

Effects of hypo- and hypersalinity on photosynthetic performance of *Sargassum fusiforme* (Fucales, Heterokontophyta)

X.J. XIE^{*,***}, X.L. WANG^{*,***,****}, L.D. LIN^{**}, L.W. HE^{*,***}, W.H. GU^{*,***}, S. GAO^{*,***}, X.F. YAN^{**}, G.H. PAN[#], M.J. WU^{*,*,+}, and G.C. WANG^{*,***,†}

Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, 266071 Qingdao, China^{*}

Zhejiang Provincial Key Lab for Water Environment and Marine Biological Resources Protection, College of Life and Environment Science, Wenzhou University, 325035 Wenzhou, China^{**}

Laboratory of Marine Biology and Biotechnology, Qingdao National Laboratory for Marine Science and Technology, 266071 Qingdao, China^{***}

University of Chinese Academy of Sciences, 100039 Beijing, China^{****}

Tianjin Key Laboratory of Marine Sources and Chemistry, College of Marine Science & Engineering, Tianjin University of Science & Technology, 300457 Tianjin, China[#]

Abstract

Photoprotection mechanisms protect photosynthetic organisms, especially under stress conditions, against photodamage that may inhibit photosynthesis. We investigated the effects of short-term immersion in hypo- and hypersalinity sea water on the photosynthesis and xanthophyll cycle in *Sargassum fusiforme* (Harvey) Setchell. The results indicated that under moderate light [$110 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$], the effective quantum yield of PSII was not reduced in *S. fusiforme* fronds after 1 h in hyposalinity conditions, even in fresh water, but it was significantly affected by extreme hypersalinity treatment (90‰ sea water). Under high light [HL, $800 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$], photoprotective mechanisms operated efficiently in fronds immersed in fresh water as indicated by high reversible nonphotochemical quenching of chlorophyll fluorescence (NPQ) and de-epoxidation state; the quantum yield of PSII recovered during the subsequent relaxation period. In contrast, fronds immersed in 90‰ sea water did not withstand HL, barely developed reversible NPQ, and accumulated little antheraxanthin and zeaxanthin during HL, while recovery of the quantum yield of PSII was severely inhibited during the subsequent relaxation period. The data provided concrete evidence supporting the short-term tolerance of *S. fusiforme* to immersion in fresh water compared to hypersalinity conditions. The potential practical implications of these results were also discussed.

Additional key words: aquaculture; chlorophyll *a* fluorescence; dithiothreitol; rapid light curve; violaxanthin.

Introduction

Light energy is essential for photosynthesis; however, it may also result in potential damage of photosynthetic organisms. High light (HL) may result in production of reactive oxygen species (ROS) and destruction of reaction

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[†]Corresponding authors; fax: (86)0532-82898574, e-mail: wmj@wzu.edu.cn (M.J. Wu), gcwang@qdio.ac.cn (G.C. Wang)

Abbreviations: AL – actinic light; Ax – antheraxanthin; Chl – chlorophyll; DEPS – de-epoxidation state; DTT – dithiothreitol; F – fluorescence under illumination; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_m' – maximal fluorescence yield of the light-adapted state; F_v/F_m – maximal quantum yield of PSII photochemistry; HL – high light; I_k – light saturation coefficient; NPQ – nonphotochemical quenching; rETR – relative electron transport rate; $rETR_{\text{max}}$ – maximal relative electron transport rate; RLC – rapid light curve; ROS – reactive oxygen species; SP – saturation pulse; Vx – violaxanthin; Zx – zeaxanthin; Φ_{PSII} – effective quantum yield of PSII.

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centers of photosystems and thylakoid membranes (Niyogi 1999). It is generally accepted that excessive light energy results in overreduction at the stromal side of chloroplast and in accumulation of reduced electron carriers in photosynthetic electron transport chain (Niyogi 2000, Shikanai 2007), and this, as a result, causes oxidative stress. Thus, the balance between the light capturing and light utilization in photosynthesis is essential to avoid HL-induced photodamage, and thus it should be finely adjusted. Numerous studies have demonstrated that it is not HL *per se* that results in light stress, but the high ratio of photon flux density to photosynthesis (Demmig-Adams and Adams III 1992). Thus, when photosynthesis decreased after exposure to any stress conditions, even moderate light illumination may become excessive.

Extreme hypersalinity conditions or desiccation on air cause severe inhibition of the PSII activities in red algae (Lin *et al.* 2009), brown algae (Harker *et al.* 1999, Gévaert *et al.* 2002, 2003), and also in green algae (Gao *et al.* 2011). Prolonged immersion under hypersalinity conditions may cause a dissociation of oxygen evolving complex of PSII (Allakhverdiev and Murata 2008). Under such circumstance, the xanthophyll cycle, which is one of the most important photoprotection processes in algae and plants involved in dissipation of excessive energy *via* modulating the architecture of LHCII (García-Plazaola *et al.* 2012, Jahns and Holzwarth 2012, Ruban *et al.* 2012), could be activated in most desiccation-tolerant algal species (Harker *et al.* 1999, Gévaert *et al.* 2003, Fernández- Marín *et al.* 2011a,b; Xie *et al.* 2013). In contrast, the effects of

hyposalinity conditions on photosynthesis and the operation of the xanthophyll cycle in marine algae are relatively less investigated, though such conditions also often occur in estuarine and intertidal zone, *e.g.*, due to mixing of sea water with rain, melt water, or fresh water from rivers.

Sargassum fusiforme (Harvey) Setchell (Fucales, Heterokontophyta) is one of the most economically important macroalgae inhabiting the northwest coast of the Pacific Ocean (Tseng 1990), and currently is industrially cultivated in Japan, Korea, and China. Although numerous studies have focused on its nutrition composition (Kolb *et al.* 1999, Dawczynski *et al.* 2007), pharmaceutical potential (Jung *et al.* 2007, Yoon *et al.* 2011), and heavy metal metabolism (Yokoi and Konomi 2012, Zou *et al.* 2014), few studies have been performed to study their tolerance to environmental stresses, especially to changes in seawater salinity and HL. The information on the tolerance of salinity and light may be potentially useful for industrial production.

In this study, *S. fusiforme* were subjected to various salinity stress treatments; the photosynthetic properties of PSII and photoprotective capabilities after treatments were investigated. The aims of this contribution were to examine: (1) if *S. fusiforme* could endure hypo- and hypersalinity stresses under HL; (2) if the xanthophyll cycle remains active under such stress conditions, and its relationship with NPQ. Based on these results, we tried to provide practical advices for the management in aquaculture of *S. fusiforme*.

Materials and methods

Plant material: Fronds of *S. fusiforme* were collected from the Dongtuo County (27°50' N, 121°10' E), Zhejiang Province, China. The fronds were temporarily cultivated in the laboratory in a tank containing sea water at temperature *ca.* 16°C. The sea water was refreshed every day during the experimental period. The fronds were cut into lengths of 2–3 cm for the experimental treatments.

Hypo- and hypersalinity treatments: To investigate the effects of hypo- and hypersalinity conditions on the photosynthetic properties of *S. fusiforme*, the cut fronds were immersed in sea water of various salinities in 12-well plate for 1 h in darkness. The salinity of the sea water was adjusted by adding NaCl or diluting with ddH₂O to sterilized natural sea water of 0, 15, 30, 50, and 90‰.

Treatment	Salinity [‰ of the sea water]
S0	0
S15	15
S30	30
S50	50
S90	90

To inhibit the xanthophyll cycle, dithiothreitol (DTT) was added to the sea water at a final concentration of 3 mM. After treatments, fronds were subjected to light treatments and Chl fluorescence measurements. The normal salinity of sea water was 30‰. Groups treated in normal sea water was set as control.

Light treatments and chlorophyll (Chl) *a* fluorescence measurements: Hypo- and hypersalinity-treated fronds in 12-well plates were placed on the sample platform of *Imaging-PAM* with a *Maxi-head* (Walz GmbH, Effeltrich, Germany), and then illuminated with moderate actinic light [AL, 110 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$] provided by the instrumental LEDs (450 nm), and Chl fluorescence was simultaneously determined. The determination procedures are briefly described as follows: the treated fronds were first illuminated under moderate AL for approximately 5 min, the effective quantum yield of PSII was calculated as: $\Phi_{\text{PSII}} = (F_m' - F)/F_m'$, where F_m' represents the maximal fluorescence under illumination assessed by saturation pulse [SP, 2700 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$], and F is the fluorescence under illumination (Genty *et al.* 1989). The fronds were then illuminated with AL of various intensities for 30 s to assess the rapid light curve (RLC). The relative

electron transport rate was calculated as: $rETR = \Phi_{PSII} \times PAR$, where PAR represents the photosynthetically actinic radiation, and Φ_{PSII} was the effective quantum yield of PSII under a given PAR (Schreiber 2004). The measured RLCs were fitted with a model from Walsby (1997) to calculate the maximal $rETR$ ($rETR_{max}$) and light-saturation coefficient (I_k). Since the inhibition factor may cause overestimation of $rETR_{max}$, the final results of $rETR_{max}$ were adjusted according to Nitschke *et al.* (2012). For fronds in each well, at least ten circle areas were selected, their fluorescence parameters were averaged and considered as being representative.

The effects of hypo- and hypersalinity on the photoprotective capabilities of *S. fusiforme* were also investigated. The treated fronds were initially exposed to measuring modulated light of very low intensity to assess the minimal fluorescence (F_0), and then to SP to assess the maximal fluorescence (F_m). This was followed by HL illumination of $800 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ (450 nm) for approximately 30 min and subsequently to low light of $55 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ for approximately 30 min. SP was triggered to assess the quenching of Chl fluorescence at an interval of 1 min during the measurements. The potential maximal quantum yield of PSII was calculated as: $F_v/F_m = (F_m - F_0)/F_m$. Nonphotochemical quenching (NPQ) was calculated as: $NPQ = (F_m - F_m')/F_m'$ (Maxwell and Johnson 2000).

Separation and quantification of pigments of xanthophyll cycle: Fronds treated with various salinity conditions or HL were immediately frozen in liquid nitrogen and stored at -80°C for extraction and analysis of pigments. Pigments were extracted with precooled mixture of methanol:acetone (1:1) as introduced in a previously published method (Xie *et al.* 2013).

An Agilent 1200 HPLC equipped with an Rx-C18 analytical column ($4.6 \times 250 \text{ mm}$) (Agilent Technologies

Inc., Santa Clara, CA, USA) was used for the separation and quantification of xanthophyll cycle pigments. The elution procedures followed those outlined in previous reports (Thayer and Björkman 1990, Enriquez *et al.* 2010) with minor modifications. Specifically, for the first 15 min of the linear gradient, the eluents were transferred from 15% water, 30% methanol, and 55% acetonitrile to 15% methanol and 85% acetonitrile. This was followed by a 2-min linear gradient to 15% water, 15% methanol, 35% acetonitrile, and 35% acetoacetate. From 17 to 27 min, the linear gradient run to 8% water, 22% methanol, 20% acetonitrile, and 50% acetoacetate, and then it continued isocratically until the end of the 30-min separation period. The flow rate was 0.75 ml min^{-1} , and the column oven temperature was 50°C . The pigments were detected at 432 nm, and the peak areas were used to calculate the pigments content according to the standard curve. The Chl *a* and zeaxanthin (Zx) standards were obtained from Sigma (St. Louis, MO, USA); violaxanthin (Vx) and antheraxanthin (Ax) were obtained from the International Laboratory USA (South San Francisco, CA, USA). The concentrations of xanthophyll cycle pigments were normalized to Chl *a*. De-epoxidation state (DEPS) was used as an indicator of the relative content of de-epoxidized xanthophylls in the xanthophyll cycle pigments pool, and was calculated as $(Ax + Zx)/(Vx + Ax + Zx)$.

Statistical analysis: All results were presented as mean value \pm SD of three to four independent experiments. The IBM SPSS Statistics 19 package (IBM Co., Armonk, NY, USA) was used to perform statistical analysis. One-way analysis of variance (ANOVA) and Tukey's post-hoc test ($\alpha=0.05$) were used to determine whether significant differences in photosynthetic performances and xanthophyll cycle pigment contents exist between various salinity treatment groups. Graphs were constructed using Origin Pro 8.5.0 SRI (OriginLab Co., Northampton, MA, USA).

Results

Effects of hypo- and hypersalinity treatments on photosynthesis: Fronds of *S. fusiforme* treated with sea water of various salinities in darkness for 1 h were first subjected to stepwise increasing light in order to assess briefly the effects of the hypo- and hypersalinity treatments on the photosynthetic properties of PSII. For the control group (S30), the Φ_{PSII} value under AL of $110 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ was approximately 0.23 ± 0.05 , while for moderate hypo- and hypersalinity stress, *i.e.*, S15 and S50, the Φ_{PSII} was 0.26 ± 0.04 and 0.18 ± 0.04 , respectively (Fig. 1). Interestingly, in the extreme hypersalinity-stressed fronds, the Φ_{PSII} value showed the opposite trend; the Φ_{PSII} value under S0 increased significantly to 0.32 ± 0.04 , and under S90 decreased significantly to 0.08 ± 0.05 (Fig. 1).

It is obvious that both hypo- and hypersalinity stresses decreased the convexity of RLCs. The $rETR$ in hypersaline sea water (S50 and S90, Fig. 2A) remarkably decreased in

comparison with those in normal sea water (S30), especially under higher PAR. In particular, in the samples under S90, the photosynthetic electron transports were almost completely inhibited. In contrast, hyposaline stress (S15 and S0) influenced $rETR$ less (Fig. 2A). Similarly, hypersalinity stresses (S50 and S90) significantly attenuated the $rETR_{max}$ and I_k (Fig. 2B).

Hypersalinity stresses in darkness induced a decrease in F_v/F_m , *i.e.*, 0.68 ± 0.01 and 0.64 ± 0.01 for the samples under S50 and S90, respectively, significantly lower than the control samples, the F_v/F_m for the latter was 0.71 ± 0.01 . The F_v/F_m in the fronds immersed in fresh water increased to 0.74 ± 0.01 . For S15, there was no significant difference in the value of F_v/F_m compared with the control.

To assess the photoprotective capabilities, hypo- and hypersalinity-stressed fronds were exposed to HL of $800 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ for 30 min, and then to low light

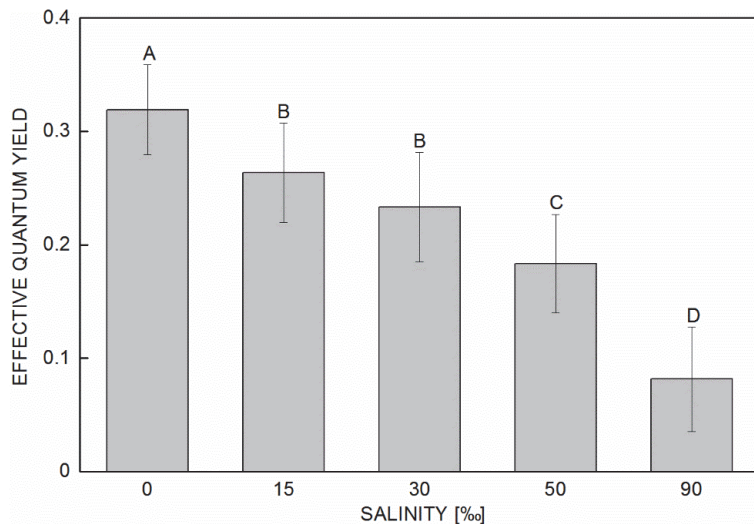


Fig. 1. Effects of hypo- and hypersalinity treatments on the effective quantum yield of PSII (Φ_{PSII}) in *Sargassum fusiforme* under moderate illumination. Fronds were treated with sea water of various salinities in darkness for 1 h. Then, Φ_{PSII} was determined under actinic light of $110 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$. Letters above the columns represent statistical differences of the Φ_{PSII} value for the fronds under various salinity conditions based on one-way ANOVA and Tukey's post-hoc test ($\alpha=0.05$). Data are presented as means \pm SD of three to four independent experiments.

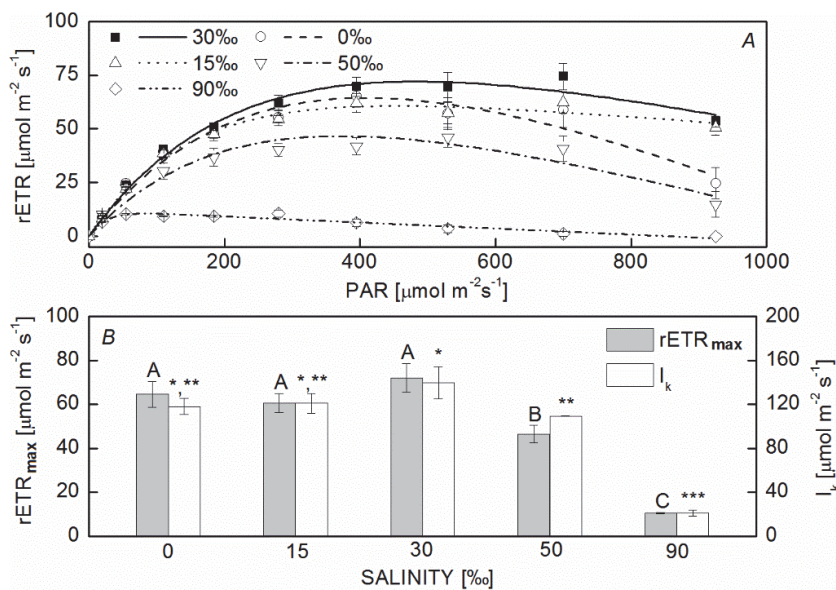


Fig. 2. Rapid light curve of *Sargassum fusiforme* treated with sea water of various salinities where fronds were immersed in sea water of various salinities in darkness for 1 h. After treatment, the fronds were exposed to actinic light of $110 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ for approximately 5 min, and then subjected to RLC measurements (A). The quantum yields were assessed with saturation pulses at intervals of 30 s. Two important photosynthetic parameters, i.e., maximal relative electron transport rate ($rETR_{max}$) and light saturation coefficient (I_k) were deduced from the RLC according to Walsby (1997) and Nitschke (2012) (B). Letters and stars above the columns represent statistical differences of the $rETR_{max}$ and I_k value for the fronds under various salinity conditions based on one-way ANOVA and Tukey's post-hoc test ($\alpha=0.05$). Data are presented as means \pm SD of three to four independent experiments.

of $55 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ for a further 30 min to recover. During HL, the value of Φ_{PSII} was 0 for all five groups (data not shown). When the HL was turned off, the recovery of Φ_{PSII} under low light may reflect the degree of photodamage. In the control samples, the Φ_{PSII} recovered to 0.53 ± 0.01 at the end of 30-min relaxation, accounting for 74.6% of F_v/F_m (Fig. 3). For samples under S0, S15, and S50, Φ_{PSII} recovered by approximately 71.6, 72.2, and 70.6%, respectively, compared with the control. In contrast, the samples treated with S90 were severely damaged by HL and Φ_{PSII} recovered to 0.17 ± 0.05 , only accounting for 26% of original F_v/F_m values.

In the control samples, the maximal NPQ after 30 min of HL was 4.59 ± 0.53 , and it was 0.25 ± 0.06 at the end of 30-min relaxation (Fig. 4). For the moderate hypo- and hypersalinity stressed fronds, i.e., S15 and S50, the maximal NPQ was 4.37 ± 0.49 and 3.99 ± 0.45 , respectively. After 30 min of recovery, NPQ was 0.38 ± 0.08 and

0.25 ± 0.21 , respectively. The fronds stressed by extremely hypersaline sea water (S90) barely developed reversible NPQ; the maximal NPQ was only 1.13 ± 0.2 at the end of HL period, the quenching of Chl fluorescence hardly recovered during the relaxation period, and the NPQ value was 0.91 ± 0.29 at the end of the relaxation period. In contrast, samples immersed in fresh water developed extremely high NPQ during HL stress, the maximal NPQ was 6.54 ± 0.51 . This value returned to 0.54 ± 0.16 after 30 min of relaxation (Fig. 4).

It was demonstrated that the induction of NPQ in brown algae can be blocked by DTT (García-Mendoza and Colombo-Pallotta 2007). After 30 min of HL, the maximal NPQ was only 1.13 ± 0.16 in the fronds immersed in fresh water in the presence of DTT (Fig. 5); the percentage inhibition by DTT was 82%. The inhibitory effects of DTT on NPQ were also seen in the other three groups (Fig. 5).

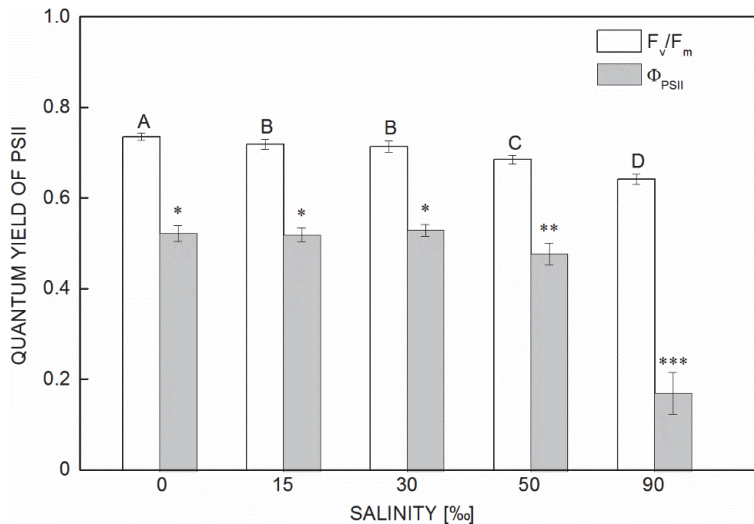


Fig. 3. Effects of hypo- and hypersalinity treatments on the quantum yield of PSII in *Sargassum fusiforme*. The maximal quantum yield of PSII photochemistry (F_v/F_m) was measured following treatment with sea water of various salinities in darkness for 1 h. The fronds were then exposed to actinic light (AL) of $800 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ for 30 min, followed by exposure to AL of $55 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ for 30 min. The effective quantum yield of PSII (Φ_{PSII}) was measured at the end of low light illumination period. Statistical differences are indicated by *different letters* or *stars* above the columns based on one-way ANOVA and Tukey's post-hoc test ($\alpha=0.05$). Data are presented as means \pm SD of three to four independent experiments.

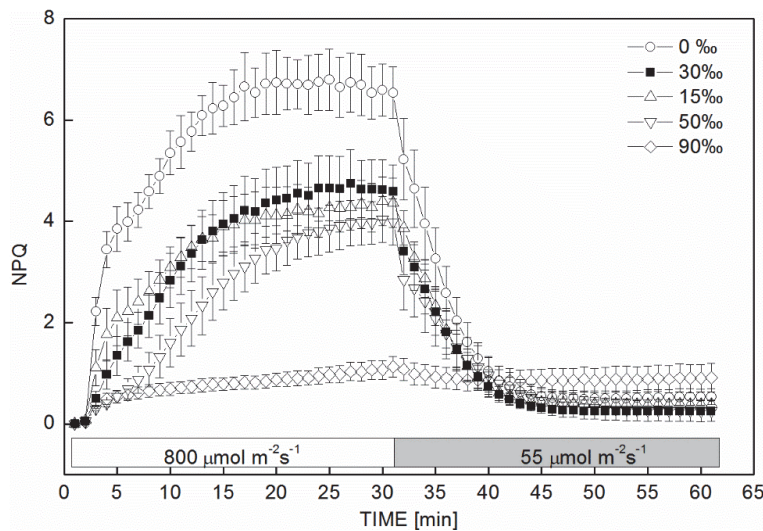


Fig. 4. Effects of hypo- and hypersalinity treatments on the development and relaxation of nonphotochemical quenching (NPQ) in *Sargassum fusiforme*. Hypo- and hypersalinity stressed fronds were illuminated with actinic light of $800 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ for approximately 30 min, and then with $55 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ for approximately 30 min. NPQ was assessed by saturation pulses at intervals of 1 min. Data are presented as means \pm SD of three to four independent experiments.

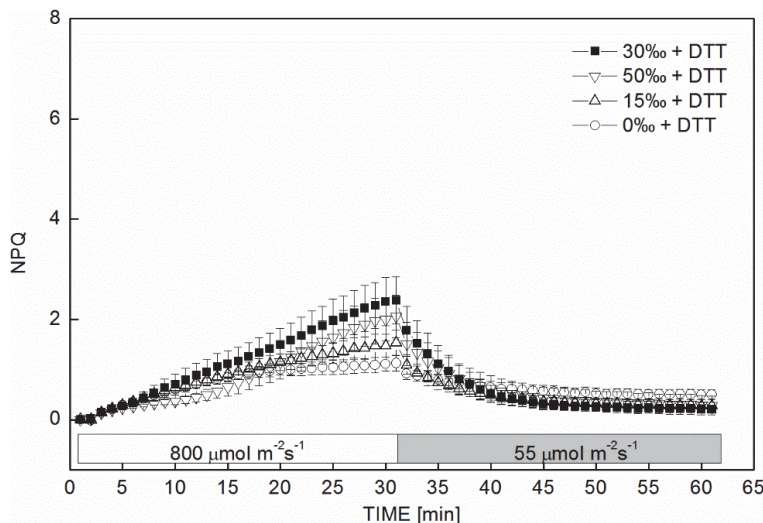


Fig. 5. Inhibitory effects of dithiothreitol (DTT) on the development and relaxation of nonphotochemical quenching (NPQ) in *Sargassum fusiforme*. DTT was supplemented at a final concentration of 3 mM during the last 30 min of hypo- or hypersalinity stress in darkness (this also applies to Fig. 6). Fronds treated with sea water of 90‰ salinity barely developed reversible NPQ (Fig. 4), and thus were not subjected to treatment with DTT. Data are presented as means \pm SD of three to four independent experiments.

Effects of hypo- and hypersalinity stresses on the xanthophyll cycle: The changes in relative contents of xanthophyll cycle pigments were investigated in hypo- and

hypersalinity-stressed fronds of *S. fusiforme* before and after HL stress. Before HL stress, there was no difference in the DEPS value among five treated groups. Xanthophyll

cycle pigments were mainly present in the form of Vx as the value of DEPS was 0. Immersion in hypo- and hypersalinity conditions did not result in remarkable accumulation of Ax and Zx. However, after 30 min of HL, the DEPS value increased dramatically. In the control sample, HL caused an increase in DEPS to 0.2 ± 0.04 (Fig. 6). Hypersalinity stress negatively influenced the accumulation of Ax and Zx, especially, under S90, and DEPS increased only to 0.1 ± 0.02 , significantly lower than that of the control group. In contrast, hyposalinity stress stimulated the accumulation of Ax and Zx. Under S0, the DEPS value increased to 0.41 ± 0.02 , almost twice that of the control. HL induced accumulation of Ax and Zx that was mostly re-epoxidized to Vx after 30 min of recovery (Fig. 6).

The effect of DTT on the xanthophyll cycle was also

investigated. In the presence of DTT, the accumulation of Ax and Zx was severely inhibited during HL. For the S0 fronds, the DEPS only increased to 0.02 ± 0.01 . This inhibitory effect of DTT was also seen in the other treated groups. Due to the strong inhibitory effects of extreme hypersalinity stress on the xanthophyll cycle (Fig. 6), the fronds of S90 treatment were not subjected to treatment with DTT.

HL induced both the development of NPQ (Fig. 4) and the accumulation of Ax and Zx (Fig. 6), and during subsequent low-light illumination, NPQ decreased (Fig. 4), and the accumulated Ax and Zx were re-epoxidized to Vx (Fig. 6). DTT strongly inhibited both the development of NPQ (Fig. 5) and the accumulation of Ax and Zx during HL (Fig. 6). Thus, the development of NPQ and the accumulation of Ax and Zx were closely correlated (Fig. 7).

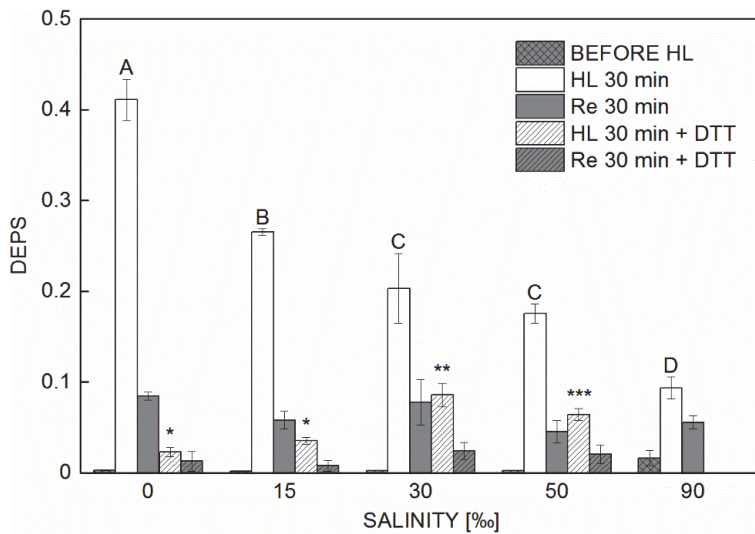


Fig. 6. Effects of hypo- and hypersalinity treatments on the xanthophyll cycle of *Sargassum fusiforme*. Hypo- and hypersalinity stressed fronds were illuminated with actinic light of $800 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ for 30 min, and then with $55 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ for 30 min to recover. Samples were collected before and after high light (HL) stress, and after recovery. Statistical differences are indicated by different letters above the columns based on one-way ANOVA and Tukey's post-hoc test ($\alpha=0.05$). Data are presented as means \pm SD of three to four independent experiments. Re – recovery; DTT – dithiothreitol.

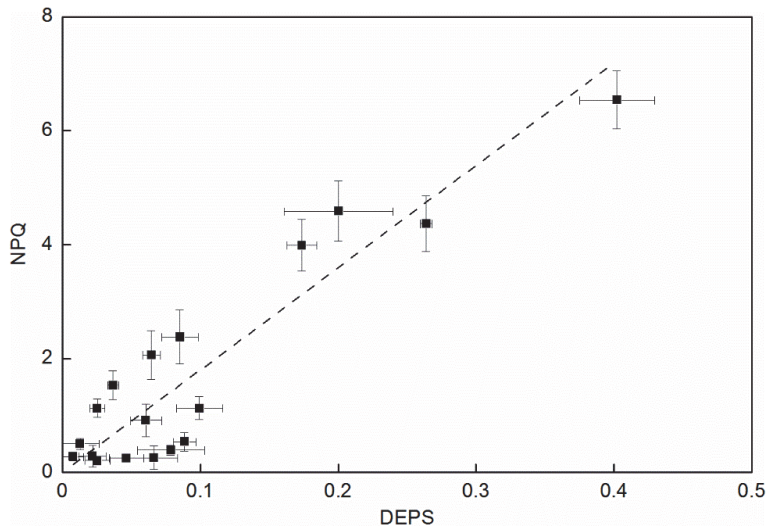


Fig. 7. The positive correlation between the relative contents of antheraxanthin and zeaxanthin and nonphotochemical quenching (NPQ) in *Sargassum fusiforme*. The adjusted R-square is 0.95. Data were obtained from Figs. 4, 5, 6. Vertical and horizontal bars represent SD for $n = 3$ or 4.

Discussion

Hypersalinity inhibits xanthophyll cycle in *S. fusiforme*: Intertidal algae frequently experience fluctuations in

seawater salinities. Hyposalinity conditions may exert manifold effects on micro- and macroalgae, including

inhibition of extracellular carbonic anhydrase (Liu *et al.* 2012), inducing ions efflux, *e.g.*, iodine (Nitschke and Stengel 2014) and K^+ (Reed 1984). The endurance of marine algae to hyposaline condition often differs from species to species. It was summarized that Phaeophyceae generally possess higher tolerance to hyposalinity treatment than Rhodophyceae and Chlorophyceae (Kirst 1990). Whether species within Phaeophyceae tolerate hyposalinity stress, it depends mostly on the horizontal distribution (intertidal or subtidal zone) and species-specific acclimation potentials (Karsten 2007). *S. fusiforme* inhabits an intertidal zone and inevitably suffers from direct exposure to air during low tide, and possible concomitant hypo- or hypersalinity stresses. Our results indicate that immersion in hyposaline conditions (fresh water and sea water of 15‰) in darkness for 1 h did not induce reduction of Φ_{PSII} (Fig. 1) and F_v/F_m (Fig. 3). Nevertheless, the results of RLC and derived photosynthetic parameters, *i.e.*, $rETR_{max}$ and I_k , indicated that the PSII activities were slightly affected under HL (Fig. 2). The effects of hyposalinity conditions on photosynthesis of *S. fusiforme* became apparent after exposure to HL. Especially the freshwater group, where NPQ value reached 6.8 ± 0.6 under HL for 30 min. Such high NPQ value has also been observed in other species of Heterokontophyta, *e.g.*, *Pelvetia canaliculata* (Harker *et al.* 1999), *Macrocystis pyrifera* (Colombo-Pallotta *et al.* 2006), and *Phaeodactylum tricornutum* (Lavaud *et al.* 2002), by contrast to green lineage, in which NPQ value is often between 2 and 4 (Niyogi *et al.* 1997, Niyogi *et al.* 1998, Goss and Jakob 2010). Based on the results of NPQ and variations in xanthophyll cycle pigments under HL and following relaxation (Figs. 4, 5, 6A), it could be concluded that though the photosynthetic performance of PSII was slightly inhibited under HL by hyposalinity (Fig. 2), the operation of the xanthophyll cycle seemed to be stimulated. Thus, as a response to combined stresses of hyposalinity and HL, the fronds immersed in fresh water developed higher NPQ and accumulated more Zx than those in normal sea water.

By contrast to hyposalinity stresses, hypersalinity stresses (50‰ and 90‰) resulted in the significant reduction of PSII activities (Figs. 1–3). RLC results showed that both $rETR_{max}$ and I_k were significantly reduced (Fig. 2B), suggesting the endurance of *S. fusiforme* to HL was weaker under hypersalinity conditions. This inhibition effects of hypersalinity stress to photosynthetic performance were also observed in most investigated macroalgal species (Kirst 1990). It was reported that the reduction of PSII activity may be the result of feedback control or of photoinhibition (White and Critchley 1999). According to the results shown in Fig. 4, *e.g.*, the development of NPQ under hypersalinity and HL was depressed, we could deduce that the photoprotection mechanisms responsible for quenching of Chl fluorescence were severely inhibited

by hypersalinity conditions. It was demonstrated that the induction of NPQ in brown algae was directly correlated with the amount of Zx (García-Mendoza and Colombo-Pallotta 2007). Our results also showed that the conversion of Zx under HL was also inhibited by hypersalinity. Inhibition of the Zx accumulation by DTT (Fig. 6) resulted in reduction of NPQ (Fig. 5). Thus, it is reasonable to assume that the low level of NPQ under HL in the fronds immersed in 90‰ sea water mainly resulted from the inhibition effects of hypersalinity on the conversion of Vx to Zx.

It is well known that the inhibitory effects of hypersalinity could be divided into two components: dehydration of algal cells due to intra- and extracellular water potential difference, and influx of toxic ions, *i.e.*, Na^+ and Cl^- , into cells affecting the metabolism (Kirst 1990, Karsten 2012). The immersion treatment in 90‰ sea water lasted only 2 h in total in our present work, thus, too short time to achieve new homeostasis by synthesis of osmolytes, *e.g.*, mannitol (Reed *et al.* 1985, Rousvoal *et al.* 2011). This requires time from several hours to 2–3 d, so dehydration of *S. fusiforme* cells was inevitable during HL stress. However, it seems not likely that the inhibition of the xanthophyll cycle resulted from dehydration, since *S. fusiforme* could recover even after 6 h of desiccation in air (Pang *et al.* 2007), and the xanthophyll cycle was rarely ceased during desiccation in most desiccation-tolerant algae (Fernández-Marín *et al.* 2009, Fernández-Marín *et al.* 2011a,b). Thus, we propose that the inhibitory effects of hypersalinity on the accumulation of Zx during HL could mainly result from the influx of toxic ions, *e.g.*, Na^+ and Cl^- . However, further investigations in order to evaluate the long-term acclimation of *S. fusiforme* to hypo- and hypersalinity stresses are needed.

Potential implications of the endurance of *S. fusiforme* in fresh water for aquaculture: In East Asia, *S. fusiforme* is widely used as a food source (Tseng 1990). A market demand for this alga is increasing. In China, the majority of *S. fusiforme* currently supplied to the market comes from aquaculture. However, many issues limiting the massive aquaculture production of *S. fusiforme* remain to be resolved. One of the most difficult issues is contamination by epiphytic algae, *e.g.*, diatoms, species of red or green algae. These contaminating algae compete with *S. fusiforme* for nutrition and light, and retard its growth and production (Asaeda *et al.* 2004). Some cultivators treat these contaminated fronds of *S. fusiforme* with fresh water overnight (personal experience). According to our results, we propose that freshwater treatment might be combined with HL for some stubborn epiphytes. The duration of this combined treatment depends on the sensitivities of the contaminating species to fresh water and HL, and also requires further investigations.

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