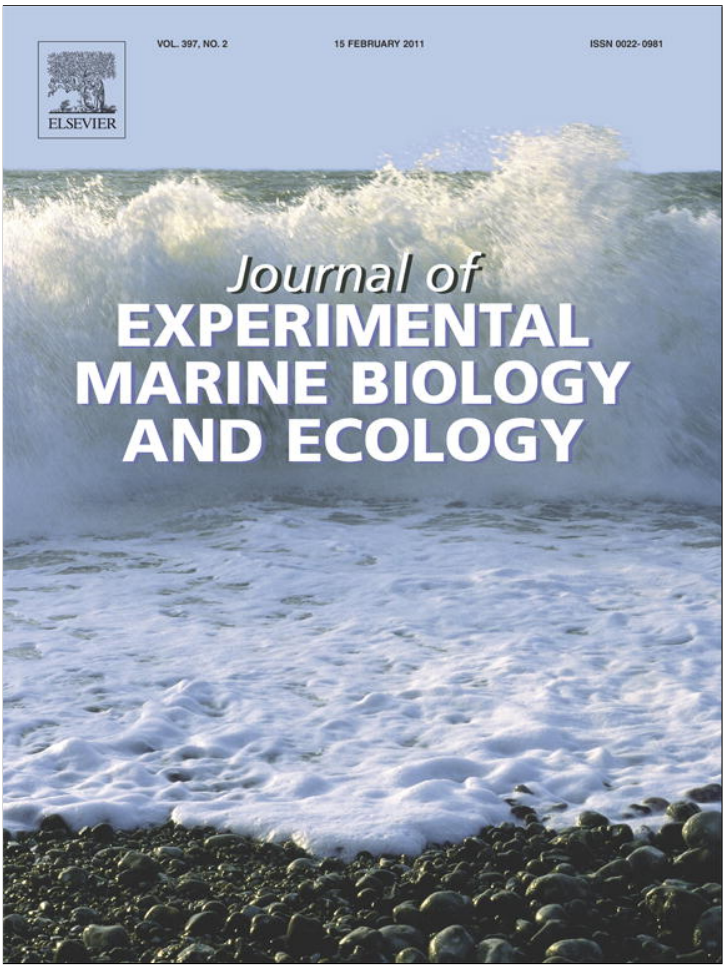

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Impacts of chlorination and heat shocks on growth, pigments and photosynthesis of *Phaeodactylum tricornutum* (Bacillariophyceae)

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ABSTRACT

The main impacts of cooling water from thermal (nuclear) power plants on aquatic organisms were caused by chlorination and temperature increase. In this study, we investigated the impacts of residual chlorine and short-term heat shocks on growth, pigment contents and photosynthesis of *Phaeodactylum tricornutum*. Growth of *P. tricornutum* was completely inhibited; Chlorophyll *a* and carotenoids contents deceased about 63.3% and 61.4% in 24 h treated with 0.2 mg L^{-1} chlorine. The negative effects of chlorination increased with enhanced concentration and prolonged exposure time. Relative electrode transfer rate (rETR) of *P. tricornutum* was significantly suppressed when treated with 0.2 mg L^{-1} residual chlorine for 24 h. Furthermore, the effective quantum yield (F_v'/F_m') decreased first but then recovered with prolonged exposure when residual chlorine ranged between 0.1 and 0.2 mg L^{-1}. The cells were less sensitive to heat shocks compared with chlorination: the rETR and F_v'/F_m' was suppressed only when the temperature exceeded 35 °C for 1 h. When *P. tricornutum* was exposed to chlorination combined with heat shocks, the rETR was further inhibited at 35 °C. It indicated that both chlorination and heat shocks had negative impacts on the primary producers living in discharging coastal waters; furthermore, there were synergistic effects of heat shocks on chlorination toxicity.

1. Introduction

Steam electric plants generally are located on seacoasts due to convenient using of abundant seawater for condenser cooling. In addition to elevated temperature, chlorine is widely used for controlling fouling organisms in cooling systems of coastal power plants (Carpenter et al., 1972). Chlorination of seawater might lead to the formation of chlorination byproducts which potentially inhibit microbial growth (Huang et al., 1999; Choi et al., 2002). Generally, concentrations of residual chlorine ranged between 0.1 and 0.2 mg L^{-1} in thermal discharges (Allonier et al., 1999), with a temperature enhancement of 6 to 12 °C and lasted for about 30 min (Bamber, 1995; Choi et al., 2002; Poornima et al., 2006). Phytoplankton, the major primary producers supporting the coastal marine food chain, are drawn into the cooling circuit of power plants, it is likely that they would be affected by the physical and chemical stress factors.

Temperature is a major environmental factor that plays a critical role in regulating growth, reproduction, and metabolism of the phytoplankton (Coutant and Suffern, 1979; Davison, 1991; Iguchi and Ikeda, 2005), influencing the species composition, species dominance, succession pattern and phytoplankton communities in aquatic ecosystem (Langford, 1990; Manush et al., 2004; Kent et al., 2007). The impacts of thermal discharge in receiving waters may aggravate the local ecosystem because the average temperature of the earth's atmosphere and water has increased in recent decades due to global warming (Stenevik and Sundby, 2007). In tropical waters, any further temperature increase in the ambient waters due to the discharge of heated effluents may seriously affect the growth and productivity of phytoplankton, which in turn, has the potential to influence other trophic levels through energy transfer.

Field investigations have generally shown negative impacts of chlorination on phytoplankton abundance (Hamilton et al., 1970; Brook and Baker, 1972; Eppley et al., 1976). Responses of bacteria and heterotrophic nanoflagellates to chlorination and elevated water temperature of thermal discharges in coastal waters have been reported (Choi et al., 2002; Shiah et al., 2006). However, few studies have been carried out to determine interactive impacts of elevated water temperatures and chlorination on phytoplankton productivity in tropical waters (Saravanane et al., 1998; Poornima et al., 2005). As an important component of the coastal marine food chain, the possible impacts of thermal effluents on phytoplankton need to be understood in detail.

Diatoms are unicellular photosynthetic eukaryotes which contribute close to 20%–25% of global primary productivity due to their ecological success in the world's oceans (Mann, 1999; Montsant et al.,

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2005). Therefore, these microorganisms may play an important role in sustain the healthy ecosystem. *Phaeodactylum tricornutum* is one of the most widely utilized model systems for studying the ecology, physiology, biochemistry and molecular biology of diatoms (Apt et al., 1996). In this study, we used *P. tricornutum*, a model diatom species that has been intensively studied in the previous works, to investigate the impacts of chlorination individually and in combination with heat shocks on growth, pigments content and photosynthesis of phytoplankton.

2. Materials and methods

2.1. Experimental organisms

P. tricornutum, a unicellular diatom, was obtained from state key laboratory of marine environmental science (Xiamen University). The samples were grown in f/2 medium (Guillard and Ryther, 1962) at 20 °C and illuminated with cool-white fluorescent light at a level of 40 μmol $m^{-2} s^{-1}$ (12L: 12D). The cultures were routinely shaken (2–3 times per day) and while at exponential growth the cells (~10^5 cells mL^{-1}) were harvested at the end of dark period and used for all the experimentations.

2.2. Preparation of residual chlorine solution

To completely consume the chlorine required materials in seawater, certain amount of NaClO was added to the filtered seawater to reach 2 mg L^{-1} before the experiments performance according to documents (Huang et al., 1999; Jiang et al., 2008). The chlorine concentration in seawater was measured using a residual chlorine analyzer (HANNA HI93701, Italy). When the chlorine concentration attenuated to 0.01 mg L^{-1}, certain volume of 1 g L^{-1} NaClO solution was added to adjust it to the needed chlorine concentration.

2.3. Experiments to test impacts of chlorination on growth, pigments content and photosynthesis

To test the effects of residual chlorine on physiological activities and biochemical components of *P. tricornutum*, the cells (~0.8×10^5 cells mL^{-1}) were treated with different concentration of residual chlorine for 24 h at 20 °C and 40 μmol $m^{-2} s^{-1}$. The concentration of residual chlorine was set to 0 (control), 0.1, 0.2, 0.3, 0.4, 0.6 and 0.8 mg L^{-1} (triplicates for each level). It was measured again at the end of treatments to get the average chlorine levels throughout. Cell concentration and pigment contents were determined at the end of exposures, however, the relative electrode transfer rate (rETR) and effective quantum yield (F_v'/F_m') were determined at intervals.

2.4. Determination of growth and pigment contents

Growth of *P. tricornutum* was expressed as changing in cell concentration and pigment contents. The cells at the end of exposures were homogenously mixed and 2 mL cultures were used for counting with a Z2™ Counter (Beckman Coulter, Buckinghamshire, UK). To measure the contents of chlorophyll *a* (Chl-*a*) and carotenoids, 20 mL cultures were filtered on Whatman GF/F filter (Ø 25 mm) and extracted with 10 mL absolute methanol overnight in darkness at 4 °C. The absorbance spectrum of supernatant was measured with a spectrophotometer (Shimadzu, UV 2501-PC) after centrifuging for 5 min at 5000 *g*. The Chl-*a* content was estimated according to equation of Porra (2002) and that of carotenoids was determined based on equation of Parsons and Strickland (1963).

2.5. Determination of photosynthetic activities

To examine the impacts of chlorination on the photosynthetic capacity, the F_v'/F_m' and rETR were determined with a dual-PAM-100 (Walz, Effeltrich, Germany), with the actinic light set to 40 μmol photons $m^{-2} s^{-1}$and the saturating pulse of 5000 μmol photons $m^{-2} s^{-1}$ lasting for 0.8 s. Parameters of the rETR vs. I curves were analyzed according to Eilers and Peeters (1988) as follows: $rETR = I/(aI^2 + bI + C)$, where E is the irradiance (μmol $m^{-2} s^{-1}$), a, b and c are the adjustment parameters. The initial slope (i.e. α), the maximum rETR ($rETR_{max}$) and the light saturation parameters (I_k) are expressed as a function of the parameters a, b and c: $I_k = (c/a)^{1/2}$, $\alpha = 1/c$, $rETR_{max} = 1/[b + 2(ac)^{1/2}]$.

2.6. Experiments to test impacts of heat shocks on photosynthesis

To examine the effects of temperature gradients on the physiological activities of *P. tricornutum*, the cells (~0.8×10^5 cells mL^{-1}) were transferred to 50 mL plugged transparent plastic bottles, then the bottles were maintained in a big water tank and the temperature was controlled with a circulating cooler (Eyela, CAP-3000, Tokyorikakikai Co. Ltd., Tokyo, Japan). The temperature gradients were set to 15, 20, 25, 30, 35 and 40 °C, respectively. The rETR of the cells at each temperature was measured as above mentioned methods after 1 h exposure.

2.7. Experiments to test effects of chlorination combined with heat shocks on photosynthesis

The plankton in the discharging waters often suffered from both toxicities of chlorination and heat shocks. The cells (~0.8×10^5 cells mL^{-1}) were transferred to 50 mL plugged transparent plastic bottles; the residual chlorine concentration was adjusted to 0.15 mg mL^{-1} (which has less negative effects on its growth according to former experiment at 20 °C). Then they were cultured for 1 h with above mentioned temperature gradients. The rETR of the cells was measured and the photosynthetic parameters were determined according to above mentioned methods.

2.8. Statistical analysis

One-way ANOVA, non-parametric analysis (Kruskal–Wallis analysis) and Tukey tests were used to establish differences among treatments, with a significant level set at 5% ($p = 0.05$).

3. Results

Cell concentration increased to 2.18 (±0.03), 2.06 (±0.04) and 0.96 (±0.06)×10^5 cells mL^{-1} from 0.8 (±0.01)×10^5 cells mL^{-1} in 24 h incubations with initial residual chlorine concentration of 0, 0.1 and 0.2 mg L^{-1}, respectively. Further increased chlorine concentration led to decreased cell concentration and there were significant ($p<0.05$) differences among the treatments (Fig. 1). The Chl-*a* concentration in the cultures with 0 and 0.1 mg L^{-1} chlorine at the beginning of the incubations increased to 0.78 (±0.02) and 0.74 (±0.02) μg mL^{-1} in 24 h from 0.35 (±0.03) μg mL^{-1} (Fig. 2A). However, it changed little in cultures with 0.2 mg L^{-1} chlorine and completely disappeared at 0.4 mg L^{-1} chlorine. Similar changes were found in carotenoids content, it increased to 0.035 (±0.007), 0.034 (±0.005) μg mL^{-1} from 0.017 (±0.002) μg mL^{-1} when cultured with 0 and 0.1 mg L^{-1} chlorine and decreased with enhanced residual chlorine concentration (Fig. 2B).

Chlorination inhibited the photosynthesis of *P. tricornutum* also, especially when the chorine concentration exceeded 0.2 mg L^{-1}. The $rETR_{max}$, α and I_k of the cells were significantly ($p<0.01$) inhibited when treated with 0.2 and 0.4 mg L^{-1} residual chlorine for 24 h at 20 °C (Table 1). The $rETR_{max}$, α and I_k decreased about 11%, 14% and

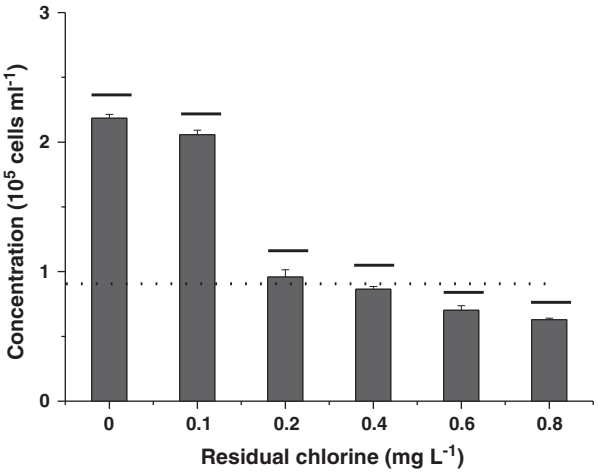

Fig. 1. Effects of chlorination on growth of *P. tricornutum* in 24 h at 20 °C and 40 μmol m^{-2} s^{-1} cool-white fluorescent light. The dotted line denoted the cell concentration at the beginning of inoculations. The means and standard errors were based on triplicate incubations. Horizontal bars at different levels above the columns indicate significant ($p<0.05$) differences among the treatments.

21% in 24 h at 0.2 mg L^{-1} residual chlorine and decreased 74%, 86% and 65% in cultures with 0.4 mg L^{-1} chlorine (Fig. 3). No photosynthetic activities were detected at further increased chlorine concentration.

The Fv'/Fm' of *P. tricornutum* decreased rapidly and reached the minimum yield in 2 h incubation with residual chlorine more than 0.2 mg L^{-1} (Fig. 4A). Chlorine of 0.1 mg L^{-1} had no suppression on the Fv'/Fm' compared with the control (0 mg L^{-1} chlorine). The Fv'/Fm' of the cells could recover to the initial values in 24 h though it decreased rapidly at the beginning of exposure at 0.2 mg L^{-1} chlorine. However, the Fv'/Fm' decreased to the lowest values and could not

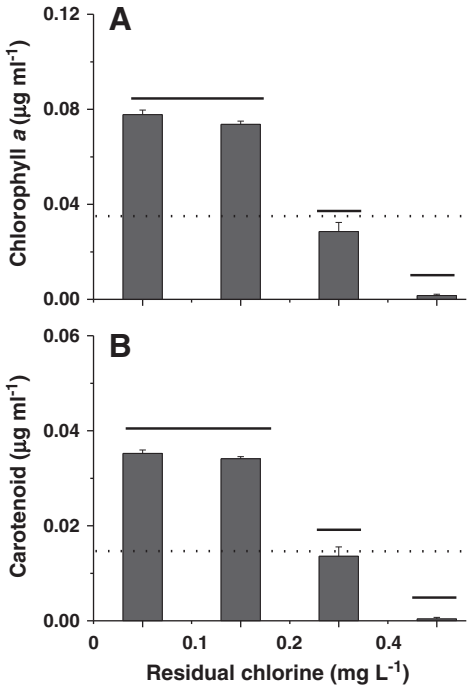

Fig. 2. Effects of chlorination on chlorophyll *a* (A) and cartenoids contents of *P. tricornutum* in 24 h at 20 °C and 40 μmol m^{-2} s^{-1} cool-white fluorescent light. The dotted lines denoted the pigment concentration at the beginning of inoculations. The means and standard errors were based on triplicate incubations. Horizontal bars at different levels above the columns indicate significant ($p<0.05$) differences among the treatments.

Table 1
Photosynthetic parameters derived from the rETR-I curves for *P. tricornutum* cells treated with different concentration of residual chlorine (mg L^{-1}) for 24 h at 20 °C. $rETR_{max}$, the maximum relative electron transfer rate; α, the relative electron transfer efficiency; I_k, light saturation point (μmol m^{-2} s^{-1}). The means and standard errors were based on triplicate incubations.

Residual chlorine	$rETR_{max}$	α	I_k
0	68.82 ± 4.26^a	0.28 ± 0.02^a	1701.60 ± 177.32^a
0.1	65.74 ± 3.65^a	0.25 ± 0.02^a	1455.65 ± 172.47^a
0.2	61.90 ± 2.15^b	0.24 ± 0.01^b	1336.17 ± 80.27^b
0.4	18.02 ± 8.43^c	0.04 ± 0.02^c	603.58 ± 175.56^c
0.6	- -	- -	- -
0.8	- -	- -	- -

"- -" indicates no data was gained during the experiments and the different superscript letters in the same column indicate significant ($p<0.05$) differences among the treatments.

recover at further increased chlorine concentration. The 24 h medium inhibition concentration (IC_{50}) of chlorination on Fv'/Fm' of *P. tricornutum* was 0.26 mg L^{-1}, and it was gained by plotting the Fv'/Fm' at 24 h to chlorine concentration at the beginning of inoculations (Fig. 4B).

There were no significant ($p>0.05$) differences among rETRs of *P. tricornutum* in cultures ranged between 15 and 25 °C, however, the rETRs were significantly ($p<0.01$) suppressed when the temperature increased to 35 °C and further increased temperature led to enhanced suppression (Fig. 5). Temperature enhancement showed similar effects on Fv'/Fm' of the cells: there were no significant ($p>0.05$) differences when the temperature ranged from 15 to 30 °C, but the Fv'/Fm' significantly ($p<0.01$) decreased at 35 and 40 °C (Fig. 6). Furthermore, adding 0.15 mg L^{-1} chlorine to the cultures led to further inhibition at 35 °C but not at 40 °C.

The rETRs of *P. tricornutum* cultured for 1 h at different temperatures with or without chlorine were shown in Fig. 7. Adding 0.15 mg L^{-1} chlorine to the cells did not led to further inhibition except for that at 35 °C. The α and I_k of the cells were further suppressed by 58% and 44% by 0.15 g L^{-1} chlorine at 35 °C (Table 2).

4. Discussion

The growth and photosynthesis of *P. tricornutum* was suppressed and its photosynthetic pigments were bleached when cultured with chlorine more than 0.2 mg L^{-1}. The Fv'/Fm' and rETR were not affected by heat shocks ranged between 15 and 30 °C but were significantly ($p<0.05$) suppressed when the temperatures increased

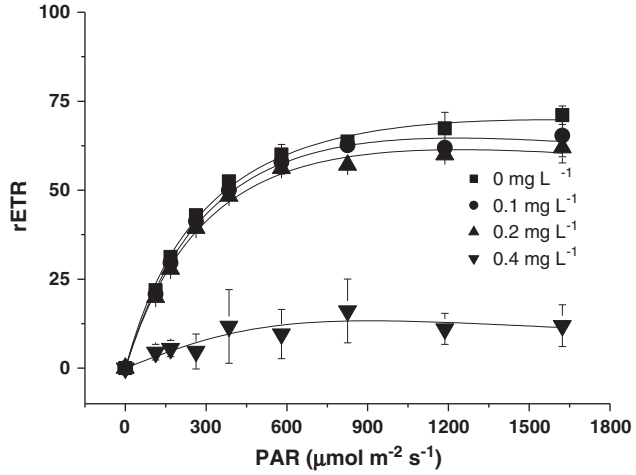

Fig. 3. Effects of chlorination on relative electron transfer rate (rETR) of *P. tricornutum* in 24 h at 20 °C and 40 μmol m^{-2} s^{-1} cool-white fluorescent light. The means and standard errors were based on triplicate incubations.

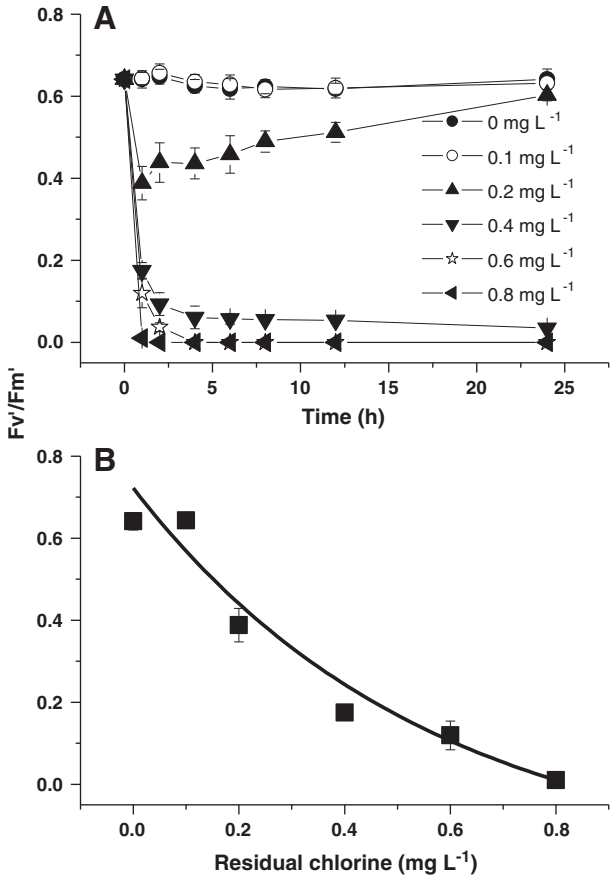

Fig. 4. Changes in effective quantum yield (F_v'/F_m') of *P. tricornutum* treated with chlorination for different intervals (A) and 24 h (B) at 20 °C and 40 μmol m^{-2} s^{-1} cool-white fluorescent light. The means and standard errors were based on triplicate incubations.

to 35 and 40 °C. Adding 0.15 mg L^{-1} chlorine to the cultures led to further inhibition at 35 °C but not at other temperatures.

An earlier laboratory study on the marine phytoplankton species *Chlamydomonas* sp. and *Skeletonema costatum* concluded that high temperatures (35–40 °C) and residual chlorine (1.5–2.3 mg L^{-1}) greatly damaged them (Hirayama and Hirano, 1970). In this study, we found that chlorination (>0.2 mg L^{-1}) and heat shocks (≥35 °C) suppressed the growth and photosynthesis of *P. tricornutum* separately (Figs. 1–5). It was consistent to the studies performed at a power plant in tropical coastal waters (Ahamed et al., 1993; Poornima et al., 2005). Chuang et al. (2009) also found that high water temperature (40 °C) in summer largely suppressed phytoplankton photosynthesis, even for a short period of 20 min as it passed through the cooling condenser. In general, this species showed good tolerance to growth at temperatures between 15 and 25 °C; however, for temperatures above 30 °C, its growth was usually inhibited (Rousch et al., 2004; Goldman and Ryther, 1976). Our results also demonstrated that the impacts of residual chlorine and high water temperatures were dose dependent. It was indicated that chlorination caused a greater reduction in phytoplankton productivity compared to thermal stress at a tropical power station (Choi et al., 2002; Poornima et al., 2006). Our results further approved that phytoplankton photosynthesis was more suppressed by chlorination than by elevated water temperature (Tables 1 and 2; Figs. 1–5). The further damage caused by chlorination than by short-term heat shocks may be due to the destruction of photosynthetic pigments as a result of chlorination (Fig. 2A, B).

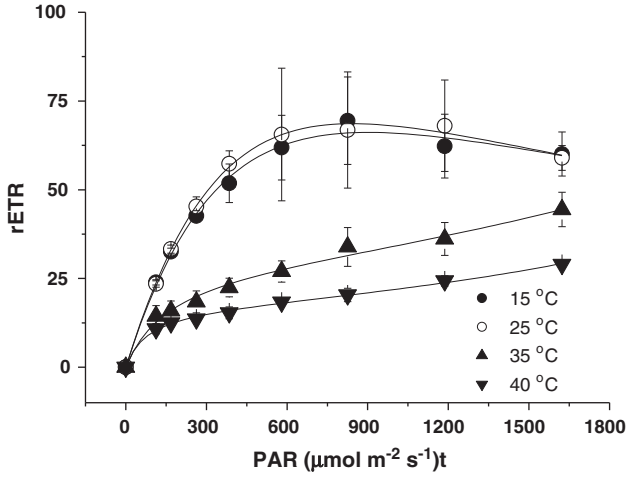

Fig. 5. Effects of 1 h heat shocks on relative electron transfer rate (rETR) of *P. tricornutum*. The means and standard errors were based on triplicate incubations.

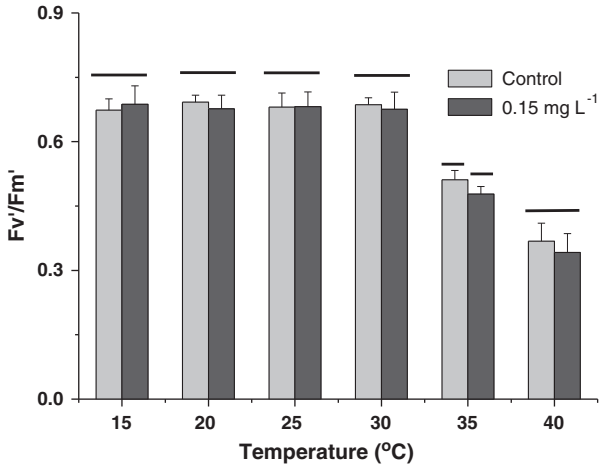

Fig. 6. Effects of 0.15 mg L^{-1} chlorination combined with heat shocks on effective quantum yield (F_v'/F_m') of *P. tricornutum* in 1 h. The means and standard errors were based on triplicate incubations. Horizontal bars at different levels above the columns indicate significant ($p<0.05$) differences among the treatments.

Brook and Baker (1972) proved that phytoplankton photosynthesis decreased 40% at 0.2 mg L^{-1} chlorine in temperate waters. Carpenter et al. (1972) found that the lowest dosage of 0.1 mg L^{-1} caused a 71% decline in phytoplankton productivity in a power plan. Chuang et al. (2009) indicated 0.2 mg L^{-1} chlorine completely suppressed phytoplankton photosynthesis regardless of the heat shocks. In this study, we found that 0.2 mg L^{-1} chlorine in 24 h incubation decreased the rETR, α and I_k by 11%, 14% and 21%, respectively (Table 1). The differences among above researches may be caused by species dependent resistance to chlorination. It can be included that the continuous impacts of 0.2 mg L^{-1} chlorine will be lethal for phytoplankton.

The rETR and Fv'/Fm' of the cells changed little when temperature ranged from 15 to 30 °C but were significantly suppressed over 35 °C (Figs. 5 and 6). Furthermore, exposure to heat shocks combined with low chlorination (0.15 mg L^{-1}) only led to further inhibition at 35 °C (Fig. 7). Though the rETR was further suppressed at 40 °C (Fig. 6), adding low dosage chlorine (0.15 mg L^{-1}) to the heat shock caused little suppression (Table 2; Fig. 7); this may be due to the faster decomposition rate of hypochlorous acid (HOCl) with increased water temperature (Davis and Coughlan, 1983).

Phytoplankton photosynthesis is an enzyme controlled and temperature-dependent process, electron transport to the enzyme Rubisco during light reactions through photosynthetic system II is generally sensitive to temperature (Raven and Geider, 1988). The

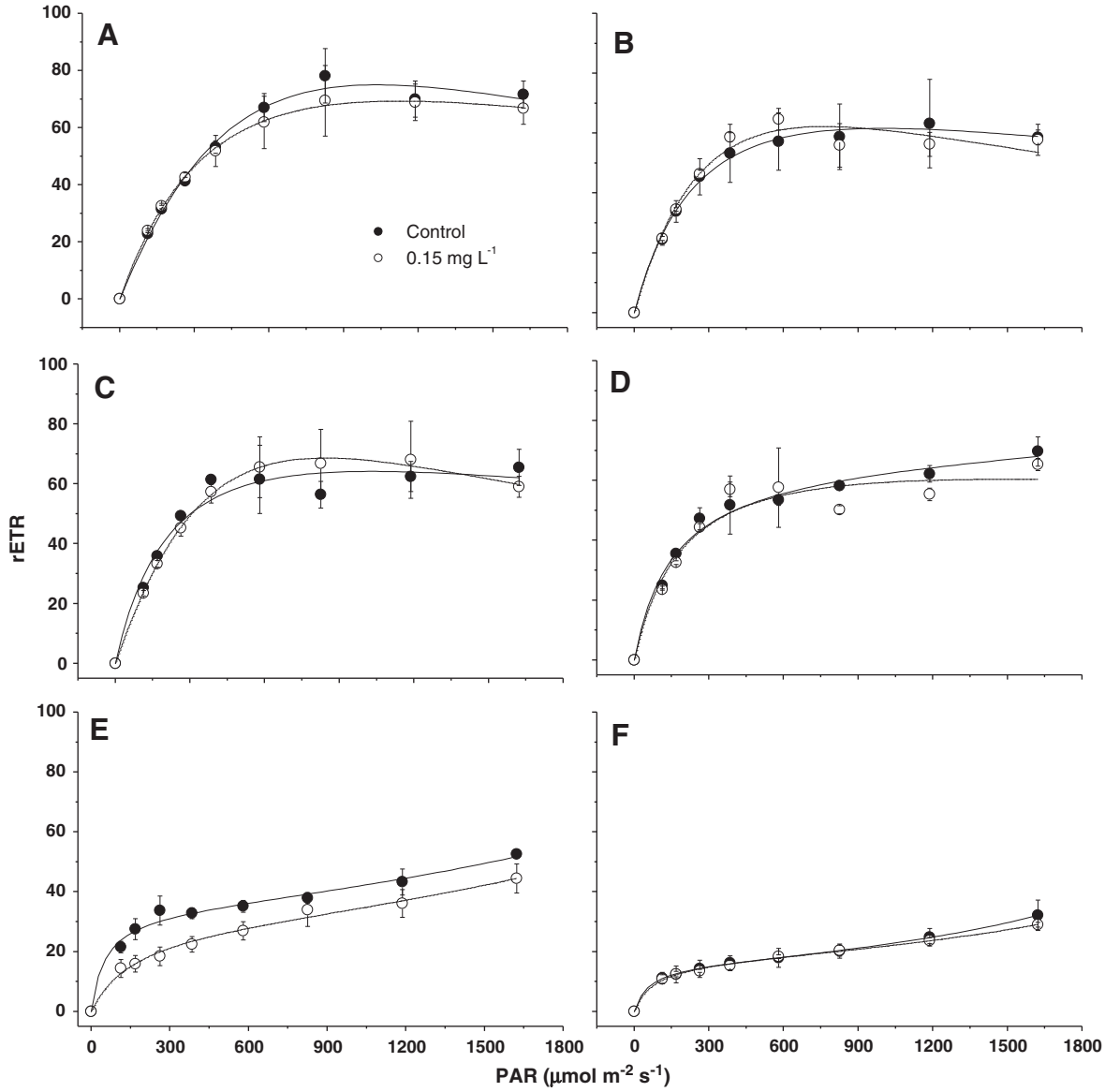

Fig. 7. Effects of 0.15 mg L^{-1} chlorination combined with heat shocks on relative electrode transfer rate (rETR) of *P. tricornutum* in 1 h. The temperature gradients at panels A, B, C, D, E and F were 15, 20, 25, 30, 35 and 40 °C, respectively. The means and standard errors were based on triplicate incubations.

Table 2
Photosynthetic parameters derived from the rETR-I curves for *P. tricornutum* cells treated with residual chlorine (0.15 mg L^{-1}) at different temperature levels for 1 h. $rETR_{max}$, the maximum relative electron transfer rate; α, the relative electron transfer efficiency; I_k, light saturation point (μmol $m^{-2}s^{-1}$). The means and standard errors were based on triplicate incubations.

Temperature (°C)	Chlorine (mg/L)	rETR	α	I_k
15	0	75.70 ± 5.39	0.22 ± 0.05	1098.95 ± 189.70
	0.15	69.03 ± 9.33	0.27 ± 0.08	1426.85 ± 771.79
20	0	60.58 ± 12.08	0.33 ± 0.05	1078.99 ± 248.89
	0.15	62.43 ± 2.71	0.31 ± 0.07	799.84 ± 128.23
25	0	66.07 ± 4.27	0.38 ± 0.07	1387.74 ± 596.57
	0.15	68.38 ± 8.78	0.25 ± 0.06	844.42 ± 28.98
30	0	58.76 ± 10.49	0.43 ± 0.02	2014.65 ± 426.34
	0.15	57.66 ± 7.98	0.39 ± 0.08	1329.04 ± 514.20
35	0	28.45 ± 1.52	0.43 ± 0.04**	903.01 ± 125.64**
	0.15	23.03 ± 4.66	0.18 ± 0.02**	502.23 ± 87.74**
40	0	13.45 ± 4.59	0.27 ± 0.02	491.75 ± 247.04
	0.15	13.71 ± 2.10	0.27 ± 0.16	545.36 ± 229.47

The superscript symbol "**" in the same column indicate significant ($p<0.01$) differences among the treatments.

rETR will be higher at a higher water temperature (Falkowski and Raven, 2007). However, high temperatures over 30–35 °C will lead to a decrease in activity of photosynthetic enzymes (Raven and Geider, 1988; Davison, 1991); this may be the crucial reason for the suppression in rETRs and Fv'/Fm' of *P. tricornutum* over 35 °C (Figs. 5–7). According to the results based on *P. tricornutum*, a dominant diatom in nature, the chlorination would greatly suppress the phytoplankton productivity in the inlet and outlet region. Furthermore, the heat shocks that often over 30 °C, especially in summer in the subtropical region would aggravate the suppression.

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