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QIU, Xuchun

Institute of Environmental Health and Ecological Security, School of the Environment and
Safety Engineering, Jiangsu University

WU, Min

Department of Environmental Sciences and Industrial Development, Faculty of Postgraduate
Studies for Advanced Sciences, Beni-Suef University

MUKAI, Koki

Laboratory of Marine Environmental Science, Faculty of Agriculture, Kyushu University

SHIMASAKI, Yohei

Laboratory of Marine Environmental Science, Faculty of Agriculture, Kyushu University

他

<https://doi.org/10.5109/2340991>

出版情報：九州大学大学院農学研究院紀要. 64 (2), pp.293-300, 2019-09-02. Faculty of
Agriculture, Kyushu University

バージョン：

権利関係：



Effects of Elevated Irradiance, Temperature, and Rapid Shifts of Salinity on the Chlorophyll *a* Fluorescence (OJIP) Transient of *Chattonella marina* var. *antiqua*

Xuchun QIU¹*, Min WU¹, Koki MUKAI, Yohei SHIMASAKI and Yuji OSHIMA

Laboratory of Marine Environmental Science, Faculty of Agriculture, Kyushu University,
Motooka 744, Nishi-ku, Fukuoka City, 819-0395, Japan

(Received May 7, 2019 and accepted May 8, 2019)

Chattonella spp. often experience a broad variety of environmental changes in coastal areas. However, insights into their stress responses to shifts of environmental factors remains largely unknown. In this study, we investigated responses in the OJIP transient of *C. marina* var. *antiqua* grown under various culture conditions. Short-term irradiance shifts experiment showed that a 4-h exposure to elevated irradiance (EI, $1100 \pm 50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) significantly decreased the F_v/F_m ratio, PI_{ABS} , and ETo/RC , while increased the ABS/RC , TRo/RC , and Dio/RC of *Chattonella* cells. When the cells were shifted back to the control irradiance (CI, $110 \pm 10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), all the above parameters recovered to normal status within 3 h. During batch cultures, repeated irradiance shifts (exposure to EI for 4 h per day) significantly decreased the maximum cell densities of *C. marina* var. *antiqua* (to 64% of that in control), but did not affect the maximum growth rate. The decrease of PI_{ABS} could recover to normal status at the next morning at the exponential phase, suggesting the importance of weak light intensity in morning and evening for the recovery from photoinhibition and consequent algal growth in actual environment. But PI_{ABS} level did not completely recover at the stationary phase. The interaction of temperature and rapid salinity shifts played significant roles in mediating growth rate, F_v/F_m ratio, and PI_{ABS} of *C. marina* var. *antiqua*. Despite water temperatures, rapid salinity shifts from 30 to 10 tended to notably decrease growth and photosynthetic activity of *C. marina* var. *antiqua*.

Key words: *Chattonella marina* var. *antiqua*; irradiance shifts; Chlorophyll *a* fluorescence transient (OJIP); photosynthesis, salinity shifts

ABBREVIATIONS: ABS/RC : light absorption per active reaction center; Dio/RC : dissipation energy per active reaction center; ETo/RC : the electron transport per active reaction center; F_v/F_m ratio: maximum quantum yield of Photosystem II; OJIP: chlorophyll *a* fluorescence transient; PI_{ABS} : performance index on absorption basis; PS: photosystem; Q_A : primary quinone; RC: reaction center; TRo/RC : trapping of excitation energy per active reaction center

INTRODUCTION

Over the past several decades, raphidophytes *Chattonella* spp. have frequently formed harmful algal blooms (HABs) and caused serious ecological damages and economic losses in the coastal waters around the world (Imai and Yamaguchi, 2012; Pérez-Morales *et al.*, 2017; García-Mendoza *et al.*, 2018). Because a massive increase in cell number is essential for the occurrences of *Chattonella* HABs, numerous studies have been conducted to investigate the effects of ambient factors on their growth and bloom ecology (Imai and Yamaguchi, 2012). In general, the irradiance, water temperature, salinity, and nutrients are the most significant factors for the growth and their bloom formation of *Chattonella* spp. (Nakamura, 1985; Yamaguchi *et al.*, 1991; Yamatogi *et al.*, 2006; Katano *et al.*, 2012; Katano *et al.*, 2014), while some biological factors also play important roles in affecting their bloom dynamics (Qiu *et al.*, 2011; Qiu *et al.*, 2014; Park *et al.*, 2016).

On the other hand, HABs species may undergo rapid

shifts of environmental factors, especially in the coastal areas. For example, the irradiance of surface water can increase from 0 to $>1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ within 8 hours in summer (Marshall and Hallegraeff, 1999), and the salinity of surface water can rapidly decrease from more than 30 to less than 10, due to large amount of freshwater inflow after heavy rain (Katano *et al.*, 2012). Adaptation to those unavoidable environmental stress is crucial for the survival and growth of algae. It has been shown that exposure to elevated irradiance may cause photooxidative damage of *Chattonella* species (Warner and Madden, 2007; Mukai *et al.*, 2018), and rapid decrease in salinity may affect their diel vertical migration (DVM) behavior and accumulation in surface water (Katano *et al.*, 2012; Shikata *et al.*, 2014). However, insights into the responses of *Chattonella* to rapid shifts of environmental factors remains largely unknown.

Photosynthesis is the basic and essential process for the growth, cell division, and other vital functions of all photosynthetic organisms (Baker, 2008; Schaum *et al.*, 2017; Slattery *et al.*, 2017). Thus, photosynthetic parameters have been widely used to reflect and monitor algal growth and health (Kruskopf and Flynn, 2010; Stirbet and Govindjee, 2011; Larkum *et al.*, 2012). For example, the F_v/F_m ratio, which reflects the efficiency of photochemical conversion of light energy, has been found to be significantly correlated to the growth rate

¹ Institute of Environmental Health and Ecological Security, School of the Environment and Safety Engineering, Jiangsu University, Zhenjiang, Jiangsu 212013, P. R. China

* Corresponding author: e-mail: xuchunqiu@agr.kyushu-u.ac.jp (X. Qiu)

and/or growth phase of phytoplankton species (Kruskopf and Flynn, 2010; Oukarroum, 2016), including *Chattonella* (Qiu *et al.*, 2013; Qiu *et al.*, 2016). Recently, the Chlorophyll *a* fluorescence transient (OJIP) test has become an important tool in monitoring the photosynthetic events and physiological state of plant and algae (Strasser *et al.*, 2004; Kruskopf and Flynn, 2010). It has been proved that the OJIP analysis not only can reflect the impacts of various stresses to algae, but also can provide reliable interpretation for the mechanisms involved in the stress responses (Hockin *et al.*, 2012; Žak and Kosakowska, 2015).

In this study, therefore, we investigated variations in OJIP parameters of *C. marina* var. *antiqua* grown under various culture conditions, i.e., short-term irradiance shifts, repeated irradiance shifts, and rapid salinity shifts at various temperatures. The objective of this study is to reveal the stress responses of *Chattonella* species to shifts of those environmental factors, from the viewpoint of its photosynthetic activity.

MATERIALS AND METHODS

Species and general culture conditions

An axenic strain of *C. marina* var. *antiqua* (NIES-1) was obtained from the National Institute of Environmental Studies (NIES, Japan). Stock cultures were maintained using modified SWM-3 medium (Yamasaki *et al.*, 2007) adjusted to a salinity of 30 at 25°C under $110 \pm 10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ of cool-white fluorescent illumination with a 14:10 h light: dark cycle. Semicontinuous cultures of *C. marina* var. *antiqua* were conducted under the same conditions as stock culture. These cultures were diluted daily by fresh media to maintain a constant cell density ($8 \times 10^3 \text{ cells ml}^{-1}$), and, after acclimation, a constant growth rate ($0.59 \pm 0.08 \text{ div. d}^{-1}$). Those cell suspensions were used for the following experiments.

Effects of short-term irradiance shifts

Effects of short-term irradiance shifts on OJIP parameters were assessed over the course of a day during the semicontinuous culture under the control irradiance (CI, i.e., $110 \pm 10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). About 50 ml cell suspension was incubated to a new 70 ml sterile flask ($n = 3$) and then shifted from control irradiance to an elevated irradiance (EI) at $1100 \pm 50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ from 11:00 (6 h after lights-on), and subsamples for OJIP test were taken at 15:00 (4 h after shift to EI). Subsequently, 30 ml cell suspension exposed to EI were incubated to a new 70 ml sterile flask ($n = 3$) and then shifted to CI for recovery, respectively. Samples to assess recovery of OJIP parameters were taken at 16:00 (1 h after recovery), 17:00, and 18:00. As the control, OJIP parameters of cell suspensions in the semicontinuous culture were measured at the necessary time points described as above.

Effects of repeated irradiance shifts

To assess effects of repeated exposure to elevated

irradiance (EI), batch cultures of *C. marina* var. *antiqua* were started by inoculating cells suspension in semicontinuous culture into 70-ml sterile flask (Nunc; $n = 3$) containing 50 ml modified SWM-3 medium, at an initial cell density of $1 \times 10^3 \text{ cells ml}^{-1}$. *C. marina* var. *antiqua* was grown under the same conditions as stock culture, except the irradiance condition described as follows: (i) control, the irradiance was set at $110 \pm 10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ during the 14-h light period (Fig. 1A); (ii) repeated exposure to elevated irradiance (REI), the irradiance was elevated to $1100 \pm 50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ from 11:00 to 15:00, and was set at $110 \pm 10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ during the rest times of the 14-h light period (Fig. 1B). Batch cultures of *C. marina* var. *antiqua* were conducted for 7 days, and all flasks were gently mixed by hand twice a day. Measurement of cell number and OJIP test were conducted daily at 10:30 (4.5 h after the start of the photoperiod), and OJIP parameters in REI treatment were also measured at 15:00 (just after 4-h exposure to EI) and 18:00 (after 3-h recovery in CI).

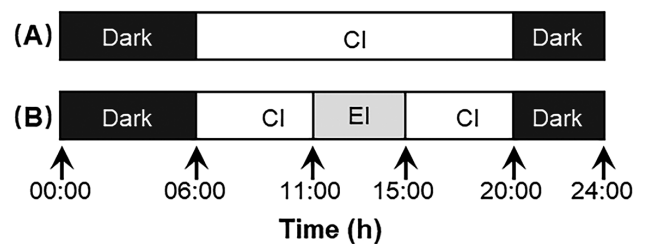


Fig. 1. Diagram showing the setting of light intensity for testing effects of repeated elevated irradiance (REI) exposure on the growth and OJIP-fluorescence parameters of *Chattonella marina* var. *antiqua* during batch cultures. CI: control irradiance at $110 \pm 10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$; EI: elevated irradiance at $1100 \pm 50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$.

Combined effects of temperatures and rapid shifts of salinity

To test the effects of rapid shifts in salinity at different temperatures, *C. marina* var. *antiqua* cells in semicontinuous culture were preconditioned to each temperature (i.e., 30, 25, or 20°C) for a minimum of 2 weeks before experiment. Five test salinities (30, 25, 20, 15, and 10) of modified SWM-3 medium were adjusted by mixing the modified SWM-3 media of salinities 30 and 0, accordingly. After acclimation, *C. marina* var. *antiqua* cells in semicontinuous were inoculated into 8-ml sterile culture tubes (Evergreen Scientific, Los Angeles, CA) containing 5 ml of a prepared modified SWM-3 medium with different salinities, and grown under corresponding temperatures. The initial cell density was $1 \times 10^3 \text{ cells ml}^{-1}$, and other culture conditions (except temperature and salinity) were the same as those of stock cultures. Each experimental group had three replicates. Cultivations were conducted for 3 days, and cell growth was measured daily by *in vivo* fluorescence (model 10-AU-005-CE fluorometer; Turner Designs, Sunnyvale, CA). The OJIP test were conducted at the end of culti-

vation (i.e., 72 h after the salinity shifts).

OJIP test and calculation of growth rate

Unless noted otherwise, OJIP tests were conducted at the same times between 10:00 (4 h after the start of the photoperiod) and 11:00 am to minimize effects of diel periodicity in algal physiological factors. The OJIP test was conducted using an AquaPen-C portable fluorometer (Photon Systems Instruments, Czech Republic). The AquaPen-C was set to maximum saturation pulse intensity of $3000 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ of red light (625 nm) for 2 s and OJIP curves were recorded using the supplied FluorPen software (Photon Systems Instruments). The OJIP features of the fluorescence induction curve were defined following the equations described by Strasser *et al.* (2004).

The growth rate (GR, divisions d^{-1}) were determined as $\text{GR} = \ln(N_2/N_1) / [\ln(2) * (t_2 - t_1)]$, where N_1 and N_2 are defined as values of cell densities or *in vivo* fluorescence at time 1 (t_1) and time 2 (t_2), respectively (Guillard, 1973). The maximum growth rate was determined as the maximum GR from 3 consecutive data points during the entire cultivation.

Statistical analysis

The experimental data were checked for assump-

tions of homogeneity of variance across treatments using Levene's test, and data were normalized using square root or square arcsine transformation to meet the requirements of analysis of variance (ANOVA). For data of subsection 2.2, an independent sample t-test was used to test for differences between the irradiance shifts treatment and control groups. For data of subsection 2.3, one-way ANOVA followed by Dunnett's pairwise multiple comparison t-test was used to test differences between treatments and control. For data of subsections 2.4, two-way ANOVA was applied in analyzing the effects of temperature, salinity and their interactions on the growth and OJIP parameters. Once a significant interaction was detected, a simple effect analysis was conducted to examine the difference between various salinity shifts groups within each level of temperature. All statistical analyses were performed using SPSS Advanced Models 11.0J software (SPSS Japan, Tokyo, Japan).

RESULTS

Effects of short-term irradiance shifts

During the light period of experiment, the ranges of average F_v/F_m ratio, PI_{ABS} , ABS/RC , TRo/RC , ETo/RC , and Dlo/RC of *C. marina* var. *antiqua* grown at control irra-

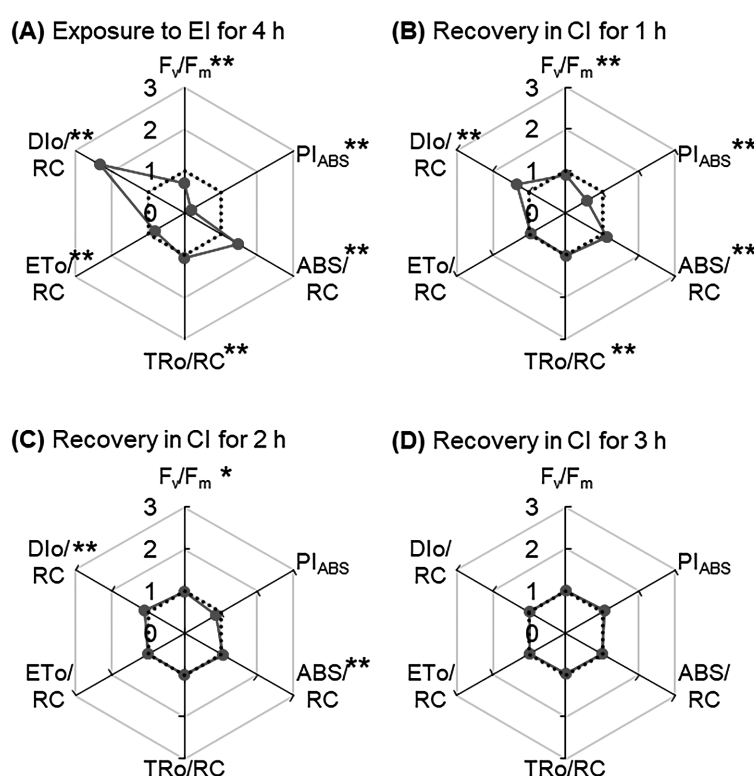


Fig. 2. Spider diagrams showing variations in OJIP-fluorescence parameters of *Chattonella marina* var. *antiqua* exposed to elevated irradiance (EI, $1100 \pm 50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 4 h (A), and subsequently recovered at control irradiance (CI, $110 \pm 10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 1 h (B), 2 h (C), and 3 h (D). Data of irradiance shifts treatment group (solid lines with circle symbol) are shown as the values after normalization to respective values obtained in the control (dotted lines). Asterisks following the parameter labels indicates significant between treatment and control (* $p < 0.05$; ** $p < 0.01$).

diance were 0.67–0.70, 0.97–1.42, 2.47–2.71, 1.74–1.78, 1.03–1.07, and 0.73–0.94, respectively. As shown in Fig. 2A, exposure to EI for 4 h significantly decreased the F_v/F_m ratio (72% of control), PI_{ABS} (18% of control), and ETo/RC (82% of control), while increased the ABS/RC (145% of control), TRo/RC (107% of control), and DIo/RC (234% of control). When the EI exposed cells were shifted back to the CI, all the parameters derived from the OJIP analysis rapidly recovered to the level of those in the control group. The ETo/RC recovered within 1 h (Fig. 2B), the PI_{ABS} and TRo/RC recovered within 2 h (Fig. 2C), and the F_v/F_m ratio, ABS/RC , and DIo/RC recovered within 3 h (Fig. 2D).

Effects of repeated irradiance shifts

Despite the irradiance conditions of batch cultures, *C. marina* var. *antiqua* cells began to grow well from day 1 and reached early stationary phase from day 4 onwards (Fig. 3A). There was no significant difference in the maximum growth rates of *C. marina* var. *antiqua* cultured in CI (1.07 ± 0.04 divisions d^{-1}) and REI (0.98 ± 0.04 divisions d^{-1}). However, REI treatment significantly reduced the maximum cell densities of *C. marina* var. *antiqua*, which were decreased to 64% ($p < 0.01$) of that in control cultures. As shown in Fig. 3B, the F_v/F_m ratio of *C. marina* var. *antiqua* was significantly decreased to 61–92% of that in control cultures after 4-h EI exposure, and those inhibitions recovered to the level of control (97–101%) at the next morning. The PI_{ABS} of *C. marina* var. *antiqua* was significantly decreased to 5–60% of that in control cultures after 4-h EI exposure, and those inhibitions recovered to the level of control (94–96%) at the next morning at the exponential phase (i.e., day 1 to day 3, Fig. 3B). When the cultures reached stationary phase (i.e., from day 4 onwards), however, the inhibitions of PI_{ABS} could not completely recover to the level of control (Fig. 3C).

Effects of temperature and rapid shifts of salinity

The maximum growth rates of *C. marina* var. *antiqua* grown at different combinations of temperature and salinity are shown in Fig. 4A. The highest maximum growth rate (0.96 ± 0.04 divisions d^{-1}) was observed at 25°C and a salinity of 20. Two-way ANOVA indicated significant effects of temperature, salinity shifts, and their interactions on the growth rate, F_v/F_m ratio, and

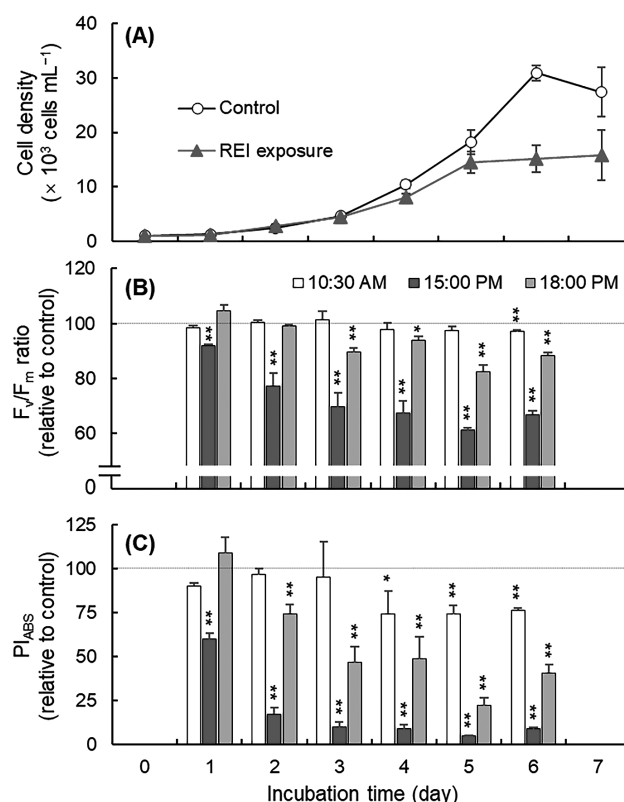


Fig. 3. Daily variations in cell density (A), F_v/F_m ratio (B) and PI_{ABS} (C) of *Chattonella marina* var. *antiqua* during batch cultures at different irradiance conditions. Control irradiance (CI): $110 \pm 10 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ during the 14-h light period; REI: the irradiance was elevated to $1100 \pm 50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (EI) from 11:00 to 15:00, and was set at CI during the rest times of light period. OJIP parameters were measured at 10:30 (just before EI exposure), 15:00 (just after the 4-h exposure to EI), and 18:00 (3h after the end of EI exposure). Data of F_v/F_m ratio and PI_{ABS} are mean \pm SD ($n = 3$), which are shown as the values after normalization to respective values obtained in the control (dotted lines). Asterisks indicates significant between treatment and control (* $p < 0.05$; ** $p < 0.01$).

PI_{ABS} of *C. marina* var. *antiqua* at the $p < 0.001$ level (Table 1). Therefore, simple effect analysis was conducted to examine the difference among salinity shifts within each level of temperature. At 30°C, only the salinity shifts from 30 to 10 significantly decreased the maximum growth rate, F_v/F_m ratio, and PI_{ABS} of *C.*

Table 1. Summary of results of two-way ANOVA testing the effects of temperature, salinity shifts and their interaction on the maximum growth rate, F_v/F_m ratio, and PI_{ABS} of *C. marina* var. *antiqua*^a

Factors	df	Maximum growth rate					F_v/F_m ratio				PI_{ABS}			
		Type III-SS	Mean S	F-value	p-value		Type III-SS	Mean S	F-value	p-value	Type III-SS	Mean S	F-value	p-value
Intercept	1	2.2e+01	2.2e+01	1.2e+04	<0.001		2.4e+01	2.4e+01	2.0e+06	<0.001	2.3e+02	2.3e+02	8.8e+03	<0.001
Temperature	2	6.9e-01	3.4e-01	1.9e+02	<0.001		5.0e-02	2.5e-02	2.0e+03	<0.001	3.6e+01	1.8e+01	7.1e+02	<0.001
Salinity	4	8.2e-01	2.1e-01	1.1e+02	<0.001		3.3e-03	8.3e-04	6.7e+01	<0.001	2.0e+00	4.9e-01	1.9e+01	<0.001
Interaction	8	8.3e-02	1.0e-02	5.8e+00	<0.001		8.7e-04	1.1e-04	8.8e+00	<0.001	1.4e+00	1.8e-01	7.1e+00	<0.001
Error	30	5.4e-02	1.8e-03				3.7e-04	1.2e-05			7.6e-01	2.5e-02		

^a df: degree of freedom; Type III-SS: Type III sum of squares; Mean S: mean square; bold value is statistically significant

marina var. *antiqua* (Fig. 4). At 25°C, the acute salinity shifts from 30 to 10 also tended to significantly decrease the maximum growth rate, F_v/F_m ratio, and PI_{ABS} (Fig. 4), and the acute salinity shifts from 30 to 20–25 tended to significantly increase the PI_{ABS} (Fig. 4C). At 20°C, acute salinity shifts from 30 to 10 or 15 tended to significantly decrease the maximum growth rate and F_v/F_m ratio of *C. marina* var. *antiqua* (Fig. 4A and B), while no significant differences were observed among their PI_{ABS} values (Fig. 4C).

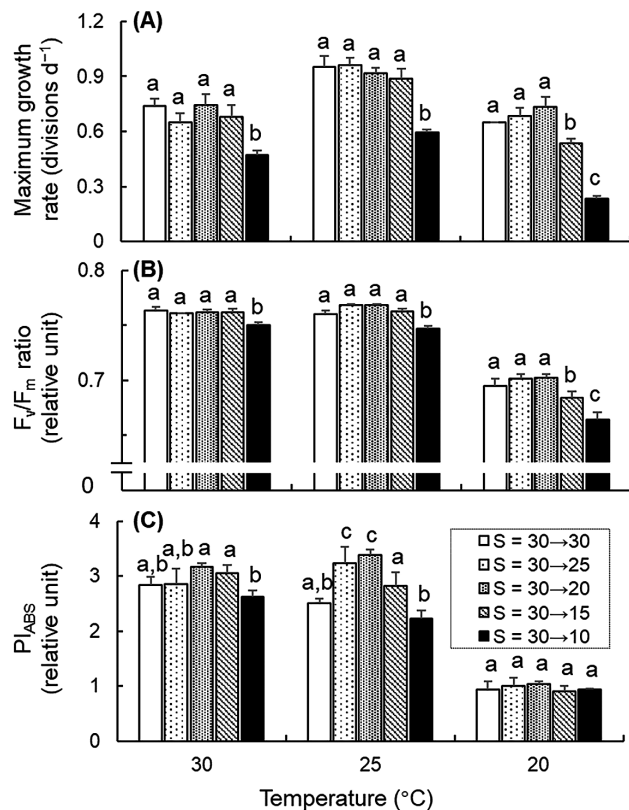


Fig. 4. Effects of rapid salinity shifts on the maximum growth rate (A), F_v/F_m ratio (B) and PI_{ABS} (C) of *Chattonella marina* var. *antiqua* grown at different temperatures. Data are mean \pm SD of triplicate measurements, and values not sharing a common letter are significantly different at $p < 0.05$.

DISCUSSION

Chlorophyll *a* fluorescence transient (OJIP) analysis suggested that *C. marina* var. *antiqua* at exponential phase has relative strong capabilities to avoid impacts of environmental stress, such as rapid shifts to elevated irradiance or to low salinity. Those capabilities may contribute to maintaining a high photosynthetic activity and growth rate of cells grown at complex and volatile environment conditions, such as in the coastal waters during summer seasons.

Our results demonstrated that short-term exposure to light intensity of $1100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ is strong enough to induce photoinhibition of *C. marina* var. *antiqua*, as indicated by the significant decreases in F_v/F_m

ratio and PI_{ABS} (Fig. 2A). When the amount of light exceeds that which can be used for photochemical energy transfer, the potential for irreversible photooxidative damage increases (Sirikhachornkit and Niyogi, 2010). However, our results suggested that *C. marina* var. *antiqua* may own strong capabilities to resist the potential damage induced high light intensity, as indicated by the rapid (within 3 h) recovery of the photosynthetic parameters, after shifting back to CI (Fig. 2B–D). Similarly, Warner and Madden (2007) also reported that exposure to a high light intensity ($600 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 1 h) notably reduced the F_v/F_m ratio of the *C. subsalsa* (rhopidophyte), while the inhibited F_v/F_m ratio could recover to normal within 6 h after shifting back to the low irradiance ($30 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). It seems that *Chattonella* spp. have strong tolerance to high light intensity exposure.

Analysis of parameters derived from the OJIP curve also well supported the conclusion that *Chattonella* has strong tolerance to irradiance shifts (Fig. 2). Under EI exposure conditions, the increase in ABS/RC could attribute to the decrease in active reaction centers and/or in active Q_A reducing centers (Strasser and Stirbet, 1998). The inhibition of re-oxidation of Q_A^- to Q_A can increase the value of TRo/RC, in turn resulting in reduced electron transport per trapping, as indicated by the reduced ETo/RC (Strasser *et al.*, 2000; Sepratoomrosh *et al.*, 2016). The notably increased Dto/RC indicates an increased non-photochemical energy dissipation from the active RCs (Strasser *et al.*, 2004). All these energy flux ratios supported that the photosynthetic efficiency was decreased due to EI exposure. However, rapid recovery of those parameters derived from the OJIP curve suggested that the regulate mechanisms are effective for acclimating to rapid changes in light intensity, which may give *Chattonella* cells some ecological advantages HABs.

It is well known that natural populations of some flagellates display diurnal vertical migration (DVM) behavior, which enable them to actively acquire light at surface layers and nutrients over a wide depth range (Watanabe *et al.*, 1995; Shikata *et al.*, 2015; Tilney *et al.*, 2015). Because *Chattonella* cells tend to accumulated in the surface water during midday in summer (Watanabe *et al.*, 1995), a strong tolerance to EI exposure is necessary for resisting the potential photooxidative damages. During the process of downward migration, the rapid recovery of photosynthetic activity may help *Chattonella* cells to store more energy and produce more organic matter for other vital functions. Those rapid photoprotective mechanisms may provide an explanation for the findings that *Chattonella* species can maximize photosynthesis and growth well under high light conditions (Warner and Madden, 2007; Qiu *et al.*, 2013; Tilney *et al.*, 2015).

During a batch culture, repeated EI exposure (REI, exposure to EI for 4h per day) did not affect the maximum growth rates but significantly reduced the maximum cell density of *C. marina* var. *antiqua* (Fig. 3A). Similar growth curves of *C. marina* var. *antiqua* and *C.*

subsalsa grown under elevated irradiances conditions have also been found by previous studies (Warner and Madden, 2007; Qiu *et al.*, 2013; Tilney *et al.*, 2015). The OJIP analysis of parameters suggests that the tolerance of *C. marina* var. *antiqua* to EI exposure was depended on their growth phases (Fig. 3). In the exponential growth phase, the inhibitions in F_v/F_m ratio and PI_{ABS} recovered to the level of control in the next morning, which may provide an explanation for the unaffected growth rate of *C. marina* var. *antiqua* cells in the REI group. These findings strongly suggested that the weak light intensity in morning and evening is important for the recovery from photoinhibition and consequent algal growth in actual environment. On the other hand, excessive light intensity can cause photooxidative stress in plants and algae, and eliminating those oxidative substances needs extra consumption of energy (Leeuwe *et al.*, 2005; Mukai *et al.*, 2018). Moreover, the reduced tolerance of *C. marina* var. *antiqua* in the stationary phase may result in an accumulation of photooxidative damage due to repeated EI exposure, which may further aggravate the extra energy consumption. Therefore, we inferred that the reduced EI-tolerance may partly contribute to the reduced maximum cell density of *C. marina* var. *antiqua* in exposure group.

The molecular mechanisms involved in the stress-tolerance of *Chattonella* to EI are still unclear. In general, excessive light energy can increase the production of reactive oxygen species (ROS) and thereby cause oxidative stress in plants and algae (Leeuwe *et al.*, 2005; Mukai *et al.*, 2018). Thus, the 2-Cys peroxiredoxin (Prx), which is a highly expressed antioxidant in *C. marina* var. *antiqua* in the exponential phase, has been considered to play important roles in protecting cells against photooxidative damage and maintaining a high growth rate (Qiu *et al.*, 2013; Mukai *et al.*, 2018; Mukai *et al.*, 2019). Indeed, the transcript expression levels of 2-Cys Prx in *Chattonella* cells was induced by EI exposure in batch cultures (Mukai *et al.*, 2018), and its protein expression levels exhibited significant positive correlations with the growth rate and F_v/F_m ratio of *Chattonella* cells during a field HAB (Qiu *et al.*, 2016). In addition, our previous study also found a significant decrease in protein expression level of 2-Cys Prx in *C. marina* var. *antiqua* at the later stationary phase (Qiu *et al.*, 2013). This finding may partly explain the reduced tolerance of *C. marina* var. *antiqua* to EI exposure, when the cells reached stationary phase.

The growth rates of *C. marina* var. *antiqua* at different combinations of temperature and salinity (Fig. 4 A) agreed well with those reported by previous studies (Yamaguchi *et al.*, 1991; Yamatogi *et al.*, 2006). For example, Yamaguchi *et al.* (1991) reported that *C. marina* var. *antiqua* can grow at temperatures from 15 to 30°C and salinity from 10 to 35, with an maximal growth rates of 0.97 divisions d^{-1} (at the combination of 25 °C and 25). Relative high growth rates (0.65–0.96 divisions d^{-1}), F_v/F_m ratios (0.76–0.77), and PI_{ABS} (2.5–3.4) were observed within the temperature range of 25–30 °C and salinity range of 15–30. Those finding are

consistent with the phenomenon that dense blooms (>1000 cells ml^{-1}) of *C. marina* var. *antiqua* often occurred at the temperatures of 25–33°C in coastal areas of Japan (Yamaguchi *et al.*, 1991; Yamatogi *et al.*, 2006; Katano *et al.*, 2012; Qiu *et al.*, 2016). However, the surface salinity sometimes suddenly decreased to <10 as a consequence of freshwater input from rainfall during *Chattonella* blooms (Katano *et al.*, 2012; Katano *et al.*, 2014). Our results showed that the acute salinity shifts from 30 to 10 significantly decreased the maximum growth rate, F_v/F_m ratio, and PI_{ABS} of *C. marina* var. *antiqua*. Some previous studies have found that *Chattonella* cells moves to avoid water with low salinity (Katano *et al.*, 2012; Katano *et al.*, 2014; Shikata *et al.*, 2014). Thus, our finding, together with those field observations, suggested that salinity shifts may play important roles in regulating the bloom dynamics of *Chattonella*.

In summary, our results indicated that the OJIP-test can provide reliable interpretation for stress responses of *Chattonella* spp. to rapid shifts of environmental factors. Among the parameters derived from the OJIP-test, the PI_{ABS} is the most integrated and sensitive one for reflecting the photosynthetic events and physiological state of *Chattonella*. Nevertheless, molecular mechanisms involved in the regulation of OJIP transients in *Chattonella* is still largely unknown. Further works are clearly needed to investigate variations in gene and protein expressions of OJIP transients in *Chattonella* cells at various environmental stress, in order to promote our understanding of their adaptation mechanisms to changed environmental factors.

AUTHOR CONTRIBUTIONS

X. Qiu designed and carried out experiments, and wrote the paper. M. Wu and K. Mukai carried out experiments and data analysis. Y. Shimasaki designed the study. Y. Oshima supervised the work. All authors assisted in editing of the manuscript and approved the final version.

ACKNOWLEDGEMENTS

This study was supported by the Japan Society for the Promotion of Science Postdoctoral Fellowship for Foreign Researchers (Grant No. P12405) and a scientific research foundation for Jinshan Distinguished Professors, Jiangsu University (Grant No. 4111370002).

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