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# Desalination effects on macroalgae (part A): Laboratory-controlled experiments with *Dictyota* spp. from the Pacific Ocean and Mediterranean Sea

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Desalination brines from direct seawater intake that get discharged to coastal areas may produce stress responses on benthic marine communities, mostly due to its excess salinity, and especially on sessile organisms; in this context, macroalgae have been understudied in desalination ecotoxicological investigations. In this study, we assessed the short- and long-term cellular tolerance responses in two brown species of the macroalgae genus *Dictyota* through controlled laboratory conditions. *Dictyota kunthii* was collected from the eastern Pacific Ocean (average salinity, ~34 psu), whereas *Dictyota dichotoma* was from the Mediterranean Sea (average salinity, ~37 psu). Each macroalgae species was exposed for up to 7 days to two conditions with increased salinity values: +2 and +7 psu above their natural average salinity. Photosynthetic parameters and oxidative stress measurements were determined. The results showed that, in both *Dictyota* species, high salinity values induced reduced photoinhibition ( $F_v/F_m$ ) but increased the primary productivity ( $ETR_{max}$ ) and light requirement ( $E_{k_{ETR}}$ ) especially after 7 days. Conversely, the photosynthetic efficiency ( $\alpha_{ETR}$ ) decreased in hypersalinity treatments in *D. dichotoma*, while there were no changes in *D. kunthii*. The reactive oxygen species hydrogen peroxide ( $H_2O_2$ ) was greater at high salinity values at 3 days for *D. dichotoma* and after 7 days in *D. kunthii*, while lipid peroxidation decreases under hypersalinity with time in both species. Despite the evident  $H_2O_2$  accumulation in both species against hypersalinity, it did not produce oxidative damage and important impairment in the photosynthetic apparatus. These results contribute to understanding the tolerance strategies at the cellular level of *Dictyota* spp., which may be considered as potential candidates for biomonitoring of desalination impacts in the field.

## KEYWORDS

desalination, hypersalinity, brown macroalgae, photosynthesis, oxidative stress

# 1 Introduction

In the recent decades, seawater desalination has been promoted as a feasible solution to cover limited freshwater availability and its higher demand at a global scale (Fernández-Torquemada and Sánchez-Lizaso, 2007; Jones et al., 2019). This scarcity of water has been induced by exponential consumption in global population, increasing demand per capita, industry expansion, and contamination, together with the global climate crisis (Schewe et al., 2014; Fernández-Torquemada et al., 2019; Jones et al., 2019).

Desalination is the process by which dissolved salts and minerals from any water source, such as seawater, brackish groundwater, surface water, and even sewage, are removed (Jones et al., 2019; Fernández-Torquemada et al., 2019). Among the available technological options for desalination, reverse osmosis (RO) has become the preferred method due to its relatively low cost and energy efficiency (Fernández-Torquemada and Sánchez-Lizaso, 2007; Fernández-Torquemada et al., 2009; Shahzad et al., 2017). However, RO desalination plants may discharge residual brine into the subtidal zone, which is composed of a hypersaline effluent nearly twice as concentrated as the input seawater. The brine may also contain trace chemical residues such as coagulants, biocides, and anti-scalants (Lattemann and Höpner, 2008; Belkin et al., 2017; Fernández-Torquemada et al., 2019; Panagopoulos and Giannika, 2022; Panagopoulos, 2022). In spite of the latter, the potential detrimental effects of desalination have been observed to be mainly associated with the osmotic stress caused by salt excess (e.g. Del Pilar Ruso et al., 2015; De la Ossa Carretero et al., 2016; Cambridge et al., 2019; Fernández-Torquemada et al., 2019; Rodríguez-Rojas et al., 2020; Panagopoulos and Haralambous, 2020a; Panagopoulos and Haralambous, 2020b; Sandoval-Gil et al., 2022; Muñoz et al., 2023).

In marine photoautotrophs, hypersaline exposure induces water imbalance within the cellular environment (Kirst, 1990). An excess of  $\text{Na}^+$  and  $\text{Cl}^-$  ions in the extracellular medium can cause cellular dehydration, ionic exchange disruption, and overproduction of reactive oxygen species (ROS) (Kumar et al., 2014; Garrote-Moreno et al., 2015a). In this context, most studies regarding the effects related with brine-derived hypersalinity have been carried out in marine angiosperms or seagrasses; these have demonstrated marked species-specific sensitivity and tolerance (e.g., Sandoval-Gil et al., 2014; Piro et al., 2015; Garrote-Moreno et al., 2015a; Panda et al., 2019; Shetty et al., 2019; Capó et al., 2020; Sandoval-Gil et al., 2022)—for instance, a controlled comparative study using artificial marine salts from 37 to 60 practical salinity units (psu) showed higher salinity tolerance in the seagrass *Cymodocea nodosa* than in *Posidonia oceanica* as evidenced by the higher ratios of  $\text{K}^+/\text{Na}^+$  and  $\text{Ca}^{2+}/\text{Na}^+$  (Garrote-Moreno et al., 2015b). Moreover, photosynthetic responses through *in vivo* chlorophyll *a* fluorescence in the seagrass *Posidonia australis* have evidenced a decrease in maximum electron transport rate ( $\text{ETR}_{\text{max}}$ ) and saturation irradiance ( $\text{Ek}_{\text{ETR}}$ ) after 4 and 6 weeks of exposure to hypersalinity (46 and 54 psu) (Cambridge et al., 2017), while in *Enhalus acoroides* hypersalinity (40 and 50 psu) declined the maximal quantum yield ( $F_v/F_m$ ) and chlorophyll content after 20 days of exposure (Kongrueng et al., 2018). Records on macroalgae and related communities are very limited, especially under brine impacts in the field or upon controlled laboratory experiments

extrapolated to desalination discharges. In this context, there are some records available under high salinity values—for instance, enhanced salinity of up to 90 psu for 4 days in the intertidal green macroalga *Ulva fasciata* produced an intense oxidative stress condition; more specifically, hypersalinity induced  $\text{H}_2\text{O}_2$  overproduction and increased lipid peroxidation together with photosynthetic inhibition compared with controls (30 psu) (Lu et al., 2006). On the other hand, the intertidal macroalga *Ulva compressa* demonstrated decreased primary productivity and photoinhibition against hypersalinity stress (32 vs. 82 psu) after 6 days (Muñoz et al., 2020). In addition, in the only field assessment in the context of desalination impacts using macroalgae as biomonitoring organisms, transplants of the model brown macroalga *Ectocarpus* were subjected to brine discharges from a desalination plant in Chile (at nearly 37 vs. 34 psu at the control site) and prompted a decrease in photosynthetic activity and a significant stress condition as evidenced by the over-accumulation of ROS and antioxidant molecules (Rodríguez-Rojas et al., 2020). Thus, considering the ecological relevance of macroalgae and their abundance and wide distribution at a global scale in areas operating and projecting for the development of the industry, these make them suitable candidates for biomonitoring purposes in the frame of the potential environmental impacts of desalination.

The Dictyotales order includes 214 brown algae species, among them the genus *Dictyota*, which is the third most diverse order after Ectocarpales. Dictyotales has a worldwide distribution from temperate waters to the subtropical and tropical zones (Bittner et al., 2008). Moreover, *Dictyota* species can be found from the intertidal to the subtidal zones, being one of the most environmentally versatile and abundant marine macroalgae—thus sustaining large ecologically and economic coastal ecosystems globally (Bogaert et al., 2020). Due to the distribution of *Dictyota* species, they have developed different strategies to persist in adverse environments and salinity shifts (Andrade et al., 2006; Macaya and Thiel, 2008; Laib and Leghouchi, 2012). In the most ambitious and globally representative macrophyte environmental monitoring experience conducted to date in the context of desalination impacts, we evaluated the responses of two species of *Dictyota*—*D. kunthii*, from the north Chilean Pacific coast, and *D. dichotoma*, from the east Spain Mediterranean coast—exposed to hypersalinity conditions.

In this study, in short-term-controlled laboratory conditions, we compared photosynthetic activity and oxidative stress response in the *Dictyota* species under increased salinity levels similar to those found nearby brine discharges. In a complementary article (Muñoz et al., 2023), similar parameters were assessed in these species under transplantation experiments in desalination plant-impacted sites.

## 2 Materials and methods

### 2.1 Macroalgae collection and experimental design

*D. kunthii* was collected from Antofagasta Bay, Chile (23°38'47" S, 70°23'53" W) at 15-m depth. The natural salinity in Antofagasta Bay oscillates between 34.32 and 35.35 psu, and the retention of water is favored by a cyclonic twist, producing a higher superficial water

temperature that varies between 14.5 and 20°C (Dávila and Valdés, 2015). On the other hand, *D. dichotoma* was collected from Alicante Bay, Spain (38°20'45.59" N, 0°29'26.47" W) at 2-m depth. High temperature and irradiance induce a negative water balance of Mediterranean Sea, producing an excess of evaporation and a minimum precipitation contribution and generating a mean salinity of 37–37.5 psu and seawater temperature of 12–28°C (Naranjo et al., 2015). After collection, the individuals were stored in a cooler and immediately transported to the laboratory to start the acclimation process.

Both species were acclimated for 24 h in plastic flasks with seawater obtained from their natural habitat and exposed to constant aeration, 50  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  light, and 12:12-h light/dark photoperiod; considering the environmental conditions in their natural habitats, temperatures of  $17 \pm 1^\circ\text{C}$  and  $20 \pm 1^\circ\text{C}$  were used for *D. kunthii* and *D. dichotoma*, respectively, for acclimation and experimental purposes. After acclimation, both species were exposed to hypersalinity treatments, increasing in 2 (+2) and 7 (+7) psu from their natural baseline ranges (controls; CTRL). In this regard, the experiments for *D. kunthii* consisted in 35 (CTRL), 37, and 42 psu, each one with three biological replicates. On the other hand, the experimental conditions for *D. dichotoma* salinity were 37 (CTRL), 39, and 44 psu, each one with three biological replicates. Increasing values of salinity were chosen considering the real saline plume profiles generated from brine discharges of desalination plants located in Antofagasta (Rodríguez-Rojas et al., 2020) and Alicante (Fernández-Torquemada et al., 2009). The desired salinity values were obtained adding artificial sea salts (Instant Ocean<sup>®</sup> for *D. kunthii* and Reef Salt<sup>®</sup> for *D. dichotoma*) mixed with filtered seawater. Salinity monitoring was performed with a multiparametric probe (YSI model Professional Plus<sup>®</sup>). Approximately 300 g of each species (~10 of *D. kunthii* and ~15 individuals of *D. dichotoma*) was distributed separately in lid-free polycarbonate chambers with 500 ml of seawater with different salinity values in three biological replicates. Subsamples of algae thallus, avoiding the fixing disk, were collected after 3 and 7 days to analyze photosynthetic performance, while for biochemical analyses the tissue was frozen with liquid nitrogen and stored at  $-80^\circ\text{C}$ .

## 2.2 Photosynthetic performance

Photosynthetic measurements corresponding to the maximum quantum yield of photosystem II (PSII;  $F_v/F_m$ ), electron transport rate (ETR), estimator of photosynthetic efficiency ( $\alpha_{\text{ETR}}$ ), saturation irradiance for ETR ( $E_{K_{\text{ETR}}}$ ), and non-photochemical quenching (NPQ) were registered with a pulse-amplitude modulated chlorophyll *a* fluorometer (JUNIOR PAM, Walz GmbH, Effeltrich, Germany) with the Win Control-3.2 software. All calculations were performed according to Celis-Plá et al. (2014); Celis-Plá et al. (2020); Rodríguez-Rojas et al. (2020). ETR and NPQ were calculated according to Schreiber et al., 1995.

## 2.3 Concentration of hydrogen peroxide

The levels of  $\text{H}_2\text{O}_2$  were determined using a spectrophotometer according to Rodríguez-Rojas et al. (2020) but with modifications for *D.*

*kunthii* and *D. dichotoma*. For both species, ~100 mg fresh weight biomass was ground with liquid nitrogen and mixed with 100  $\mu\text{l}$  of 10% trichloroacetic acid (TCA) in the presence of glass beads (3 mm), 100  $\mu\text{l}$  of 10 mM potassium phosphate buffer (pH 7.0), and 100  $\mu\text{l}$  of lysis buffer. In the case of *D. kunthii*, lysis buffer from E.Z.N.A. total RNA kit I was used (Omega Bio-tek), while in *D. dichotoma*, the lysis buffer described by Pearson et al. (2006) that contained 100 mM Tris, 50 mM EDTA (pH 7.5), 2 M NaCl, and 2% CTAB was applied. The subsequent protocol steps are described elsewhere (Rodríguez-Rojas et al., 2020).

## 2.4 Concentration of thiobarbituric acid reactive substances

The thiobarbituric acid reactive substance (TBARS) levels were quantified using a spectrophotometer according to Rodríguez-Rojas et al. (2020) with modifications for each species to evaluate lipid peroxidation. For *D. kunthii*, fresh biomass (~100 mg) was ground with liquid nitrogen and mixed with 300  $\mu\text{l}$  of 10% TCA. For *D. dichotoma*, ~150 mg fresh biomass was ground with liquid nitrogen and mixed with 450  $\mu\text{l}$  of 10% TCA. Both extracts were vortexed for 20 min. Subsequently, the mixture was centrifuged at  $17,800 \times g$  for 15 min at  $4^\circ\text{C}$ . The protocol steps performed further were as described elsewhere (Rodríguez-Rojas et al., 2020).

## 2.5 Statistical analyses

Statistical analyses related to the effects of salinity on photosynthetic activity and oxidative stress were analyzed by two-way ANOVA followed by *a posteriori* test of Student–Newman–Keuls test (Underwood, 1997). The level of significance used was 95% confidence interval ( $p = 0.05$ ). Prior to the statistical analysis, the homogeneity of variances was tested using Cochran test and a visual inspection of the residuals in terms of normality (Underwood, 1997). Statistical software version 7 (Stat Soft Inc., Tulsa, OK, USA) and statistical package SPSS v.21 (IBM, USA) were applied. Principal component analysis (PCA) was carried out to visualize patterns among all parameters through PERMANOVA+ with PRIMER6 package. The PCA was built with all the photosynthetic and oxidative stress variables to calculate the percentage of variation by each of the axes in a multidimensional scale (Anderson et al., 2008).

## 2.6 Data availability

All datasets obtained to perform statistical analysis of the entire manuscript correspond to raw numerical data that is not publicly available. Nevertheless, the authors agree to share the raw data if required or when necessary.

# 3 Results

## 3.1 Photosynthetic activity

Salinity induced a significant photoinhibition (measured  $F_v/F_m$ ) in *D. kunthii* after 7 days at +2 psu, (Figure 1A). In *D. dichotoma*,

significant photoinhibition was observed in the highest salinity (+7 psu) only at 3 days, but it returned to baseline levels at day 7 (Figure 1B). The maximum electron transport rate ( $ETR_{max}$ ) increased significantly in *D. kunthii* during exposure to +7 and +2 psu after 3 and 7 days, respectively (Figure 1C). A similar behavior was observed for *D. dichotoma*, in which  $ETR_{max}$  increased significantly with salinity at both experimental times, mostly at +7 psu (Figure 1D).

The photosynthetic efficiency ( $\alpha_{ETR}$ ) of *D. kunthii* suffered no changes (Figure 2A). In contrast,  $\alpha_{ETR}$  in *D. dichotoma* subjected to high salinity values always decreased compared with baseline levels, especially under +7 psu at 3 days (Figure 2B). The saturation of irradiance ( $E_{k_{ETR}}$ ) in *D. kunthii* only increased significantly under +2 psu at 7 days (Figure 2C). On the other hand, in *D. dichotoma*,  $E_{k_{ETR}}$  significantly increased under hypersalinity at both experimental times, although with greater levels at 3 days (Figure 2D).

On the other hand, the maximal non-photochemical quenching ( $NPQ_{max}$ ) in *D. kunthii* showed a sustained significant decrease with increasing salinity levels at day 3 (Figure 3A). The pattern of  $NPQ_{max}$  was similar in *D. dichotoma*, although a significant decrease with respect to the controls was only detected at +7 psu after 3 days (Figure 3B).

## 3.2 Quantification of $H_2O_2$ and TBARS content

While the accumulation of  $H_2O_2$  in *D. kunthii* displayed a decrease only at +2 psu after 3 days, at 7 days there was an increase with respect to controls, especially at +2 psu, suggesting an oxidative

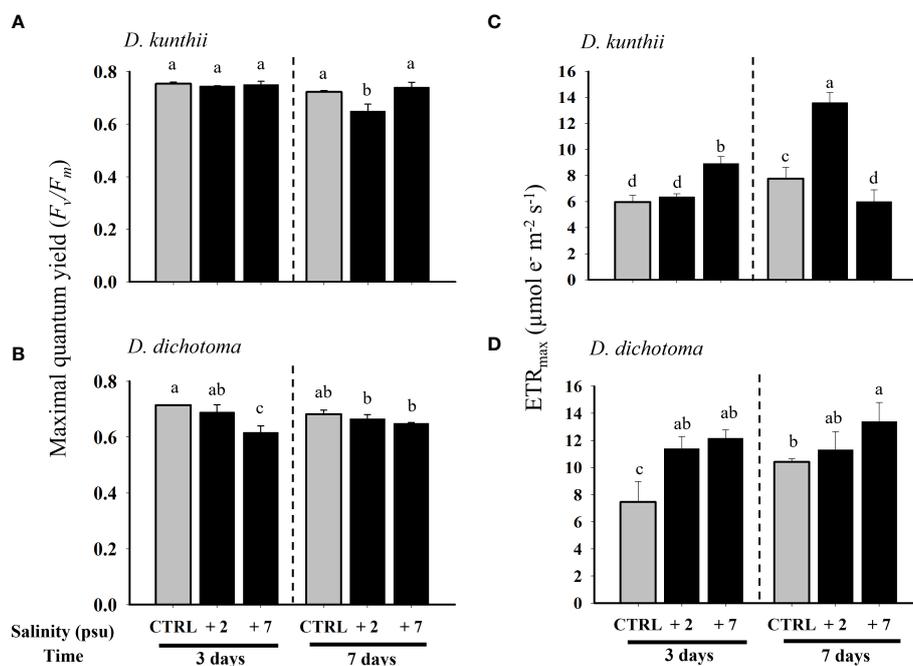
stress condition (Figure 4A). Almost an inverted pattern compared with *D. kunthii* was observed in *D. dichotoma*, where  $H_2O_2$  was largely accumulated at +2 psu and decreased significantly after 7 days in comparison to the control values, reflecting that ROS detox mechanisms were activated (Figure 4B). As in lipid peroxidation, TBARS decreased in all treatments with respect to controls at day 3 in *D. kunthii*, although without a significant difference among them at 7 days, suggesting that, although there was an increase in ROS, there was no oxidative damage (Figure 4C). In contrast, in *D. dichotoma*, no differences were detected between treatments, with the exception of a significant decrease at +7 psu after 7 days, which was in agreement with the low ROS levels (Figure 4D).

## 3.3 Principal component analysis

We used PCA to identify potential patterns of response to high salinity exposure based on photosynthetic ( $F_v/F_m$ ,  $\alpha_{ETR}$ ,  $ETR_{max}$ ,  $E_{k_{ETR}}$ , and  $NPQ_{max}$ ) and oxidative stress ( $H_2O_2$  and TBARS) parameters (Figure 5). The first dimension of the PCA represented 98.9% of the total variation and was mainly characterized by  $H_2O_2$ . This first axis allowed to segregate the two studied species. The second dimension represented 0.9% of the total variation and mainly represented  $ETR_{max}$  and  $E_{k_{ETR}}$ .

## 4 Discussion

In the present investigation, we evaluated the effects of hypersalinity under laboratory-controlled experiments emulating



**FIGURE 1**  
Maximal quantum yield ( $F_v/F_m$ ) and maximum electron transport rate ( $ETR_{max}$ ) in *D. kunthii* (A, C) and *D. dichotoma* (B, D) exposed to high salinity values. For *D. kunthii*, the salinity values were 35 psu (control), 37 (+2) psu, and 42 (+7) psu; for *D. dichotoma*, these were 37 psu (control), 39 (+2) psu, and 44 (+7) psu. The samples were analyzed after 3 and 7 days. Data corresponds to mean  $\pm$  SD ( $n = 3$ ). Letters represent statistical difference at 95% confidence interval (Student–Newman–Keuls test,  $p < 0.05$ ).

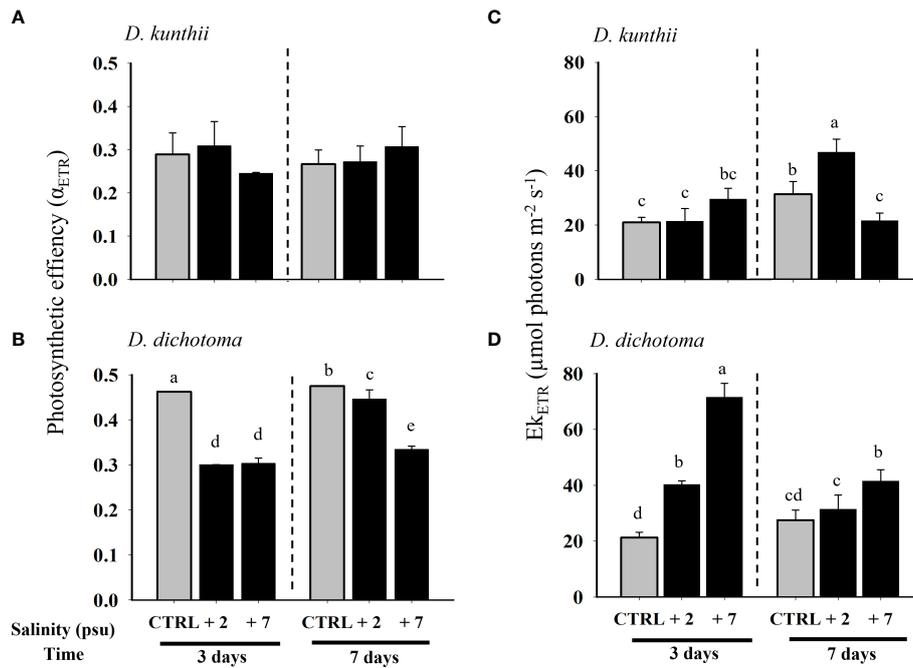


FIGURE 2

Photosynthetic efficiency ( $\alpha_{ETR}$ ) and saturation of irradiance ( $E_{k_{ETR}}$ ) in *D. kunthii* (A, C) and *D. dichotoma* (B, D) exposed to high salinity values. For *D. kunthii*, the salinity values were 35 psu (control), 37 psu (+2), and 42 (+7) psu; for *D. dichotoma*, these were 37 psu (control), 39 (+2) psu, and 44 (+7) psu. The samples were analyzed after 3 and 7 days. Data corresponds to mean  $\pm$  SD ( $n = 3$ ). Letters represent statistical difference at 95% confidence interval (Student–Newman–Keuls test,  $p < 0.05$ ). No letters mean no significant differences.

desalination impacts by assessing the photosynthetic and oxidative stress responses of the macroalgae *D. kunthii* (Chile) and *D. dichotoma* (Spain). Our results evidenced the effects of excess salinity in both species, although with interspecific differences.

In terms of photosynthesis, the maximal quantum yield ( $F_v/F_m$ ), used as indicator of the photoinhibition of photosynthetic activity in PSII (Celis-Plá et al., 2016; Celis-Plá et al., 2020), demonstrated low impairment only in *D. dichotoma* at 3 days, while in *D. kunthii* it remained almost unaltered. These results demonstrate that excess salinity caused photoinhibition of photosynthesis only at early exposure in the case of *D. dichotoma*, with a recovery process at 7 days as observed. In contrast, other brown macroalgae species like *Ectocarpus* showed a radical decrease of  $F_v/F_m$  after 3 and 7 days with similar salinity levels, although exposed to brine at a desalination discharge site (Rodríguez-Rojas et al., 2020). Similarly, Lu et al. (2006) reported a marked decrease in  $F_v/F_m$  when *U. fasciata* was exposed to hypersalinity treatments (60–150 psu) compared with the controls (30 psu), while in the green macroalga *U. compressa* the increment of salinity (42, 62, and 82 psu) showed no effects on  $F_v/F_m$  after 7 days of exposition.  $E_{TR_{max}}$  or photosynthetic productivity displayed an increase in *D. dichotoma* with respect to *D. kunthii* under hypersalinity conditions at the end of the experimental period. This is in agreement with the results observed in other macroalgae species such as *U. compressa* exposed to 42 psu after 6 days in laboratory conditions (Muñoz et al., 2020). Conversely, the brown macroalga *Ectocarpus* showed a severely impaired  $E_{TR_{max}}$  after 7 days when the alga was transplanted in a brine-impacted site (36 psu) compared with the control (34 psu) (Rodríguez-Rojas et al., 2020). These ecophysiological responses are related with the mechanism for the light harvesting complex associated with PSII; as these responses were different

between species, the information suggest that the size of the antenna complex was bigger in *D. kunthii* than in *D. dichotoma* (Grzymiski et al., 1997). In consequence, *D. dichotoma* demonstrates a sun-type algal behavior, i.e., a concomitant increase of  $E_{TR_{max}}$  and irradiance of saturation or  $E_{k_{ETR}}$  (Celis-Plá et al., 2014). Therefore, although both *Dictyota* species are phylogenetically related, they displayed different photoinhibition and photoacclimation capacities under excessive salinity levels, showing a more marked adapted response phenotype in *D. dichotoma* than in *D. kunthii*. In this regard, macroalgae are a highly diverse group with complex functional phenotypes with a variety of ecological roles, even within similar phylogenetic backgrounds; these aspects determine their morphological plasticity and mechanisms to withstand environmental threats, indeed also mediating their capacity to acclimate to global change scenarios (Falkenberg et al., 2013; Gao et al., 2019).

Regarding our data, the higher salinity levels had no apparent effects on the photosynthetic efficiency ( $\alpha_{ETR}$ ) of *D. kunthii*, while in *D. dichotoma* a notorious decrease was observed. Therefore, hypersalinity may have contributed to diminish the light harvesting capacity of *D. dichotoma* since this process has been observed to partially damage or deactivate the reaction center of the photosystems (Li et al., 2016; Cambridge et al., 2017)—for example, in the seagrass *Halophila johnsonii*, the increase in salinity (above 40 psu) induced the decrease of the photosynthetic efficiency ( $\alpha_{ETR}$ ) regarding the controls after 15 days (Fernández-Torquemada et al., 2005). Similar results of decreased  $\alpha_{ETR}$  were observed in the green macroalga *U. compressa* exposed to 82 psu during 6 days in the laboratory (Bittner et al., 2008). *Ectocarpus* also exhibited a  $\alpha_{ETR}$  reduction at 36 psu after 7 days under brine discharges in the field transplants (Rodríguez-Rojas et al., 2020). In contrast, the results of

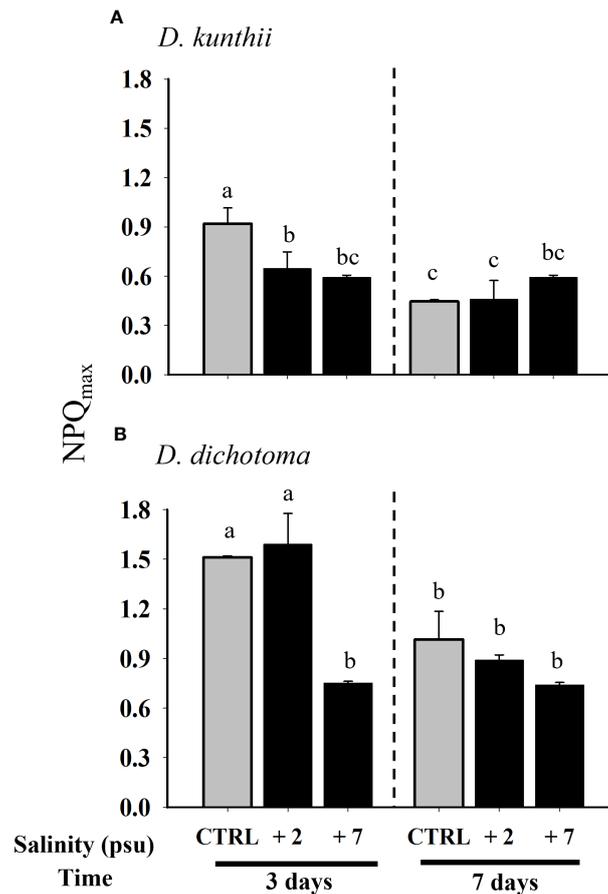


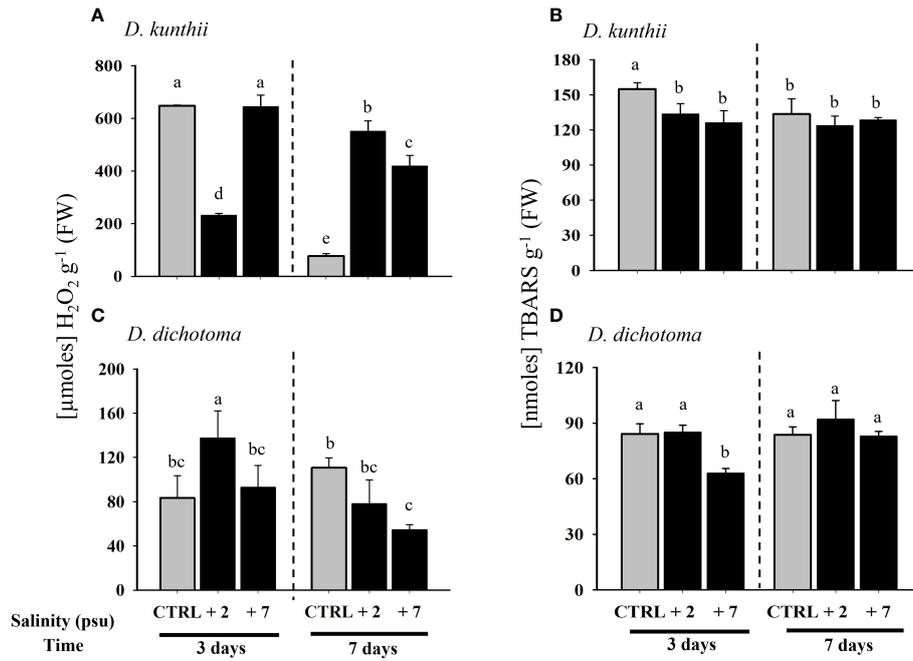
FIGURE 3

Maximal non-photochemical quenching (NPQ<sub>max</sub>) in *D. kunthii* (A) and *D. dichotoma* (B) exposed to high salinity values. For *D. kunthii*, the salinity values were 35 psu (control), 37 (+2) psu, and 42 (+7) psu; for *D. dichotoma*, these were 37 psu (control), 39 (+2) psu, and 44 (+7) psu. The samples were analyzed after 3 and 7 days. Data corresponds to mean  $\pm$  SD (n = 3). Letters represent statistical difference at 95% confidence interval (Student–Newman–Keuls test, p < 0.05).

EkETR showed a positive correlation with ETR<sub>max</sub> in both *Dictyota* species, in which light requirements increased as salinity was greater, thus saturating the ETR; this was reflected in the observed photoinhibition processes. On the other hand, our results showed a decrease in NPQ<sub>max</sub> at higher salinity levels in both species after 3 days, with a return to control levels at day 7. NPQ is an effective mechanism to dissipate excessive energy through heat during high light intensity or when light is paired with other present stressors, playing a role in photoprotection (Celis-Plá et al., 2016). In this sense, excess salinity can induce detrimental damage to the photosynthetic apparatus, and NPQ can aid in diminishing the adverse effects of hypersalinity (Zheng et al., 2019). However, it has been also reported that salt excess may damage the antenna complex, altering the light harvesting system of the photosystem and reducing the energy transferred to PSII, consequently inducing a decrease in NPQ<sub>max</sub> (Sudhir et al., 2005; Zheng et al., 2019); the latter may be behind the decrease in NPQ<sub>max</sub> in *D. kunthii* and *D. dichotoma* at day 3. The effects of salinity on photoprotection have already been reported—for instance, Singh et al. (2019) observed that excess salinity of up to 34 psu with respect to the control (29 psu) caused the decrease of NPQ<sub>max</sub> in the microalga *Dunaliella salina*. Our results also showed a recovery process in NPQ<sub>max</sub> at 7 days in both *Dictyota* species, probably demonstrating the activation of photoprotector processes. In this

regard, the brown macroalga *Sargassum polycystum* showed no changes in NPQ when exposed for 2 weeks to hypersalinity of up to 40 psu vs. the controls at 32 psu, demonstrating strong photoprotection mechanisms to withstand salinity excess (Zou et al., 2018).

Although considered as a molecule with moderate reactivity, H<sub>2</sub>O<sub>2</sub> is the precursor, through Fenton and Haber–Weiss reaction, of the highly toxic hydroxyl radical (HO·). The latter is a highly oxidizing ROS and responsible for the process of lipid peroxidation (Girotti, 2001; Lesser, 2006; Kumar et al., 2014; Moenne et al., 2016). Additionally, it has been determined that accumulation of H<sub>2</sub>O<sub>2</sub> may cause a disruption of electron chains in the mitochondria and chloroplast, eventually affecting the photosynthetic activity (Xia et al., 2014; Hajiboland, 2014). While in *D. dichotoma* H<sub>2</sub>O<sub>2</sub> accumulated mostly in +2 psu at 3 days, in *D. kunthii* this occurred in both hypersalinity conditions after 7 days. However, this was not reflected in an increase of lipid peroxidation or photosynthetic impairment as well, suggesting good management in the production or detoxification of this molecule within these species. Similarly to *D. kunthii*, the field transplants of the brown alga *Ectocarpus* showed a significant accumulation of H<sub>2</sub>O<sub>2</sub>, although it was not correlated with an increase in lipid peroxidation (Rodríguez-Rojas et al., 2020). On the other hand, experiments on the red macroalga *Pyropia haitanensis* submitted to an extremely high salinity condition (~110 psu vs. the

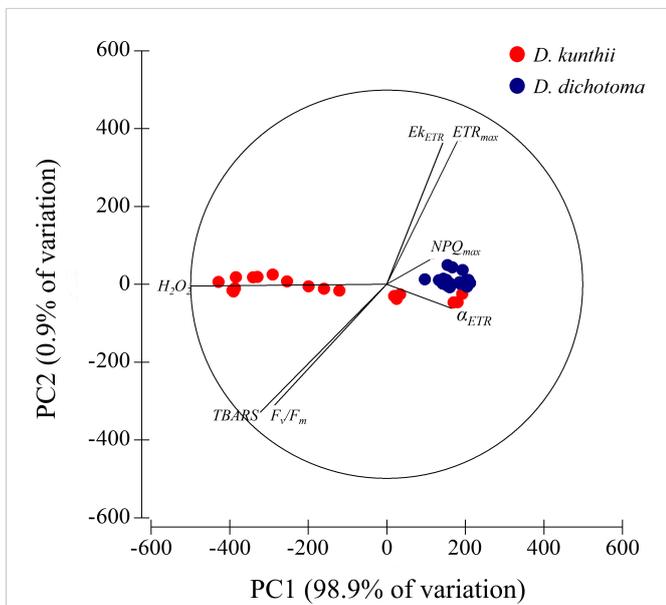


**FIGURE 4** Concentration of H<sub>2</sub>O<sub>2</sub> and thiobarbituric acid reactive substance in *D. kunthii* (A, C) and *D. dichotoma* (B, D) exposed to high salinity values. For *D. kunthii*, the salinity values were 35 psu (control), 37 (+2) psu, and 42 (+7) psu; for *D. dichotoma*, these were 37 psu (control), 39 (+2) psu, and 44 (+7) psu. The samples were analyzed after 3 and 7 days. Data corresponds to mean ± SD (n = 3). Letters represent statistical difference at 95% confidence interval (Student–Newman–Keuls test, p < 0.05).

control at ~30 psu) demonstrated an over-production of H<sub>2</sub>O<sub>2</sub> at 30 min of exposure (Wang et al., 2020). In the green macroalga *Ulva prolifera*, the exposition to high salinity values of ~60 psu induced twice the H<sub>2</sub>O<sub>2</sub> concentration in relation to the control at ~30 psu after 6 days (Luo and Liu, 2011). During the field studies by Capó

et al. (2020) in *P. oceanica* nearby a brine discharge at nearly 41 psu, an increase of lipid peroxidation compared with the control area at 38 psu was evidenced. Moreover, in a comparative study between the green macroalga *Ulva australis* and red seaweed *Pyropia yezoensis*, inter-specific responses were observed, with higher lipid peroxidation in *P. yezoensis* with respect to *U. australis* under 55 psu vs. the controls at 30 psu after 7 days (Samanta et al., 2019). Taken together, our results suggest that the *Dictyota* species studied have efficient response mechanisms to cope with photodamage and oxidative stress mediated by hypersalinity conditions.

Based on our results, *Dictyota* species seem to have a certain tolerance to salinity increments in the short term (within 7 days), although with timeline differences and a degree of connection that may indicate ecophysiological affection mediated by oxidative stress. In this regard, it has been observed that drastic fluctuations in environmental conditions, including salinity, can induce an oxidative stress condition through different metabolic pathways, although most of them end up disrupting electron transport chains in the chloroplast and transferring excess energy to oxygen; the latter can, in turn, mediate an excess of ROS that can affect metabolic and physiological processes such as photosynthesis (Moenne et al., 2016; Muñoz et al., 2020). This process may have occurred in both species under salinity excess—for instance, when H<sub>2</sub>O<sub>2</sub> increased in *D. kunthii*, it was accompanied by a decrease in *F<sub>v</sub>/F<sub>m</sub>* at 7 days; a similar pattern was observed in *D. dichotoma* at 3 days of exposure to excessive salinity levels. In spite of the latter, no specific effects were detected in terms of lipid peroxidation or main photosynthetic impairment, thus indicating efficient tolerance mechanisms to withstand excessive salinity levels that may be attributed to desalination discharges in both *Dictyota* species.



**FIGURE 5** Principal component analysis linked to photosynthetic (*F<sub>v</sub>/F<sub>m</sub>*, α<sub>ETR</sub>, ETR<sub>max</sub>, Ek<sub>ETR</sub>, and NPQ<sub>max</sub>) and oxidative stress (H<sub>2</sub>O<sub>2</sub> and thiobarbituric acid reactive substance) parameters in both times (3 and 7 days), measured in each species: *D. kunthii* (Sp1) and *D. dichotoma* (Sp2).

## 5 Conclusion

Our results showed that *D. kunthii* and *D. dichotoma* showed photoinhibition in higher salinity treatments but a gradual augmentation of primary productivity ( $ETR_{max}$ ) and irradiance of saturation ( $E_{k_{ETR}}$ ) at all times. However, a marked decrease of photosynthetic efficiency ( $\alpha_{ETR}$ ) was observed in *D. dichotoma*, but there were no changes in *D. kunthii*. NPQ decreased in both *Dictyota* spp. in higher salinity levels, making the photosynthetic apparatus damage evident. Although the  $H_2O_2$  accumulation was triggered by the hypersalinity condition in both species, this was not manifested in severe impairment of photosynthetic activity or oxidative damage. In contrast, lipid peroxidation diminished in both species, which demonstrated an efficient machinery of ROS scavenging. This work supports the results obtained from field experiments to determine the magnitude of the impact from the artificial increase of salinity compared with hypersalinity from brine discharge from desalination plants on both species with differing latitudinal distribution. This also contributes relevant information to understand about cellular tolerance strategies from macroalgae so as to confront hypersalinity in *Dictyota* spp., which, given their responses and tolerance, may be recommended as efficient candidates to address the impacts of desalination.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors without undue reservation.

## Author contributions

PM: conceptualization, field work, methodology, formal analysis, writing—original draft, and visualization. FR-R: field work, methodology, funding acquisition, and writing. PC-P: field work, methodology, and formal analysis. AL-M: field work. FB-M:

manuscript review and editing. Iván Sola: manuscript review and editing. CL: writing, formal analyses, and manuscript review. FV: field work and formal analyses. RO: field work and formal analyses. JLS-L: conceptualization, formal analysis, methodology, field work, investigation, resources, review, and editing. CS: conceptualization, field work, methodology, visualization, writing—review and editing, supervision, and funding acquisition. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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