



Effects of a thermal discharge from a nuclear power plant on phytoplankton and periphyton in subtropical coastal waters

Yi-Li Chuang, Hsiao-Hui Yang, Hsing-Juh Lin*

Department of Life Sciences, National Chung Hsing University, Taichung 402, Taiwan, ROC

ARTICLE INFO

Article history:

Received 16 September 2008

Received in revised form 9 December 2008

Accepted 6 January 2009

Available online 16 January 2009

Keywords:

Community composition

Elevated water temperature

Photosynthetic intensity

Productivity

Residual chlorine

ABSTRACT

Effects of elevated water temperatures and residual chlorine from a thermal discharge at a coastal nuclear power plant on the biomass and productivity of periphyton and phytoplankton were determined in subtropical Taiwan. Phytoplankton chlorophyll *a*, but not productivity, was significantly lower in the outlet region than in the intake region. Periphyton chlorophyll *a* was significantly greater in the outlet region than in the intake region. Nevertheless, periphyton productivity was negatively correlated with water temperature in the outlet region. A distinct difference in periphyton community composition was also detected between the two regions. Chlorination experiments showed that a chlorine concentration of 0.2 ppm greatly suppressed phytoplankton productivity, regardless of whether the water temperature was elevated or not. However, periphyton productivity was little influenced by a chlorine concentration of <0.5 ppm. Our results suggest that phytoplankton productivity was greatly affected by residual chlorine, but periphyton productivity was more affected by elevated water temperatures.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

There is great concern about the ecological impacts of coastal power plants on coastal waters (Kennish, 1992). Elevated water temperatures and residual chlorine (anti-fouling agent) are major threats from thermal discharges of coastal power plants (Langford, 1990; Krishnakumar et al., 1991). Previous impact studies of thermal discharges from coastal power plants focused on phytoplankton abundance. However, those results were inconsistent (Morgan and Stross, 1969; Hirayama and Hirano, 1970; Brook and Baker, 1972; Briand, 1975; Lo et al., 2004). Effects of the thermal discharges on the abundance, productivity, and community composition of phytoplankton and periphyton in tropical waters are scanty (Clark, 1989; Suresh et al., 1993; Dawes, 1998; Lo et al., 2004; Poornima et al., 2005, 2006).

Studies of the impacts of thermal discharges on periphyton, which is often a primary producer in coastal ecosystems, are very limited (Dell'Anno et al., 2002). Water temperature is an important environmental factor influencing the survival rate, growth ability, and reproduction of aquatic organisms (Langford, 1990; Davison, 1991). It is generally a major factor driving seasonal succession of aquatic communities. Thermal stress from thermal discharges on freshwater benthic algal communities has been reported (Stockner, 1967; Krenkel and Parker, 1971; Langford, 1990). Hawkes (1969) summarized a wide range of optimal growth temperatures for important algal species.

Devinney (1980) found that the abundance of benthic macroalgae decreased with increasing water temperature in the temperate zone.

Chlorine is a widely used biocide for controlling fouling organisms in cooling systems of coastal power plants. Chlorination of seawater in coastal power plants might lead to the formation of chlorination by-products which potentially inhibit microbial growth (Langford 1990; Choi et al., 2002). In general, concentrations of residual chlorine range from 0.1 to 0.2 ppm in thermal discharges (Allonier et al., 1999). Laboratory experiments applying different concentrations of chlorine showed little effect on marine phytoplankton abundance (Hirayama and Hirano, 1970). However, field investigations have generally shown negative impacts of chlorine on phytoplankton abundance (James, 1967; Hamilton et al., 1970; Holmes, 1970; Brook and Baker, 1972; Carpenter et al., 1972; Fox and Moyer, 1975; 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 effects of elevated water temperatures and chlorination on phytoplankton and periphyton productivity in tropical waters (Saravanane et al., 1998; Poornima et al., 2005).

The objectives of this study were (1) to compare the biomass and productivity of phytoplankton and periphyton between the outlet and intake regions of the Second Nuclear Power Plant (NPP II) in subtropical Taiwan; (2) to determine the effects of a gradient of chlorine concentrations on phytoplankton and periphyton productivities, and (3) to determine interactive effects of high water temperatures and residual chlorine on phytoplankton and periphyton productivities.

* Corresponding author. Tel.: +886 4 22840416; fax: +886 4 22874740.

E-mail address: hjlin@dragon.nchu.edu.tw (H.-J. Lin).

2. Methods

2.1. Study sites

Biomass sampling and productivity incubations were carried out every 3 months in February (winter), April (spring), July (summer), and October (Autumn) of 2001–2003 in the vicinity of the NPP II which is located in Kuosheng Bay, northern Taiwan (Fig. 1). Kuosheng Bay has a 8-km² surface area with a mean depth of 15 m. The power station uses 44 m³ s⁻¹ of seawater for cooling. The thermal discharge of NPP II is modified by the addition of antifouling biocides (about 0.2 ppm of residual chlorine measured in the outlet region) to maintain the efficiency of flow and heat transfer across the condensers. The surface seawater in the intake region ranges from 18 °C in winter to 30 °C in summer. An increasing temperature gradient of 8–12 °C exists in the water column of the outlet region. There was a distance of about 50 m among 3 replicate sampling sites in each region.

2.2. Environmental factors

Environmental factors in the water column were measured in the intake and outlet regions concurrently with primary productivity incubations. Surface water temperature, pH, dissolved oxygen (DO), and salinity were continuously monitoring at 1-min intervals *in situ* for 20 min using YSI 600XLM multi-parameter monitoring sensors. The light extinction coefficient (*k*) in the water column for photosynthetically active radiation (PAR) was determined by light measurements for 24 h using a LI-189 Quantum meter.

2.3. Biomass determination

Phytoplankton biomass in terms of chlorophyll *a* concentration was determined with a fluorometer (Trilogy, Tuner, *n* = 3) by immediately filtering 0.5-L water samples through 47-mm GF/F filters (Whatman) in the field and then extracting the filters in 90% acetone for 24 h at 4 °C in the dark (Stermann, 1988). Periphyton was gently scraped from randomly selected stones after the productivity incubations. Periphyton biomass was measured by extracting chlorophyll *a* in 90% acetone, then determining the concentration in a spectrophotometer (Parsons et al., 1984).

ophyll *a* in 90% acetone, then determining the concentration in a spectrophotometer (Parsons et al., 1984).

2.4. Productivity incubation

Periphyton productivity was determined in clear plastic chambers (*n* = 3 for each light intensity per site) using an oxygen evolution technique modified from Lin et al. (2005). The plastic chambers were exposed in the field to five different irradiances of 0%, 30%, 50%, 70%, and 100% shading by interposing screens with different mesh sizes. Net production (NP) and respiration rates were derived from changes in DO concentrations over time measured by a DO meter (YSI, Model 52). Phytoplankton productivity was also determined in various light intensities using a DO method modified from Parsons et al. (1984) by incubation in 300-ml BOD bottles (*n* = 3 for each light intensity per site).

The daily NP rates of phytoplankton and periphyton were respectively calculated by integrating the interpolated NP rates under various irradiances in reference to the relationships between irradiances and NP rates (P–E curve). Gross production (GP) was calculated as the sum of respiration and NP.

Each P–E curve on each occasion in each region was described by Eq. (1) of Jassby and Platt (1976) using a nonlinear curve-fitting procedure of the program SigmaPlot 8.0 as

$$P^B = P_{max}^B \tanh(\alpha^B E / P_{max}^B); \quad (1)$$

where P^B refers to the photosynthetic rate normalized to chlorophyll *a*, P_{max}^B is the maximum photosynthesis normalized to chlorophyll *a* in the absence of photoinhibition under optimal light, α^B is the initial slope of the line at low light, and *E* is the light intensity, as the PAR presented as $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The parameter, α^B , characterizes the slope of the light-saturation curve at low irradiance levels when photosynthesis is assumed to be proportional to the photon density. α^B also indicates the efficiency with which incident light energy is converted into photosynthetic production by the algal community. The maximum photosynthetic rate is denoted as P_{max}^B at optimal illumination, which can also be interpreted as the photosynthetic capacity. The light-saturation onset parameter (E_k) refers to the light

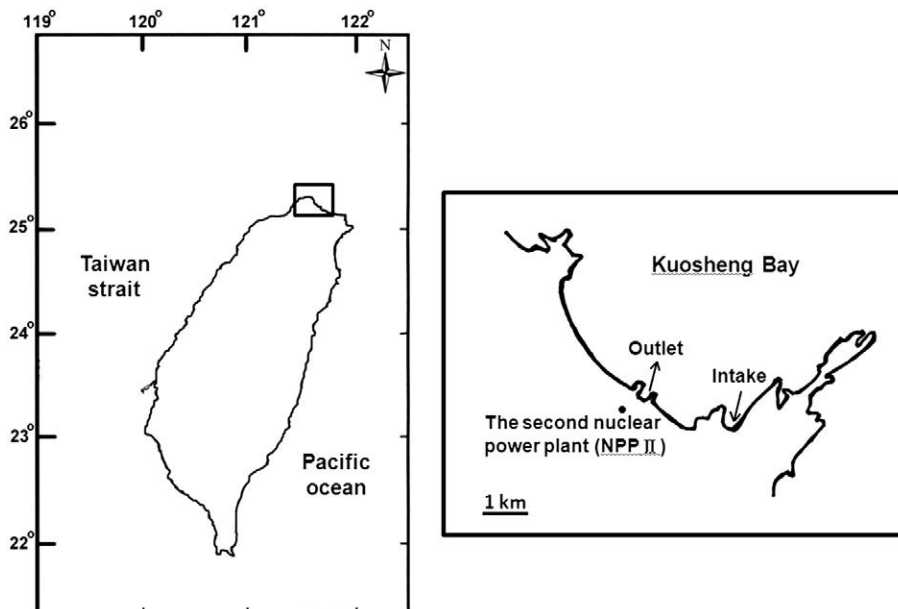


Fig. 1. Study sites of Kuosheng Bay showing the intake and outlet regions of the Second Nuclear Power Plant in northern Taiwan.

Table 1Environmental variables (mean \pm SE, $n = 12$) measured every three months in the intake and outlet regions of the Second Nuclear Power Plant in Kuosheng Bay during 2001–2003.

Site	Intake				Outlet			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Water temperature ($^{\circ}\text{C}$)	18.7 \pm 0.5	24.4 \pm 2.0	28.0 \pm 2.1	24.2 \pm 1.3	28.2 \pm 0.6	32.3 \pm 1.7	38.8 \pm 1.0	33.8 \pm 2.1
Salinity (psu)	33.0 \pm 1.0	35.0 \pm 0.0	32.6 \pm 1.4	33.2 \pm 1.6	35.0 \pm 0.0	35.3 \pm 0.3	32.7 \pm 0.9	34.0 \pm 0.1
DO (mg L^{-1})	7.6 \pm 0.2	7.3 \pm 0.5	6.1 \pm 0.2	6.7 \pm 0.4	6.8 \pm 0.2	6.0 \pm 0.4	5.7 \pm 0.2	6.0 \pm 0.2
pH	7.8 \pm 0.0	7.3 \pm 0.0	8.2 \pm 0.0	8.4 \pm 0.1	7.8 \pm 0.0	7.3 \pm 0.0	7.9 \pm 0.1	8.3 \pm 0.0
Light extinction (m^{-1})	1.1 \pm 0.2	1.1 \pm 0.2	1.4 \pm 0.2	1.1 \pm 0.1	1.1 \pm 0.5	1.3 \pm 0.3	1.1 \pm 0.3	0.9 \pm 0.2

intensity in time when the photosynthesis reaches maximum capacity (i.e., $P_{\text{max}}^{\text{B}}$).

2.5. Periphyton community composition

The composition of the periphyton community was analyzed for an entire year from April 2003 to February 2004. After periphyton productivity incubations were completed, periphyton was scraped off using clean knives and soft brushes, homogenized, and fixed in 10% formaldehyde. At least 300 diatom frustules per slide were examined at a 400x magnification (Zeiss, JENVAL-DIC), and diatom frustules were identified to genera using the taxonomy of Round et al. (1990) and Krammer and Lange-Bertalot (1999) Lange-Bertalot. Biovolume and cell number data were counted with a hemocytometer based on the valve shape and girdle view of diatoms. Filamentous green algae were enumerated based on counting the unit amount of homogenized fragments.

2.6. Chlorination experiments

Chlorination effects were determined in the field in August 2004 (summer) and February 2005 (winter). Phytoplankton P^{B} was determined *in situ* with a gradient of chlorine concentrations (0.1, 0.2, 0.5, and 1 ppm chlorine equivalent) of sodium hypochlorite in seawater with phytoplankton collected from the intake region using a radioactive ^{14}C method modified from Parsons et al. (1984). Radioactive ^{14}C of the phytoplankton was measured by injecting 300-ml BOD bottle with 10 μCi H^{14}CO_3 , incubating the samples for 3 h, and determining the quantity of ^{14}C collected on a Whatman GF/F glass-fiber filter.

P^{B} of periphyton randomly collected from the outlet region was also determined with a gradient of chlorine concentrations (0.1, 0.2, 0.5, and 1 ppm chlorine equivalent of sodium hypochlorite). After incubation for 3 h, changes in DO concentrations over time were measured by a DO meter (YSI, Model 52).

2.7. Interactive effects of high water temperatures and chlorine

Based on the ambient water temperatures in the outlet region, seawater with phytoplankton collected from the intake region was respectively preheated to 40 $^{\circ}\text{C}$ in August 2004 (summer) and to 30 $^{\circ}\text{C}$ in February 2005 (winter) for 20 min to simulate the entrainment period during which phytoplankton passes through the condenser of the cooling system of the NPP II. Preheated samples were then divided into four different treatments ($n = 3$): 0.2 ppm chlorine with the water temperature cooled down to the ambient temperature in the intake region; 0.2 ppm chlorine with a high water temperature (40 $^{\circ}\text{C}$ in summer and 30 $^{\circ}\text{C}$ in winter), high water temperature alone (40 $^{\circ}\text{C}$ in summer and 30 $^{\circ}\text{C}$ in winter); and preheated seawater alone with the water temperature cooled to the ambient temperature in the intake region. Seawater with phytoplankton collected from the intake region was used for the controls. Phytoplankton P^{B} was measured *in situ* using a radioactive ^{14}C method modified from Parsons et al. (1984) by injecting a 300-ml BOD bottle with 10 μCi H^{14}CO_3 , incubating the samples for 3 h, and determining the quantity of ^{14}C collected on a Whatman GF/F glass-fiber filter.

2.8. Data analysis

Paired *t*-test was used to determine whether environmental factors, and the biomass and productivity of phytoplankton and periphyton significantly differed between the intake and outlet regions. Effects of a gradient of chlorination and interactive effects of high water temperatures and chlorine were compared using a one-way fixed analysis of variance (ANOVA) model. Before analyses, these values were examined using power transformations (Clarke and Warwick, 2001) to conform to normality and homogeneity of the variance assumptions. If the results of the ANOVA indicated significant main effects at the 0.05 probability level, then Tukey's studentized range (HSD) test was used to determine which means significantly differed. The relationships of chlorophyll *a*, productivity, and photo-synthetic parameters of periphyton with environmental factors were determined using Spearman rank correlations.

In order to reveal regional and seasonal patterns of periphyton communities, changes in the species composition were studied using multivariate analyses in the PRIMER (vers. 6.0) computer package (Clarke and Gorley, 2006). The Bray-Curtis coefficient was used to produce a similarity matrix of species composition between any two samples according to the biovolume of each species. The data matrix consisted of a total of 24 samples with 11 species. The similarity matrix was fourth root-transformed and then ordinated using non-metric multidimensional scaling (MDS) techniques to visualize the similarity matrix and to illustrate latent patterns in the community composition. A two-way crossed analysis of similarities (ANOSIM) was used to determine whether the effects of region and season on the community composition were significant by comparing the observed statistic to its permutation distribution for the absence of differences (Clarke and Warwick, 2001). If the results indicated significance at the 0.05 probability level, pairwise comparisons and the Bonferroni correction for the significance level were used to determine which

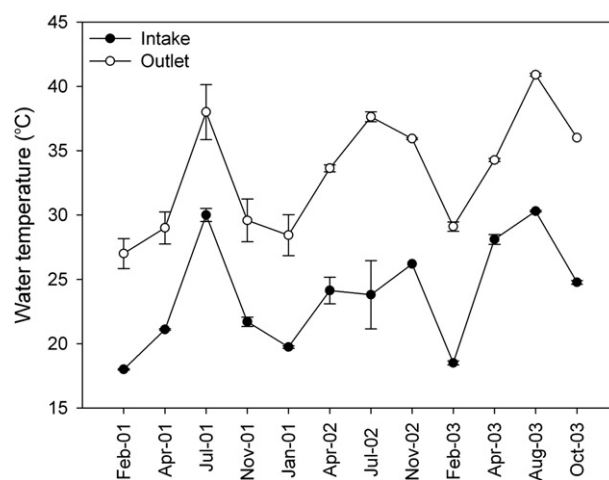


Fig. 2. Seasonal variations (mean \pm SE, $n = 3$) in water temperatures measured in the intake (●) and outlet regions (○) of the Second Nuclear Power Plant, Kuosheng Bay, Taiwan.

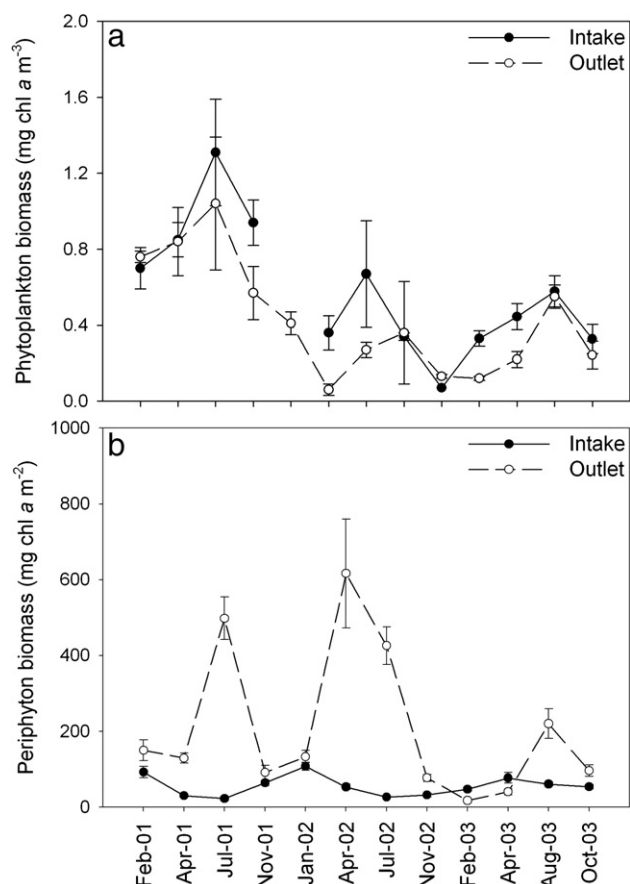


Fig. 3. Seasonal variations (mean \pm SE, $n=3$) in phytoplankton (a) and periphyton chlorophyll *a* (b) measured in the intake (●) and outlet regions (○) of the Second Nuclear Power Plant, Kuosheng Bay, Taiwan.

levels differed. The significance level was calculated by comparing the observed statistic (R , reflecting observed differences between treatments contrasted with differences among replicates within a treatment) to its permutation distribution. If the results of the global test indicated significance at the 5% probability level, pairwise R values were used to determine their separation. Communities could be clearly separated when $R>0.5$, but barely separable at all when $R<0.25$. Similarity of percentages (SIMPER) was employed to reveal the most-common species in replicate samples for each community.

3. Results

3.1. Environmental factors

Light intensity (PAR) peaked at up to $2252 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ in summer and down to $1300 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ in winter. Daily PAR values ranged from 25.9 to $51.7 \text{ mol m}^{-2} \text{d}^{-1}$. No significant difference in monitored environmental factors was detected between the intake and outlet regions, except for water temperature (Table 1). Water temperatures were always 8 – 12°C higher in the outlet region than in the intake region (paired t -test, $t = -24.19$; d.f. = 11, $p < 0.001$). Water temperature showed similar seasonal patterns in the intake and outlet regions. The highest temperature (41°C) was recorded in August 2003 in the outlet region and the lowest temperature (18°C) in February 2001 in the intake region (Fig. 2).

3.2. Biomass

3.2.1. Phytoplankton

Phytoplankton chlorophyll *a* showed a clear spatial-temporal pattern (Fig. 3a) with higher values in summer. Phytoplankton chlorophyll *a* was significantly greater in the intake region than in the outlet region (paired t -test, $t = 2.78$; d.f. = 11, $p < 0.01$). Differences between the intake and outlet regions were greater in spring. The mean value of phytoplankton chlorophyll *a* reached 0.67 mg m^{-3} in the intake region, compared to 0.44 mg m^{-3} in the outlet region.

3.2.2. Periphyton

In contrast to phytoplankton, periphyton chlorophyll *a* was significantly greater in the outlet region than in the intake region (paired t -test, $t = -2.55$; d.f. = 11, $p = 0.01$). Periphyton chlorophyll *a* reached 616 mg m^{-2} in the outlet region, which was about 3.8 times that in the intake region (Fig. 3b). Mean periphyton chlorophyll *a* ranged 5.5 – 107.6 mg m^{-2} in the intake region with peaks in early spring. In the outlet region, however, the seasonal pattern of periphyton chlorophyll *a* peaked in summer and shifted to low values in winter.

3.3. Periphyton community composition

In total, 11 algal genera were observed from the intake region and 8 genera were defined from the outlet region (Table 2). Macroalgal periphyton was dominated by the filamentous green algae, *Ulva linza* and *Ulothrix flaccida*, in the intake region, but the dominance shifted to the green alga *Ulva intestinalis* in the outlet region subjected to elevated water temperatures. Microalgal periphyton was all composed of diatoms, in which the genera *Epithemia* and *Mastogloia* occurred

Table 2
Comparison of community composition of periphyton collected every three months in the intake and outlet regions the Second Nuclear Power Plant in Kuosheng Bay from February 2003 to October 2004.

		Spring		Summer		Autumn		Winter	
		Intake	Outlet	Intake	Outlet	Intake	Outlet	Intake	Outlet
Chlorophyta	<i>Ulva intestinalis</i>		28.8		79.9		37.4		
	<i>Ulva linza</i>	86.2		55.5				78.2	37.4
	<i>Ulothrix flaccida</i>					66.9			
	<i>Navicula</i> spp.	1.0	5.2	9.1	2.7	0.9	13.1	0.6	13.1
Bacillariophyta	<i>Nitzschia</i> spp.	6.5	38.7	24.4	11.5	21.6	24.8	4.4	24.8
	<i>Achnanthes</i> spp.	2.2	16.2	7.2	3.7	8.0	21.1	14.2	21.1
	<i>Amphora</i> spp.	0.5	2.8	1.5	0.9	0.2	3.1	1.2	3.1
	<i>Cocconeis</i> spp.	1.2	7.3	2.4	1.3	0.7	0.5	1.4	0.5
	<i>Diploneis</i> spp.	0.8	1.0						
	<i>Mastogloia</i> spp.					0.1			
	<i>Epithemia</i> spp.	1.6				1.6			

Values are presented as averaged percentage of the total volume of periphyton in each region for each season ($n=2$).

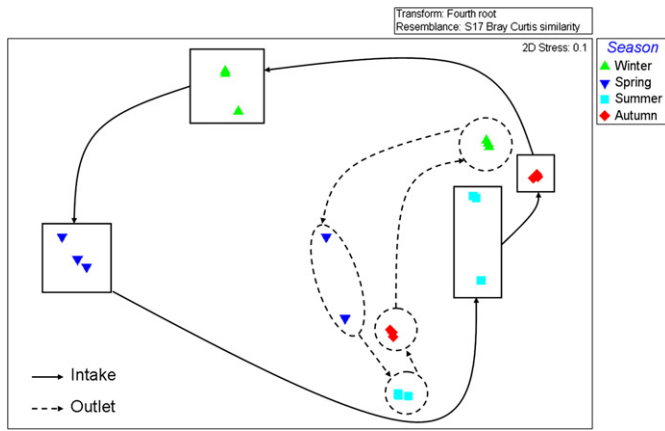


Fig. 4. MDS ordination of Bray-Curtis similarities from fourth root-transformed cell biovolumes for each species of the periphyton communities collected in the intake and outlet regions of the Second Nuclear Power Plant, Kuosheng Bay, Taiwan from April 2003 to February 2004.

only in the intake region. The genera of *Achnanthes*, *Amphora*, *Cocconeis*, *Diploneis*, *Navicula*, and *Nitzschia* occurred in both the intake and outlet regions with the most dominant diatom genus being *Nitzschia*.

The community compositions of periphyton in terms of percent cell biovolume showed distinctness between the intake and outlet regions. A higher percentage of benthic diatoms was observed in the outlet region, while *Ulva linza* was generally dominant in the intake region. In summer, however, *Ulva intestinalis* was dominant in the outlet region and contributed almost 80% to the cell biovolume of the community. SIMPER analysis revealed that *Ulva intestinalis*, *Ulva linza*, and the diatoms *Nitzschia* spp. were key species separating the periphyton communities of the intake and outlet regions.

The ANOSIM showed significant spatial and temporal effects on the periphyton community composition. Periphyton communities significantly differed between the two regions (Global $R=0.256$, $p=0.004$, Fig. 4). MDS ordination also demonstrated a significant seasonal pattern (Global $R=0.414$, $p=0.001$, Fig. 4). The seasonal pattern in the intake region was more pronounced than that in the outlet region. In the outlet region, there appeared to be a slightly compressed seasonal pattern of the periphyton community.

3.4. Productivity

3.4.1. Phytoplankton

In the outlet region, daily GP rates of phytoplankton were not necessarily lower than those of the intake region, especially in

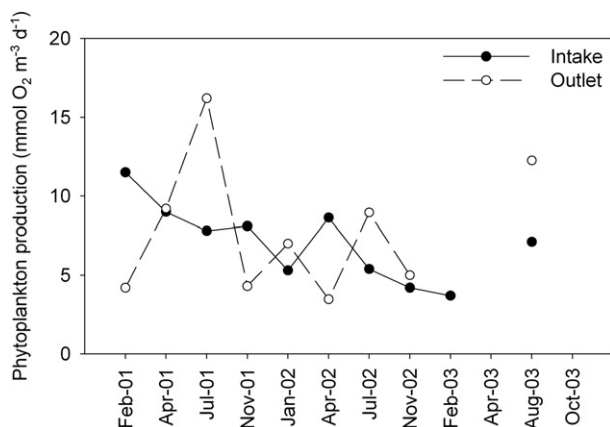


Fig. 5. Seasonal variations in the daily gross production (GP) rates of phytoplankton determined in the intake (●) and outlet regions (○) of the Second Nuclear Power Plant, Kuosheng Bay, Taiwan. Data were not available from April and October 2003.

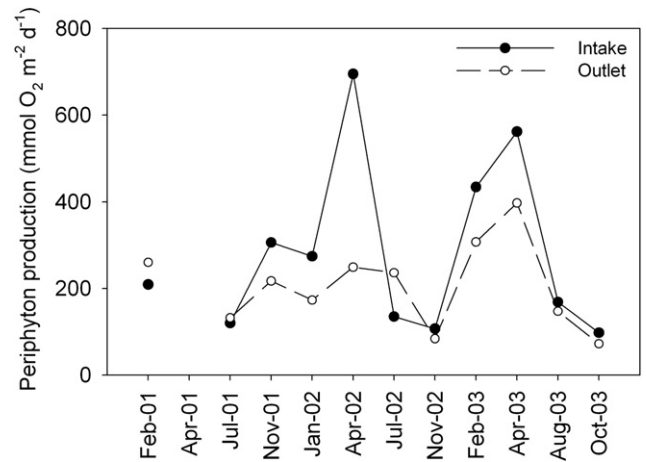


Fig. 6. Seasonal variations in the daily gross production (GP) rate of periphyton determined in the intake (●) and outlet regions (○) of the Second Nuclear Power Plant, Kuosheng Bay, Taiwan. Data were not available from April 2001.

summer (Fig. 5). Consequently, there was no significant difference in daily GP rates of phytoplankton between the intake and outlet regions (paired t -test, $t=-0.02$; d.f.=9, $p>0.05$). Mean daily GP rates of phytoplankton were 7.20 ± 0.58 mmol O_2 m^{-3} d^{-1} in the intake region and 7.06 ± 1.13 mmol O_2 m^{-3} d^{-1} in the outlet region. Some data were not available (in February, April, and October 2003) due to low light intensity that led to the absence of P_{max}^B at those times, and we could not fit the P^B values to the P-E curves to determine reliable daily GP rates.

3.4.2. Periphyton

Daily GP rates of periphyton in the intake and outlet regions showed similar seasonal patterns (Fig. 6), but were significantly greater in the intake region (paired t -test, $t=1.73$; d.f.=10, $p=0.06$). The greatest differences in daily GP rates between the intake and outlet regions occurred in spring.

P_{max}^B values of periphyton were significantly higher in the intake region than in the outlet region (paired t -test, $t=3.26$; d.f.=6, $p<0.01$). In the intake region, higher values of periphyton P_{max}^B occurred in spring (Table 3). In the outlet region, however, lower

Table 3

Comparison of water temperature (mean \pm SE, $n=3$) in the intake region, temperature elevation (ΔT), and periphyton productivity normalized by chlorophyll a (P_{max}^B) in the intake and outlet regions of the Second Nuclear Power Plant in Kuosheng Bay for a specific date in each season.

	Water temperature		P_{\max}^B		Relative change in P_{\max}^B
	Intake	ΔT	Intake	Outlet	
	(°C)	(°C)	(mmol O ₂ mg Chl a ⁻¹ h ⁻¹)		
Winter					
2001–Feb	18.0 ± 0.0	9.0	0.47	NA	NA
2002–Jan	19.7 ± 0.1	8.7	0.34	0.29	– 8
2003–Feb	18.5 ± 0.2	10.6	NA	NA	NA
Spring					
2001–Apr	21.1 ± 0.1	7.9	NA	NA	NA
2002–Apr	24.1 ± 1.0	9.5	NA	0.05	NA
2003–Apr	28.1 ± 0.4	6.2	0.84	NA	– 5
Summer					
2001–Jul	30.0 ± 0.5	8.0	0.71	0.04	– 89
2002–Jul	23.8 ± 2.6	13.8	0.50	0.05	– 82
2003–Aug	30.3 ± 0.1	10.6	0.41	0.11	– 58
Autumn					
2001–Nov	21.7 ± 0.4	7.9	0.21	0.13	– 24
2002–Nov	26.2 ± 0.0	9.7	0.58	0.21	– 47
2003–Oct	24.8 ± 0.1	11.2	0.27	0.19	– 17

NA: data not available.

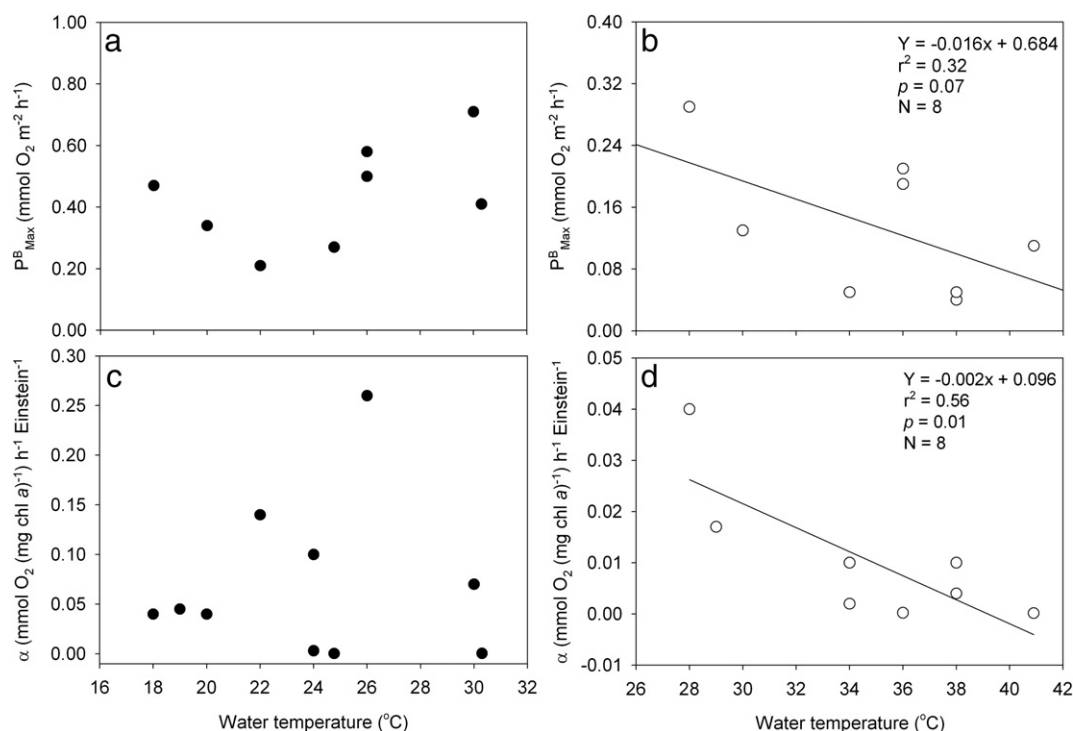


Fig. 7. Linear regression between periphyton P_{\max}^B (mmol O_2 $mg\ chl\ a^{-1}\ h^{-1}$, a and b) and initial slope α^B (mmol $O_2\ m^{-2}\ (mg\ chl\ a)^{-1}\ h^{-1}\ Einstein^{-1}$, c and d) and water temperature in the intake (●) and outlet regions (○) of the Second Nuclear Power Plant, Kuosheng Bay, Taiwan.

values of periphyton P_{\max}^B occurred in summer. The relative reduction in periphyton P_{\max}^B in the outlet region was greatest in summer.

No clear relationship was found for periphyton P_{\max}^B and α^B values with water temperatures in the intake region (Fig. 7a and c). However, they were negatively correlated with water temperatures in the outlet region (Fig. 7b and d). Spearman's rank correlation in the outlet region further demonstrated that the daily GP rate, P_{\max}^B , and α^B of periphyton were negatively correlated with only water temperature (Table 4). No correlation was detected for chlorophyll *a* or E_K of periphyton with water temperature in the outlet region.

3.5. Chlorination experiment

3.5.1. Phytoplankton

A concentration of as low as 0.1 ppm chlorine showed a suppressive effect on phytoplankton P^B when compared to the controls (Fig. 8a and b). However, there appeared to be a seasonal effect of chlorination on phytoplankton P^B . Phytoplankton P^B was completely suppressed by 0.2 ppm of chlorine in summer (one-way ANOVA, $F_{4,15} = 546.7$, $p < 0.001$). In winter, 0.1 ppm of chlorine was

observed to completely suppress phytoplankton P^B (one-way ANOVA, $F_{4,10} = 114.9$; $p < 0.001$).

3.5.2. Periphyton

Unlike phytoplankton P^B , periphyton P^B did not appear to be suppressed by chlorine concentrations of < 0.5 ppm (Fig. 9a and b). Only when the chlorine concentration exceeded a dose of 0.5 ppm was periphyton P^B marginally inhibited in summer (one-way ANOVA, $F_{4,10} = 2.95$, $p = 0.07$), or was significantly inhibited in winter (one-way ANOVA, $F_{4,10} = 4.96$, $p < 0.01$). The results for periphyton P^B were consistent with the seasonal pattern that higher periphyton production occurred in winter than in summer (Fig. 6).

3.6. Interactive effects of high water temperature and chlorine

Phytoplankton P^B was more affected by residual chlorine than by high water temperatures. The effects of high water temperatures, however, on phytoplankton P^B showed a seasonal variation. In summer, phytoplankton P^B was reduced to $< 10\%$ of the controls in the pre-heated tanks at $40\ ^\circ C$ for 20 min and in the tanks remaining at $40\ ^\circ C$ (Fig. 10a). In winter, however, phytoplankton P^B did not significantly decrease in the pre-heated tank at $30\ ^\circ C$ for 20 min, but was lower in tanks remaining at $30\ ^\circ C$ (Fig. 10b). Regardless of whether or not the tanks were incubated at high temperatures, 0.2 ppm chlorine was observed to completely suppress phytoplankton P^B in winter and summer (one-way ANOVA, $F_{3,15} = 157.5$, $p < 0.001$; $F_{3,11} = 15.53$, $p = 0.001$, respectively).

4. Discussion

In this study, the DO method was applied to determine daily GP rates of phytoplankton in the intake and outlet regions of the NPP II. The DO method was less sensitive than the radioactive ^{14}C method for the low-biomass phytoplankton in Kousheng Bay, Taiwan, which might have been due to interference by “noise” bias inside the DO meter (Williams and Jenkinson, 1982). Therefore, photosynthetic

Table 4

Summary of Spearman rank correlation coefficients for matching between periphyton chlorophyll *a* and productivity and environmental factors measured in the outlet region of the Second Nuclear Power Plant in Kuosheng Bay.

Variable	Water temperature (°C)	K (m^{-1})	DO ($mg\ L^{-1}$)	Irradiance ($\mu mol\ photon\ m^{-2}\ s^{-1}$)	Salinity (psu)
GP	−0.73***	0.18	0.53	−0.09	0.08
Chl <i>a</i>	0.20	−0.32	−0.16	0.00	0.20
P_{\max}^B	−0.63*	0.09	0.50	0.00	0.13
E_K	−0.23	−0.08	0.28	0.26	0.00
α^B	−0.58*	−0.17	0.36	−0.42	0.28

GP: daily gross production ($mmol\ O_2\ m^{-2}\ d^{-1}$); Chl *a*: periphyton chlorophyll *a* ($mg\ m^{-2}$); P_{\max}^B : photosynthetic capacity; E_K : light saturation irradiance ($\mu mol\ photon\ m^{-2}\ s^{-1}$); α^B : photosynthetic efficiency.

*Indicates the significant correlation at $p < 0.05$, and *** $p < 0.001$.

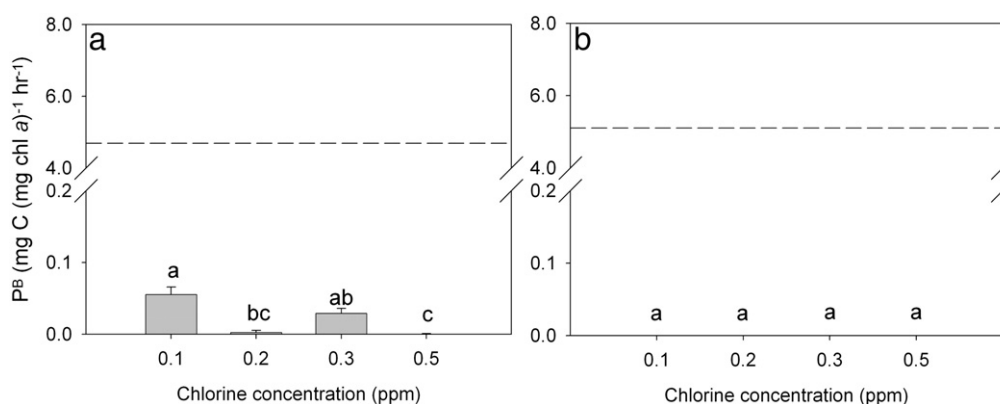


Fig. 8. Interactive effects of high water temperature and chlorine on phytoplankton P^B (mean \pm SE, $n = 3$) determined in August 2004 (winter, a) and February 2005 (summer, b). The dashed line is the mean value of phytoplankton P^B determined in the intake region of the Second Nuclear Power Plant, Kuosheng Bay, Taiwan for the controls. Means with the same letter do not significantly differ by the HSD test.

parameters of phytoplankton could not be reliably estimated from the phytoplankton P-E curves for further analyses. Despite this, the measured daily GP rates of phytoplankton were similar to the results using the radioactive ^{14}C method in the chlorination experiments and a recent study on phytoplankton productivity in the vicinity of the NPP II (Shiah et al., 2005).

An earlier laboratory study by Hirayama and Hirano (1970) on the marine phytoplankton species *Chlamydomonas* sp. and *Skeletonema costatum* concluded that high temperatures (35–40 °C) and residual chlorine should not greatly damage them. In the interactive experiments, however, high water temperatures were found to suppress

phytoplankton P^B . Our results further demonstrated that the impact of high water temperature was regulated by water temperature and exposure time. When water temperatures reached 40 °C in summer, high water temperature alone largely suppressed phytoplankton P^B , even for a short period of 20 min as it passed through the cooling condenser. This finding is consistent with a study of a power plant in tropical coastal waters by Poornima et al. (2005). However, our results are inconsistent with the results of Saravanane et al. (1998) who concluded that the recovery potential of entrained phytoplankton in a coastal power plant was unaffected by heat stress. The reason may be that their study did not consider the period during passage through the cooling condenser. We further discovered that when the water temperature was raised and maintained at 30 °C in winter, the reduction in phytoplankton P^B was smaller. Phytoplankton P^B was even less affected when phytoplankton was exposed to 30 °C for a short period of 20 min simulating entrainment in the cooling condenser of the NPP II.

Inconsistent with the results of an earlier study by Briand (1975), the interactive experiments further demonstrated that phytoplankton P^B was more affected by chlorine than by elevated water temperature (Fig. 10). Regardless of whether or not the tanks were heated, 0.2 ppm chlorine was observed to completely suppress phytoplankton P^B . Similar results have been reported in previous studies of the phytoplankton GP rate being reduced by 30%–70% when exposed to low concentrations of residual chlorine in a tropical power station (Ahamed et al., 1993). Poornima et al. (2006) indicated that chlorination caused a greater reduction in phytoplankton productivity compared to thermal stress at a tropical power station. In temperate waters, Brook and Baker (1972) also found that phytoplankton photosynthesis decreased 40% with low concentrations of chlorine of about 0.2 ppm. Carpenter et al. (1972) found that the lowest dosage of 0.1 ppm caused a 71% decline in phytoplankton productivity in a power plant. Similarly, heterotrophic nanoflagellates subjected to 40 °C for 2 h showed no damage, while significant damage was found with chlorination (Choi et al., 2002). Poornima et al. (2005) also indicated that chlorination (1–3 ppm) caused more-serious damage than short-term exposure to a high water temperature at 42 °C for 30 min. The impact can be attributed to destruction of microalgal chlorophyll as a result of chlorination (Schubel and Marcy, 1978; Brooks and Liptak, 1979; Videau et al., 1979).

Our results also demonstrated a clear seasonal effect of chlorination on phytoplankton P^B . In the chlorination experiments, the lowest dosage of 0.1 ppm chlorine concentration was found to completely suppress phytoplankton P^B in winter, but was not so severe in summer. It is likely that the decomposition of hypochlorous acid (HOCl) formed as a result of chlorination increases with an increase in water temperature (Davis and Coughlan, 1983). Poornima et al.

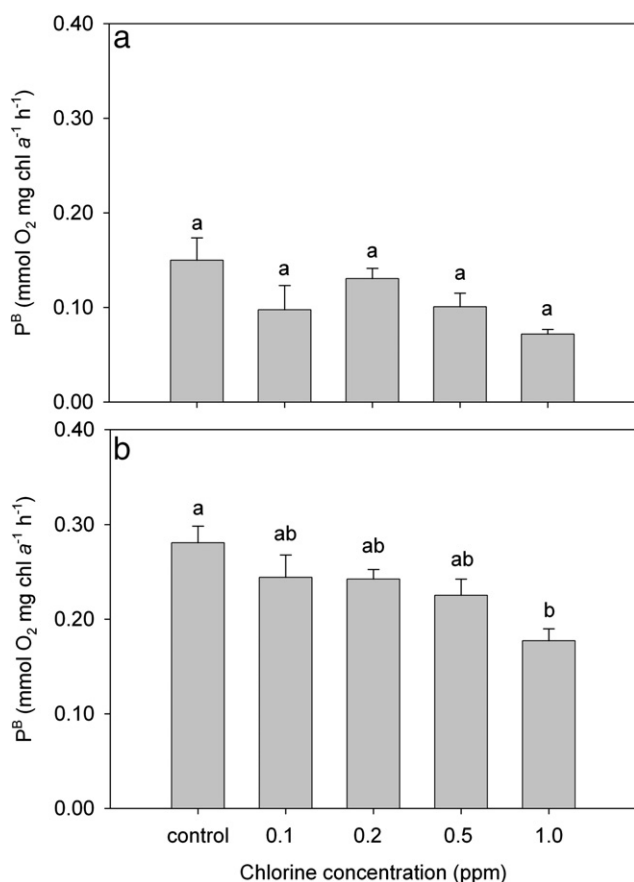


Fig. 9. Effects of a gradient of chlorination on periphyton P^B (mean \pm SE, $n = 3$) determined in August 2004 (winter, a) and February 2005 (summer, b). Means with the same letter do not significantly differ by the HSD test.

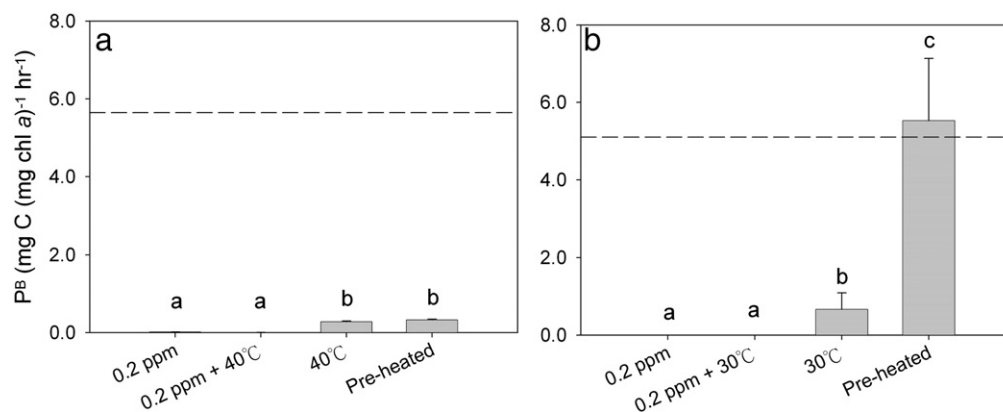


Fig. 10. Effects of a gradient of chlorination on phytoplankton P^B (mean \pm SE, $n = 3$) determined in August 2004 (winter, a) and February 2005 (summer, b). The dashed line is the mean value of phytoplankton P^B determined in the intake region of the Second Nuclear Power Plant, Kuosheng Bay, Taiwan for the controls. Means with the same letter do not significantly differ by the HSD test.

(2005) also indicated that an increase in water temperature resulted in alleviation of chlorine stress. This might be the reason that the daily GP rate of phytoplankton in the outlet region was consistently greater in summer (Fig. 5). Consequently, the difference in phytoplankton chlorophyll a between the intake and outlet regions became smaller in summer (Fig. 3a).

Mallin et al. (1994) indicated that the effect of the thermal discharge from a coastal power plant on the phytoplankton community was not significant, and largely depended on site-specific circumstances. Although the phytoplankton community composition between the outlet and intake regions were not examined in this study, a concurrent study by Lo et al. (2004) found that there were no significant differences in phytoplankton species numbers between the outlet and intake regions at the NPP II. They also indicated that the dominant phytoplankton species *Chaetoceros compressus*, *Nitzschia delicatissima*, *Skeletonema costatum*, *Thalassionema nitzschioides*, and *Lauderia borealis* were the same in both regions, although the phytoplankton species diversity was often higher in the outlet region.

In contrary to phytoplankton productivity, periphyton productivity was affected more by elevated water temperatures. This is consistent with previous studies at other coastal power plants (Boston and Hill, 1991; Sasikumar et al., 1993). Electron transport to the enzyme Rubisco during light reactions through photosynthesis system II is generally sensitive to temperature. Since the rate of photosynthesis is enzyme controlled, it is a temperature-dependent process (Raven and Geider, 1988). The electron transport rate will be higher at a higher water temperature (Falkowski and Raven, 2007). However, high temperatures above 30–35 °C will lead to a decrease in activity of photosynthetic enzymes (Davison 1991; Raven and Geider 1988). This may be the reason that periphyton P^B was suppressed in the outlet region of NPP II where water temperatures were generally >30 °C.

Despite this, greater periphyton chlorophyll a accumulated in the outlet region, especially in summer when the water temperature reached 40 °C. Similarly, higher benthic macroalgal abundance has been reported in the proximity of the thermal discharge of other coastal power plants (Verlaque, 1976). Kim (1999) reported the highest biomass of cyanobacterial mats in the proximity of an artificial thermal water outlet with wastewater. However, Suresh et al. (1993) indicated that benthic macroalgae disappeared when water temperatures exceeded 37 °C. In this study, a possible cause could be attributed to lower grazing pressure in the outlet region subjected to higher water temperatures. Feng (2002) found that the dominant herbivorous fish *Abudefduf vaigiensis* and *Heniochus acuminatus* in the coastal waters of northern Taiwan were rarely observed in the outlet region of the NPP II.

In addition to the low grazing pressure, periphyton communities occurring in the outlet region of the NPP II were dominated by

opportunistic species tolerant to high water temperatures (Devinney, 1980). While *Ulva linza* and *Ulothrix flaccida* were dominant in the intake region, *Ulva intestinalis* was dominant in the outlet region, especially in summer when water temperatures reached 41 °C. Previous studies showed that *Ulva* spp. are eurythermal and can survive at temperatures of 0–30 °C (Skinner, 1971; Fortes and Lüning, 1980; Björnstätter and Wheeler, 1990). Lewis et al. (2001) analyzed the stress proteins of *Ulva intestinalis* and indicated the ability to tolerate thermal stress. Sasikumar et al. (1989, 1993) suggested that the dominance of *Ulva intestinalis* at a thermal discharge site was relevant to the absence of herbivores and the increased algal growth rate.

High water temperatures not only alter the dominance of macroalgae, but also result in a shift in the diatom community. Nevertheless, studies on the response of the marine diatom community to elevated water temperatures are scanty (Verlaque et al., 1981). The dominant diatom genera of *Nitzschia*, *Navicula*, and *Achnanthes* observed in the periphyton of the outlet region were similar to those of freshwater periphyton tolerant of high water temperatures (Patrick, 1969). The diatom genera of *Mastogloia*, *Diploneis*, *Cocconeis*, and *Epithemia* which might not tolerate high water temperatures, were absent from the outlet region and occurred only in the intake region of the NPP II.

5. Conclusion

Phytoplankton chlorophyll a , but not productivity, was significantly lower in the outlet region than in the intake region of the NPP II. Periphyton chlorophyll a was significantly greater in the outlet region than in the intake region. However, periphyton productivity was negatively correlated with water temperatures in the outlet region, but not in the intake region. Distinct differences in the community composition of periphyton were also detected between the two regions. The chlorination experiments showed that a chlorine concentration of 0.2 ppm greatly suppressed phytoplankton productivity, regardless of whether or not the water temperature was elevated. However, periphyton productivity was little influenced by a chlorine concentration of <0.5 ppm. Our results suggested that phytoplankton productivity was greatly affected by residual chlorine, but periphyton productivity was more affected by elevated water temperatures.

Acknowledgements

This study was financially supported by Taiwan Power Company. We thank Yun-Ju Fang and Chi-Ying Huang for their assistance with the field study. Comments by the anonymous reviewer were helpful in revising the manuscript.

References

- Ahamed, M.S., Suresh, K., Durairaj, G., Nair, K.V.K., 1993. Effect of cooling water chlorination on primary productivity of entrained phytoplankton at Kalpakkam, east coast of India. *Hydrobiologia* 271, 165–168.
- Allonier, A.S., Khalanski, M., Camel, V., Bermond, A., 1999. Characterization of chlorination by-products in cooling effluents of coastal nuclear power stations. *Mar. Pollut. Bull.* 38, 1232–1241.
- Björnström, B.R., Wheeler, P.A., 1990. Effect of nitrogen and phosphorus supply on growth and tissue composition of *Ulva fenestrata* and *Enteromorpha intestinalis* (Ulvales, Chlorophyta). *J. Phycol.* 26, 603–611.
- Boston, H.L., Hill, W.R., 1991. Photosynthesis–light relations of stream periphyton communities. *Limnol. Oceanogr.* 36, 644–656.
- Briand, F.J.P., 1975. Effects of power-plant cooling systems on marine phytoplankton. *Mar. Biol.* 33, 135–146.
- Brook, A.J., Baker, A.L., 1972. Chlorination at power plants: impact on phytoplankton productivity. *Science* 76, 1414–1415.
- Brooks, A.S., Liptak, N.E., 1979. The effect of intermittent chlorination on freshwater phytoplankton. *Water Res.* 13, 49–52.
- Carpenter, E.J., Peck, B.B., Anderson, S.J., 1972. Cooling water chlorination and productivity of entrained phytoplankton. *Mar. Biol.* 16, 37–40.
- Choi, D.H., Park, J.S., Hwang, C.Y., Huh, S.H., Cho, B.C., 2002. Effects of thermal effluents from a power station on bacteria and heterotrophic nanoflagellates in coastal waters. *Mar. Ecol. Prog. Ser.* 229, 1–10.
- Clark, R.B., 1989. *Marine Pollution*. Clarendon Press, Oxford, New York, pp. 157–161.
- Clarke, K.R., Warwick, R.M., 2001. *Changes in Marine Communities: An Approach to Statistical Analysis and Interpretation*. Natural Environment Research Council, Plymouth, UK.
- Clarke, K.R., Gorley, R.N., 2006. *PRIMER v6: User Manual/Tutorial*, PRIMER–E. Plymouth, UK.
- Davis, M.H., Coughlan, J., 1983. A model for predicting chlorine concentration within marine cooling circuits and its dissipation at outfalls. In: Jolley, R.L., Brungs, W.A., Cotruvo, J.A., Cumming, R.B., Mattice, J.S., Jacobs, V.A. (Eds.), *Water Chlorination: Chemistry and Water Treatment*, vol. 4, Book 1. The Butterworth Group, Ann Arbor Science, Ann Arbor, MI, pp. 347–358.
- Davison, I.R., 1991. Environmental effects on algal photosynthesis: temperature. *J. Phycol.* 27, 2–8.
- Dawes, C.J., 1998. Human affairs and marine plants. *Marine Botany*. John Wiley & Sons, NY, p. 95.
- Dell'Anno, A., Mei, M.L., Pusceddu, A., Danovaro, R., 2002. Assessing the trophic state and eutrophication of coastal marine systems: a new approach based on the biochemical composition of sediment organic matter. *Mar. Pollut. Bull.* 44, 611–622.
- Devinney, J.S., 1980. Effects of thermal effluents on communities of benthic marine macroalgae. *Environ. Manage.* 11, 225–242.
- Eppley, R.W., Renger, E.H., Williams, P.M., 1976. Chlorine reactions with seawater constituents and the inhibition of photosynthesis of natural marine phytoplankton. *Estuar. Coast. Mar. Sci.* 4, 147–161.
- Feng, H.J., 2002. Effect of thermal seawater discharges on the community structure, diet and mortality of fishes in the waters around the Second Nuclear Power Plant in Taiwan. M.S. Thesis. National Taiwan Ocean Univ. Keelung, Taiwan.
- Falkowski, P.G., Raven, J.A., 2007. *Aquatic Photosynthesis*. Princeton Univ. Press, NJ, pp. 270–272.
- Fortes, M.D., Lüning, K., 1980. Growth rates of North Sea macroalgae in relation to temperature, irradiance, and photoperiod. *Helgol. Meeresunters.* 34, 15–29.
- Fox, J.L., Moyer, M.S., 1975. Effect of power plant chlorination on estuarine productivity. *Chesap. Sci.* 16, 66–68.
- Hamilton Jr., D.H., Flemer, D.A., Keefe, C.W., Mihursky, J.A., 1970. Power plants: effects of chlorination on estuarine primary production. *Science* 169, 197–198.
- Hawkes, H.A., 1969. Ecological changes of applied significance induced by the discharge of heated waters. In: Parker, F.L., Krenkel, P.A. (Eds.), *Engineering Aspects of Thermal Pollution*. Vanderbilt Univ. Press, TN, pp. 15–57.
- Hirayama, K., Hirano, R., 1970. Influences of high temperature and residual chlorine on marine phytoplankton. *Mar. Biol.* 7, 205–213.
- Holmes, N., 1970. Marine fouling in power stations. *Mar. Pollut. Bull.* 7, 105–106.
- James, W.G., 1967. Mussel fouling and use of exmotive chlorination. *Chem. Ind. (London)* 24, 994–996.
- Jassby, A.D., Platt, T., 1976. Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnol. Oceanogr.* 21, 540–547.
- Kennish, M.J., 1992. *Ecology of Estuaries: Anthropogenic Effects*. Marine Science Series. CRC Press, FL, pp. 398–425.
- Kim, B.H., 1999. Ecology of a cyanobacterial mat community in a Korean thermal wastewater stream. *Aquat. Ecol.* 33, 331–338.
- Krammer, K., Lange-Bertalot, H., 1999. *Subwasserflora von Mitteleuropa: Baccillariophyceae*. Volumes 1–4. Spektrum Akademischer Verlag GmbH Heidelberg, Berlin, Germany.
- Krenkel, P.A., Parker, F.L., 1971. Biological aspects of thermal pollution. *J. Appl. Ecol.* 8, 280–281.
- Krishnakumar, V., Sastry, J.S., Swamy, G.N., 1991. Implication of thermal discharges into the sea: a review. *Indian J. Environ. Prot.* 11, 525–527.
- Langford, T.E.L., 1990. *Ecological Effects of Thermal Discharges*. Elsevier Applied Science, London, UK.
- Lewis, S., Donkin, M.E., Depledge, M.H., 2001. Hsp70 expression in *Enteromorpha intestinalis* (Chlorophyta) exposed to environmental stressors. *Aquat. Toxicol.* 51, 277–291.
- Lin, H.J., Wang, T.C., Su, H.M., Hung, J.J., 2005. Relative importance of phytoplankton and periphyton on Oyster–culture pens in a eutrophic tropical lagoon. *Aquaculture* 243, 279–290.
- Lo, W.T., Hwang, J.J., Hsu, P.K., Hsieh, H.Y., Tu, Y.Y., Fang, T.H., Hwang, J.S., 2004. Seasonal and spatial distribution of phytoplankton in the waters off nuclear power plants, north of Taiwan. *J. Mar. Sci. Technol.* 12, 372–379.
- Mallin, M.A., Stone, K.L., Pamper, M.A., 1994. Phytoplankton community assessments of seven southeast US cooling reservoirs. *Water Res.* 28, 665–673.
- Morgan, R.P., Stross, R.G., 1969. Destruction of phytoplankton in the cooling water supply of a steam electric station. *Chesap. Sci.* 10, 165–171.
- Parsons, T.R., Maita, Y., Lalli, C.M., 1984. *A Manual of Chemical and Biological Methods for Seawater Analysis*. Pergamon Press, NY, pp. 115–120.
- Patrick, R., 1969. Some effects of the temperature on freshwater algae. In: Krenkel, P.A., Parker, F.L. (Eds.), *Biological Aspects of Thermal Pollution*. Vanderbilt Univ. Press, TN, pp. 161–185.
- Poornima, E.H., Rajadurai, M., Rao, T.S., Anupkumar, B., Rajamohan, R., Narasimhan, S.V., Rao, V.N.R., Venugopalan, V.P., 2005. Impact of thermal discharge from a tropical coastal power plant on phytoplankton. *J. Therm. Biol.* 30, 307–316.
- Poornima, E.H., Rajadurai, M., Rao, V.N.R., Narasimhan, S.V., Venugopalan, V.P., 2006. Use of coastal waters as condenser coolant in electric power plants: impact on phytoplankton and primary productivity. *J. Therm. Biol.* 31, 556–564.
- Raven, J.A., Geider, R.J., 1988. Temperature and algal growth. *New Phytol.* 110, 441–461.
- Round, F.E., Crawford, R.M., Mann, D.G., 1990. *The Diatoms: Biology and Morphology of the Genera*. Cambridge Univ. Press, Cambridge, UK.
- Saravanane, N., Satpathy, K.K., Nair, K.V.K., Durairaj, G., 1998. Preliminary observations on the recovery of tropical phytoplankton after entrainment. *J. Therm. Biol.* 23, 91–97.
- Sasikumar, N., Venugopalan, V.P., Azariah, J., Nair, K.V.K., 1989. After effects of a dinoflagellate bloom on the colonization of a hard bottom community in Kalpakkam coastal waters. *Mahasagar Bull. Natl. Inst. Oceanogr.* 22, 159–164.
- Sasikumar, N., Azariah, J., Nair, K.V.K., 1993. Changes in the composition of a tropical marine fouling community at power plant discharge. *Biofouling* 6, 221–234.
- Schubel, J.R., Marcy Jr., B.C., 1978. *Power Plant Entrainment: a Biological Assessment*. Academic Press, Inc., NY, pp. 19–189.
- Shiah, F.K., Tu, Y.Y., Tsai, H.S., Kao, S.J., Jan, S., 2005. A case study of system and planktonic responses in a subtropical warm plume receiving thermal effluents from a power plant. *TAO* 16, 513–528.
- Shiah, F.K., Wu, T.H., Li, K.Y., Kao, S.J., Tseng, Y.F., Chung, J.L., Jan, S., 2006. Thermal effects on heterotrophic processes in a coastal ecosystem adjacent to a nuclear power plant. *Mar. Ecol. Prog. Ser.* 309, 55–65.
- Skinner, C.E., 1971. Rapid laboratory culture of *Enteromorpha*. *Chem. Ind.* 8, 511–513.
- Sterman, N.T., 1988. Spectrophotometric and fluorometric chlorophyll analysis. In: Lobban, C.S., Chapman, D.J., Kremer, B.P. (Eds.), *Experimental Phycology: a Laboratory Manual*. Cambridge Univ. Press, UK, pp. 35–40.
- Stockner, J.G., 1967. The ecology of the Ohanapeosh hot springs, Mount Rainier National Park, Washington. Ph.D. Thesis, Univ. Washington, Seattle.
- Suresh, K., Ahamed, M.S., Durairaj, G., Nair, K.V.K., 1993. Impact of power plant heated effluent on the abundance of sedentary organisms, off Kalpakkam, East coast of India. *Hydrobiologia* 268, 109–114.
- Verlaque, M., 1976. Effect of thermal pollution from the Martigues–Ponteau station on the macrophytobenthos. *Tethys* 1, 19–46.
- Verlaque, M., Giraud, G., Boudouresque, C.F., 1981. Effects of a thermal power plant on the Mediterranean marine phytobenthos: the area of high frequency temperature changes. *Bot. Mar.* 24, 69–87.
- Videau, C., Khalanski, M., Penot, M., 1979. Preliminary results concerning effects of chlorine on monospecific marine phytoplankton. *J. Exp. Mar. Biol. Ecol.* 36, 111–123.
- Williams, P.J., Jenkinson, N.W., 1982. A transportable microprocessor controlled Winkler titration suitable for field and shipboard use. *Limnol. Oceanogr.* 27, 576–584.