

Volume 22 | Issue 5

Article 13

## DYNAMIC CHANGES IN PHYTOPLANKTON COMMUNITY STRUCTURE AFTER THERMAL SHOCK AND CHLORINATION IN A SUBTROPICAL BAY: AN EXPERIMENTAL STUDY

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Jiang, Zhi-Bing; Liao, Yi-Bo; Liu, Jing-Jing; Chen, Quan-Zhen; Zeng, Jiang-Ning; and Zhu, Gen-Hai (2014) "DYNAMIC CHANGES IN PHYTOPLANKTON COMMUNITY STRUCTURE AFTER THERMAL SHOCK AND CHLORINATION IN A SUBTROPICAL BAY: AN EXPERIMENTAL STUDY," *Journal of Marine Science and Technology*. Vol. 22: Iss. 5, Article 13. DOI: 10.6119/JMST-013-1011-2

Available at: https://jmstt.ntou.edu.tw/journal/vol22/iss5/13

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## Acknowledgements

We thank Qi-Lang Xie, Xue-Liang Chai, Jin-He Zheng, and Xiao-Yong Li for their support and for providing facilities. We also thank an anonymous reviewer for the useful comments on our manuscript. This work was supported by the National Basic Research Program of China (2010CB428903), National Marine Public Welfare Research Project (201305043-3, 201405007, and 201305009), and National Natural Science Foundation of China (41206103 and 41176142).

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# DYNAMIC CHANGES IN PHYTOPLANKTON COMMUNITY STRUCTURE AFTER THERMAL SHOCK AND CHLORINATION IN A SUBTROPICAL BAY: AN EXPERIMENTAL STUDY

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Key words: temperature increase, residual chlorine, phytoplankton, power plant cooling system.

#### ABSTRACT

Natural phytoplankton collected from the Yueqing Bay was exposed to a series of heat shock temperatures and chlorine dosages in a laboratory for simulating its passage through coastal power plant cooling systems. A short-term thermal shock (30 min) had no appreciable effect on the microalgae community for temperature elevation of 4-12°C in all seasons. However, the adverse effects of chlorination on the microalgae were considerably more severe than those of a thermal shock in terms of dominant succession, species richness, diversity, evenness, and community composition. Moreover, chlorination strongly influenced the diversity indices throughout a 15-d culture period, indicating an evident lagging effect on the injured cells of entrained microalgae. The dominance of small phytoplankton species (r-strategists, e.g., Melosira moniliformis, Nitzschia longissima, and Skeletonema costatum) gradually increased during chlorine dosage range from 1.0 to  $3.2 \text{ mg L}^{-1}$ . A typical chlorine dosage of  $1-2 \text{ mg L}^{-1}$  in cooling systems was found to influence the entrained subtropical phytoplankton community structure.

#### **I. INTRODUCTION**

Coastal power plants (CPPs) generally use seawater as a coolant for their condenser and auxiliary systems during operation, leading to an increase in the temperature of the cool-

<sup>1</sup> State Key Laboratory of Satellite Ocean Environment Dynamics, Second Institute of Oceanography, State Oceanic Administration, Hangzhou, China. ing water [1, 17]. Chlorine is widely used as an antifouling agent in cooling water for killing marine fouling organisms [22, 26, 27, 34-36]. Consequently, the plankton in cooling water, together with other marine organisms smaller than the screen mesh size (1 cm) [2, 19], experiences stresses because of a thermal shock (at the usual temperature increase of 8-12°C) and residual chlorine (at the usual chlorine dosage (CD) of 1-2 mg L<sup>-1</sup>) [1, 2, 5, 14, 31]. Typically, an 1800-2000 MW once-through CPP uses an order of 60 m<sup>3</sup> s<sup>-1</sup> of cooling water [2]. Such a large quantity of cooling water influences the plankton adjacent to the CPPs. Moreover, seawater temperature elevation caused by global warming aggravates the adverse effects of thermal pollution on entrained plankton in CPP cooling systems (CPPCSs) [13, 37].

As an important primary producer, marine phytoplankton appreciably affects the food chain and biogeochemical cycle. Once the entrained algal community structure is altered by the temperature elevation and chlorination, the composition and functioning of coastal ecosystems also change [8, 21]. Considerable attention has been paid to the effects of CPPCSs on phytoplankton. Past studies [4-7, 17, 23-27, 32, 36] have focused on comparisons of phytoplankton parameters between the intake and the outfall of CPPCSs; the parameters include the chlorophyll a (Chla) concentration, photosynthetic capability, primary productivity, community respiration, growth, species composition, abundance, and diversity. However, very few researchers have reported on the recovery of marine phytoplankton after their passage through CPPCSs. Goldman and Quinby [10] and Saravanane et al. [31] conducted laboratory experiments with water samples from CPPs in temperate and tropical zones. Chuang et al. [7] examined the effects of an elevated temperature and residual chlorine in cooling water discharged from CPPs on the biomass and productivity of periphyton and phytoplankton in subtropical Taiwan. We reported simulated experiments on the recovery of the cell density [15] and Chla [16] in entrained subtropical microalgae in CPPCSs. We found that the effects of chlorination on algal

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Para.	Spring	Summer	Autumn	Winter			
Temp	$20.0\pm0.1$	$28.0\pm0.0$	$22.0\pm0.0$	$10.0\pm0.1$			
Salinity	$25.5\pm0.2$	$20.3\pm0.1$	$27.5\pm0.1$	$21.5\pm0.1$			
pН	8.09-8.12	8.00-8.02	8.00-8.01	8.04-8.05			
COD	$1.62\pm0.12$	$1.20\pm0.06$	$0.92\pm0.05$	$1.48\pm0.05$			
PO4 <sup>3-</sup>	$0.97\pm0.05$	$1.36\pm0.09$	$1.16\pm0.05$	$1.03\pm0.06$			
NO <sub>2</sub> <sup>-</sup>	$0.57\pm0.02$	$1.71\pm0.05$	$1.07\pm0.03$	$0.86\pm0.02$			
NO <sub>3</sub>	$54.21 \pm 1.05$	$40.29 \pm 0.88$	$32.93 \pm 0.56$	$44.21\pm0.72$			
$NH_4^+$	$0.74 \pm 0.05$	$2.27\pm0.22$	$1.26\pm0.18$	$1.16\pm0.15$			

Table 1. Parameters (Para.) of experimental seawater quality. Temp: temperature (°C); COD: chemical oxygen demand (mg  $L^{-1}$ ); nutrient unit: umol  $L^{-1}$ .

biomass were considerably more severe than those of a thermal shock, although the cell density and Chla generally recovered during culture at relatively low residual chlorine levels. There are hardly any experimental studies on the response of the natural phytoplankton community structure to thermal and chlorine stresses in subtropical areas. Generally, field investigations tend to highlight the necessity of controlled laboratory tests for gaining insights into the effects of temperature elevation and chlorine addition on entrained phytoplankton [2].

In this study, owing to the operation of CPPs, phytoplankton collected from a subtropical bay (Yueqing Bay) across the four seasons were observed to show stresses because of temperature elevations ( $\Delta T$ ) and the presence of chlorine in the laboratory. Dynamic changes in the dominance, species richness, diversity, evenness, and composition of the phytoplankton community were monitored in a 15-d stable culture after subjecting the phytoplankton community to a thermal shock and chlorination. The subsequent recovery of the phytoplankton community was examined.

#### **II. MATERIALS AND METHODS**

#### 1. Materials

The experiments were carried out in spring (May 2007), summer (August 2006), autumn (September 2006), and winter (January 2007). Natural seawater associated with phytoplankton assemblage was collected from Yueqing Bay (28°19'N, 121°09'E) in the East China Sea, and zooplankton was removed using a 169-um net. An HANNA HI-93734 chlorine detector and the N, N'-diethyl-p-phenylenediamine (DPD) method (with a limitation of  $0.01 \text{ mg L}^{-1}$  and a precision of  $\pm$  $0.03 \text{ mg L}^{-1}$ ) were used to examine the residual chlorine concentration [38]. Experimental containers were dipped into a liquor of 10 mg L<sup>-1</sup> NaClO, washed with distilled water, and then dried [38]. The NaClO liquor (Antiformin) with an effective chlorine content  $\geq$  5% was diluted by adding distilled water to obtain a solution with an effective chlorine concentration of 10 g L<sup>-1</sup>; this solution was stored in a black

ture elevations ( $\Delta T$ , °C) and chlorine dosages (CDs, mg L <sup>-1</sup> ).							
Groups	$\Delta T$	CD	Groups	$\Delta T$	CD		
T1	0	0	T11	8	0		
тγ	0	1.0	T12	8	1.0		

1.8

3.2

5.6

0

1.0

1.8

3.2

5.6

T13

T14

T15

T16

T17

T18

T19

T20

8

8

8

12

12

12

12

12

Table 2.	Expe	erimental	group	desig	n at	different	tempera-
	ture	elevation	is $(\Delta T)$	', °C)	and	chlorine	dosages
	(CDs	s, mg L <sup>-1</sup> ).					

plastic	flask and	l refrigerated	at 4°C for	later use.	The water
quality	(Table 1)	) was measur	ed at the be	ginning of	the tests.

#### 2. Methods

Т3

T4

T5

T6

T7

T8

Т9

T10

0

0

0

4

4

4

4

4

Twenty tanks (44 cm  $\times$  44 cm  $\times$  28 cm, 54.2 L) were used in the experiments. Each tank was filled with 40 L natural seawater. Different  $\Delta T$  values (0, 4, 8, and 12°C) and CDs (0, 1.0, 1.8, 3.2, and 5.6 mg L<sup>-1</sup>) were set in the 20 tanks  $(4 \times 5)$ (Table 2), including the control group ( $\Delta T = 0^{\circ}$ C; CD = 0 mg  $L^{-1}$ ). The initial temperature of the tanks was the natural temperature, and several 300 W heaters were used for generating a thermal shock in the tanks. Chlorination was conducted immediately after the temperature reached the set point. The water temperature was controlled constantly for 30 min by using a WMZK-01 automatic temperature sensor (accuracy:  $\pm 0.2^{\circ}$ C). Each tank was aerated for maintaining a uniform water temperature during heating. The thermal shock was stopped after 30 min, and the tanks were cooled spontaneously. The lighting conditions were regulated by using fluorescent lamps at 500  $\pm$  50 lux in a 12 h:12 h light/dark cycle during the 15-d culture period. In view of the short life span, ranging from several hours to several days, the culture duration of marine phytoplankton was sufficiently long to reflect changes in the marine phytoplankton experiencing pollution stress [12]. The residual chlorine (total chlorine) concentration in each tank was measured after terminating the thermal shock.

#### 3. Sample Collection

The seawater (500 mL) in the control group was sampled at the beginning of the experiment. Subsequently, 500 mL of water was taken from each group after every 48 h. All the samples were preserved in 1% Lugol's iodine. After sedimentation (for at least 48 h), phytoplankton taxa were identified and counted on a scaled slide (0.1 mL) at 200× or 400× magnification with a light microscope (Olympus B42). The process was repeated thrice.

1.8

3.2

5.6

0

1.0

1.8

3.2

5.6



Fig. 1. Residual chlorine decay in each test team in different seasons.

#### 4. Data Analysis

The community diversity index (H') and evenness index (J') were calculated using the formulae of Shannon-Wiener and Pielou, respectively.

$$H' = -\sum_{i=1}^{s} P_i \ln P_i \tag{1}$$

$$J' = H'/\ln S \tag{2}$$

Here, S is the species number of the experimental group, and  $P_i$  is the ratio between the cell density of species *i* and the total cell density of the experimental group. SPSS 20.0 was used to analyze the data. Changes in the community parameters (S, H', and J') under different levels of thermal and chlorine stresses were observed over time, and the data were analyzed using repeated-measures analysis of variance (rm-ANOVA). The sphericity was assessed using Mauchly's test, and the Huynh-Feldt adjustment was used when the assumption of sphericity was rejected. Prior to ANOVA, all variables were tested for normality and homogeneity, and the data of S were log-transformed to satisfy the assumptions for ANOVA. Cluster analysis of the phytoplankton community was performed using PRIMER software (v. 6.0). The species abundance was log(x+1)-transformed before estimation using Bray-Curtis similarities between sample pairs. If the frequency was lower than 10%, the corresponding species were excluded from the cluster analysis. Two-way crossed analysis of similarity (ANOSIM) with no replication was used to examine the changes in the phytoplankton community.

#### **III. RESULTS**

#### 1. Decay of Residual Chlorine

The residual chlorine decay was difficult to compare among different seasons because of differing seawater quality (Table 1). However, the residual chlorine decayed rapidly with increasing  $\Delta T$  in all seasons (Fig. 1).



Fig. 2. Changes in *P* of the dominant phytoplankton species in spring ((a)-(d)) and summer ((e) and (f)). *P* represents the dominance of certain species in a sample. (a)-(d): Different symbols represent different  $\Delta T$ , and the dominant species in the groups with CDs of 1.0 and 3.2 mg L<sup>-1</sup> are similar to those in the groups with a CD of 1.8 mg L<sup>-1</sup>. (e) and (f): Different symbols represent different species; changes in *P* in the dominant species in Groups T5, T15, and T20 are similar to those in the Group T10.

#### 2. Changes in Dominant Phytoplankton Species

In spring, *Melosira moniliformis* became the dominant species following thermal shock and chlorination (1.0-3.2 mg L<sup>-1</sup>), whereas *Pleurosigma angulatum* dominated at a CD of 5.6 mg L<sup>-1</sup> (Figs. 2(a)-2(d)). In groups without chlorination, *P* of *M. moniliformis* varied only slightly relative to that of the control group at a  $\Delta T$  of 4-12°C (Fig. 2(a)).

In summer, the dominant succession occurred at a CD of 5.6 mg  $L^{-1}$  (Fig. 2(e)), and P of Skeletonema costatum decreased gradually. This species disappeared after the Day 13, 7, 7, and 5 at  $\Delta T$  of 0, 4, 8, and 12°C, respectively. Thus, the heat shock enhanced the toxicity of residual chlorine for S. costatum. P of Trichodesmium spp. increased gradually, reaching a maximum between Day 3 and Day 7, and then declined; finally vanished between Day 11 and Day 13. Coscinodiscus bipartitus and Cyclotella stylorum became the dominant species. The groups chlorinated with concentrations in the range 1.0-3.2 mg  $L^{-1}$  were similar to the control group (Fig. 2(e)). The dominant species did not change because S. costatum always dominated the community at a dominance of above 85% for 15 d. P of C. bipartitus and C. stylorum varied only slightly, whereas Trichodesmium spp. disappeared gradually (Fig. 2(f)).

Table 3. Results of repeated-measures ANOVA of phytoplankton community variables (*H'*, *J'*, and *S*) throughout 15 d determined by  $\Delta T$  and CD in four seasons. TBSE: tests of between-subjects effects; TWSE: tests of within-subjects effects; superscripts represent significant level; <sup>ns</sup> not significant, \* *p* < 0.05, \*\* *p* < 0.01, \*\*\* *p* < 0.001, hereafter the same.

	Variables		Spring	Summer	Autumn	Winter
	TBSE	Time	$F_{6.8,82.0} = 3.0*$	$F_{5.6,67.8} = 7.0^{***}$	$F_{8,96} = 24.4^{***}$	$F_{8,96} = 12.6^{***}$
H'	TWSE	$\Delta T$	$F_{3,11} = 1.2^{\rm ns}$	$F_{3,11} = 0.8^{\rm ns}$	$F_{3,11} = 2.8^{\rm ns}$	$F_{3,11} = 1.2^{\rm ns}$
		CD	$F_{4,11} = 6.9^{**}$	$F_{4,11} = 157.1^{***}$	$F_{4,11} = 20.0^{***}$	$F_{4,11} = 41.5^{***}$
	TBSE	Time	$F_{6.6,79.0} = 2.3*$	$F_{4.5,54.1} = 15.1 * * *$	$F_{8,96} = 14.6^{***}$	$F_{8,96} = 13.9 * * *$
J'	TWSE	$\Delta T$	$F_{3,11} = 0.6^{\text{ns}}$	$F_{3,11} = 0.4^{\rm ns}$	$F_{3,11} = 2.6^{\rm ns}$	$F_{3,11} = 1.3^{\rm ns}$
		CD	$F_{4,11} = 2.9^{\rm ns}$	$F_{4,11} = 241.53^{***}$	$F_{4,11} = 41.9^{***}$	$F_{4,11} = 59.3^{***}$
	TBSE	Time	$F_{6.6,79.0} = 90.3^{***}$	$F_{7.2,86.8} = 256.1 * * *$	$F_{6.1,72.9} = 119.4^{***}$	$F_{4.9,58.7} = 12.6^{***}$
S	TWSE	$\Delta T$	$F_{3,11} = 0.7^{\rm ns}$	$F_{3,11} = 3.9^*$	$F_{3,11} = 0.7^{\rm ns}$	$F_{3,11} = 4.2^*$
		CD	$F_{4,11} = 111.4^{***}$	$F_{4,11} = 156.0$ ***	$F_{4,11} = 245.4^{***}$	$F_{4,11} = 84.0^{***}$



Fig. 3. Succession of dominant phytoplankton species in autumn. (a) and (b): Different symbols represent different species in T1 and T10. (c) and (d): Different symbols represent changes in *N. long-issima* and *S. costatum* at CDs of 1.0-3.2 mg L<sup>-1</sup> ( $\Delta T = 0^{\circ}$ C).

In autumn, the changes in *Nitzschia longissima* and *S. co-statum* after thermal shock without chlorination were similar to those in the control group. *P* of *S. costatum* increased gradually with the reduced dominance of *N. longissima* (Fig. 3(a)). The changs in *N. longissima* and *S. costatum* in the groups for  $\Delta T = 4$ -12°C were similar to those in the groups with  $\Delta T = 0$ °C at a CD of 1.0-3.2 mg L<sup>-1</sup> (Fig. 3). *P* of *N. longissima* (Fig. 3(c)) gradually increased at a CD of 1.8-3.2 mg L<sup>-1</sup>, whereas that of *S. costatum* decreased (Fig. 3(d)). Dominant succession also occurred at a CD of 5.6 mg L<sup>-1</sup> (Fig. 3(b)), as the dominance of *N. longissima* (which vanished between Day 13 and Day 15) and *S. costatum* (which vanished between Day 7 and Day 9) gradually decreased with increasing in the dominance of *C. bipartitus* and *C. stylorum*.

In winter, the dominance of *S. costatum* decreased at a CD of  $5.6 \text{ mg L}^{-1}$ , although the species contributed more than 40%



Fig. 4. Changes in *S. costatum* at a CD of 5.6 mg L<sup>-1</sup> in winter.

to the total density (Fig. 4). *P* changes in this species varied with  $\Delta T$ , suggesting the synergistic effect of this population. The rest of the groups were similar to the control group, that is, *S. costatum* changed only slightly with a dominance of above 70%.

#### 3. Changes in S, H', and J'

The adverse effects of chlorination on the phytoplankton community were considerably more severe than those of thermal shocks in terms of S, H', and J' (Table 3). Thermal shocks appreciably affected S in winter and summer but not in spring and autumn. However, chlorination significantly influenced S in all seasons (p < 0.001) because S decreased with an increase in the CD (Fig. 5). Chlorination at 1.0 and 1.8 mg  $L^{-1}$  led to only a slight decrease in S; however, the decrease was sharp at high CDs over time, especially in groups chlorinated at 5.6 mg L<sup>-1</sup>. Thermal shocks only slightly influenced H' and J' in all four seasons, whereas chlorination significantly (p < 0.01) affected them in all seasons, except for J' in spring (Table 3). The changes in H' (Fig. 6) and J' (figure not shown but similar to that of H') varied with the CD and season. According to rm-ANOVA (Table 3), the recovery time was significantly (p < 0.05) correlated to S, H', and J'.



Fig. 7. Results of cluster analysis for different experimental groups on 15th d in different seasons.



Fig. 5. Changes in the species richness (*S*) at  $\Delta T = 12^{\circ}$ C (other  $\Delta T$  groups showed similar trends) in different seasons.



Fig. 6. Changes in the Shannon-Wiener diversity index (H') at  $\Delta T = 0^{\circ}$ C (other  $\Delta T$  groups showed similar trends). The symbols used in this figure are the same as those in Fig. 5, and the changes in the Pielou's evenness index (J') are similar to those in H'.

Table 4.	Results $(R)$ of analysis of similarity of the phyto-
	plankton community composition on Day 15 under
	stresses of different $\Delta T$ and CDs.

Variables	Spring	Summer	Autumn	Winter
$\Delta T$	$-0.07^{ns}$	-0.06 <sup>ns</sup>	0.01 <sup>ns</sup>	0.21 <sup>ns</sup>
CD	0.77**	0.91***	0.93***	0.77***

#### 4. Cluster Analysis

Cluster analysis showed that the phytoplankton community could be classified into five groups according to the CD for all seasons except spring (the similarities were 91%, 86%, and 82% in summer, autumn, and winter, respectively) (Fig. 7). The groups with CD in the range 3.2-5.6 mg L<sup>-1</sup> were considerably more different from the other groups. The ANOSIM results revealed that chlorination effects on the phytoplankton community composition were considerably more severe compared with thermal shock effects (Table 4).

#### **IV. DISCUSSION**

#### 1. Effects of Thermal Shock

Experimental results showed that thermal shocks had only a slight effect on the phytoplankton dominant succession, *S* (except in summer and winter), *H'*, *J'*, and the community composition during the 15-d laboratory culture. Apparently, the tolerance and recovery potential of subtropical phytoplankton to short-term thermal shocks are generally strong. Relatively short (30 min) exposures and the phytoplankton composition may be the reasons for these observations [29, 30]. However, if a heat shock is continuously applied, the damage caused by the thermal shock to phytoplankton will be severe [30]. Thermal shocks in summer (at a natural water temperature of 28°C) significantly (p < 0.05) reduced phytoplankton *S* compared with those in other seasons (Fig. 7) because the extremely high instantaneous temperature in summer exceeded the critical thermal maxima of several microalgal species. Ma *et al.* [23] observed that in a diatom species (*Phaeodactylum tricornutum*), both effective quantum yield  $(F_v'/F_m')$  and relative electrode transfer rate were suppressed when heat shock temperatures above 35°C were maintained for 1 h.

Phytoplankton species specificity to thermal tolerance has been previously observed in both field and laboratory studies. Briand [3] reported that the heat shock damage to diatoms caused by CPPCSs exceeded that to dinoflagellates without chlorination in Southern California. Liao *et al.* [18] performed an experiment involving a 7-d laboratory culture and found that algal species drastically decreased at temperature above  $36^{\circ}$ C in summer and above  $34^{\circ}$ C in winter for natural temperatures of 28 and 12°C, respectively. In the experiment, *S. costatum* had the maximum thermal tolerance and was dominant in the groups with a high  $\Delta T$ . *S. costatum* was also dominant in all seasons except spring. Thus, thermal shocks only slightly affected the community structure.

In view of the relatively short exposure time (usually 10-30 min) for phytoplankton in CPPCSs [2, 5, 31], the damage caused by heat shocks was minimal. Mallin *et al.* [24] and Martínez-Arroyo *et al.* [25] compared the difference in phytoplankton species composition, abundance, and diversity between the intake and outfall of CPPS, and they found that  $\Delta T$  only slightly influenced the phytoplankton community. Their results are consistent with those of the present study (Figs. 5-7).

#### 2. Effects of Chlorination

Compared with thermal shocks, chlorination caused substantial damage to subtropical phytoplankton, and the CD significantly (p < 0.01) influenced the community composition and structure (Tables 3 and 4); this result is similar to those of previous reports on temperate and tropical zones [5, 7, 11, 28]. The result is also consistent with our previous reports on the recovery of cell number [15] and Chla [16]. The recovery time was found to be an important factor that affected the succession of dominant species (Figs. 2-4) and the diversity indices (H', J', and S) and altered the phytoplankton community (Table 3) after chlorination.

*S* decreased considerably at CDs in the range  $3.2-5.6 \text{ mg L}^{-1}$  in all seasons (Fig. 5), and the family Coscinodiscaceae dominated the phytoplankton community. In the present study, the genera *Biddulphia*, *Cerataulina* and *Ditylum* of the family Biddulphiaceae died out in 15 days in all seasons, whereas both *Coscinodiscus* and *Cyclotella* of the family Coscinodiscaceae survived and dominated in the community in summer and autumn (Figs. 2 and 3). This result is consistent with that of Patil and Jagadeesan [27]. They found that *Amphiphrora*, *Navicula*, *Cylindrotheca*, and *Coscinodiscus* showed an increase in population density in natural biofilms after chlorine treatment, while *Pleurosigma*, *Amphora*, and *Thalassionema* 

did not. Thus, tolerance levels and recovery patterns are species specific, and this finding is consistent with previous reports [22, 27, 36]. Consequently, dominant succession occurred in all seasons (Figs. 2-4) except winter (the dominance of *S. costatum* gradually reduced; Fig. 4); thus, H' (Fig. 6) and J' varied with changes in the dominant species and community composition (Fig. 7) because of differences in resistance to chlorine stresses among different species.

However, chlorination has an irreversible effect on several algal species, which cannot recover (and even die out) in a relatively short period (Figs. 2-4). Table 3 shows that the recovery time significantly (p < 0.05) influences the community structure in terms of S, H', and J', thus indicating an evident lagging effect on entrained phytoplankton subjected to chlorination. Chlorine is used as a biocide to control the development of diatom biofilms (it causes mortality in diatom cells); it damages pigments (Chla and carotenoid), obstructs photosynthesis (low  $F_v/F_m$  and high  $\sigma_{PSII}$ ), and damages the cell body [23, 27, 36]. Ebenezer et al. [9] confirmed that low-dose (1-3 mg L<sup>-1</sup>) chlorination causes greater cellular damage to marine microalgae (Chlorella salina) and that the injured cells can recover in terms of chlorophyll autofluorescence, esterase activity, or productivity even after 18 h incubation in healthy media. Apparently, microalgae in CPPCSs are sensitive to chlorine and would have a considerably damaged cellular structure and function, resulting in abnormal changes in the phytoplankton community structure.

After low-CD treatment, r-strategists dominated the phytoplankton community. For example, in the present study, species with a small size [26], such as *M. moniliformis* (Fig. 2), *S.* costatum (Figs. 2-4) and N. longissima (Fig. 3), gradually became the dominant species or increased their dominance for the CD in the range  $1.0-3.2 \text{ mg L}^{-1}$ . This finding indicates that subtropical phytoplankton adjacent to CPPs might tend to be small under chlorine stress. This presumption is also supported by past laboratory and field studies. Saravanane et al. [31] conducted laboratory experiments with three water samples, collected from the intake, processing chamber, and condenser of a CPP; Thalassiosira hyaline with relatively small cells dominated the samples collected from the processing chamber and condenser, but not the sample obtained from the intake. Liu et al. [20] found that both S and abundance of net-collected phytoplankton showed an apparent decrease near the Daya Bay Nuclear Power Plant, and the composition of phytoplankton community tended to be dominated by small-sized species.

#### 3. Effects of Thermal Shock on Toxicity of Residual Chlorine

Owing to the inadequate consideration given to the experimental design, the present study could not quantitatively examine the relationship between temperature increase and chlorination through a statistical analysis, despite evidence indicating a synergistic effect in the test. Ma *et al.* [23] found a synergistic effect of chlorine and temperature increase on phytoplankton photosynthesis, but Choi *et al.* [5] and Poornima *et al.* [28] did not observe any such effect. Such difference in results is possible. Chlorine toxicity is enhanced by temperature elevation [23]. However, chlorine also decays rapidly because of temperature elevations (Fig. 1), leading to a reduction in residual chlorine [38] as well as phytoplankton exposure to chlorine. Therefore, it could not be confirmed whether thermal shocks enhance the effects of chlorine on the phytoplankton community. Further research on the synergistic effect is necessary.

#### V. CONCLUSION

The tolerance of subtropical phytoplankton to a short-term (30 min) heat shock was high, and thermal shock only slightly affected dominant succession, S, H', J', and the community composition of the phytoplankton. Given the relatively short exposure time (usually 10-30 min) of phytoplankton to CPPCSs, the effects of thermal shocks for  $\Delta T$  values in the range 8-12°C were minimal on the community structure in the subtropical region. However, the damage caused by chlorination to the subtropical phytoplankton community was considerably more severe than that inflicted by thermal shocks. Chlorination appreciably affected succession in dominance, S, H', J', and the community composition. S rapidly decreased at high CDs (3.2-5.6 mg  $L^{-1}$ ) regardless of the season. The dominance of phytoplankton with a small cell size (r-strategist) gradually increased under chlorine stress. The present data show that the typical CD (1-2 mg L<sup>-1</sup>) of CPPCSs influences the subtropical phytoplankton community structure. Furthermore, a strong lagging effect in injured cells aggravates the adverse effects.

#### ACKNOWLEDGMENTS

We thank Qi-Lang Xie, Xue-Liang Chai, Jin-He Zheng, and Xiao-Yong Li for their support and for providing facilities. We also thank an anonymous reviewer for the useful comments on our manuscript. This work was supported by the National Basic Research Program of China (2010CB428903), National Marine Public Welfare Research Project (201305043-3, 201405007, and 201305009), and National Natural Science Foundation of China (41206103 and 41176142).

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