation synergistically increase the toxicity of *Pseudo-nitzschia fraudulenta*. *PLoS ONE* 7:e32116. doi:[10.1371/journal.pone.0032116](https://doi.org/10.1371/journal.pone.0032116)
- Timmermans KR, van der Wagt B, Veldhuis MJW et al (2005) Physiological responses of three species of marine picophytoplankton to ammonium, phosphate, iron and light limitation. *J Sea Res* 53:109–120. doi:[10.1016/j.seares.2004.05.003](https://doi.org/10.1016/j.seares.2004.05.003)
- Utermöhl H (1958) Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitt Int Ver Theor und Angew Limnol* 9:1–38
- van de Poll WH, van Leeuwe MA, Roggeveld J, Buma AGJ (2005) Nutrient limitation and high irradiance acclimation reduce PAR and UV-induced viability loss in the Antarctic diatom *Chaetoceros brevis* (Bacillariophyceae). *J Phycol* 41:840–850
- Varona-Cordero F, Gutiérrez-Mendieta FJ, Rivera-Monroy VH (2014) in situ response of phytoplankton to nutrient additions in a Tropical Coastal Lagoon, (La Mancha, Veracruz, Mexico). *Estuar Coasts* 37:1353–1375. doi:[10.1007/s12237-014-9806-5](https://doi.org/10.1007/s12237-014-9806-5)
- Venrick EL (1978) How many cells to count? In: Sournia A (ed) *Phytoplankt. Man*. UNESCO, Paris, pp 167–180
- Wu Y, Gao K, Riebesell U (2010) CO₂-induced seawater acidification affects physiological performance of the marine diatom *Phaeodactylum tricornutum*. *Biogeosciences* 7:2915–2923. doi:[10.5194/bg-7-2915-2010](https://doi.org/10.5194/bg-7-2915-2010)
- Wu X, Gao G, Giordano M, Gao K (2012) Growth and photosynthesis of a diatom grown under elevated CO₂ in the presence of solar UV radiation. *Fundam Appl Limnol/Arch für Hydrobiol* 180:279–290. doi:[10.1127/1863-9135/2012/0299](https://doi.org/10.1127/1863-9135/2012/0299)
- Wu Y, Campbell DA, Gao K (2014) Faster recovery of a diatom from UV damage under ocean acidification. *J Photochem Photobiol B Biol* 140:249–254. doi:[10.1016/j.jphotobiol.2014.08.006](https://doi.org/10.1016/j.jphotobiol.2014.08.006)
- Xenopoulos MA, Frost PC (2003) UV radiation, phosphorus, and their combined effects on the taxonomic composition of phytoplankton in a Boreal Lake 1. *J Phycol* 302:291–302
- Xu Z, Gao K (2012) NH₄⁺ enrichment and UV radiation interact to affect the photosynthesis and nitrogen uptake of *Gracilaria lemaneiformis* (Rhodophyta). *Mar Pollut Bull* 64:99–105. doi:[10.1016/j.marpolbul.2011.10.016](https://doi.org/10.1016/j.marpolbul.2011.10.016)
- Zudaire L, Roy S (2001) Photoprotection and long-term acclimation to UV radiation in the marine diatom *Thalassiosira weissflogii*. *J Photochem Photobiol B Biol* 62:26–34