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Diatom Shift in a 17-year Monitoring Study of Phytoplankton Community in the Subtropical Coastal Taiwan

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Abstract

This study used 17 years of monitoring data to construct a time-series showing changes in the phytoplankton community in coastal waters receiving thermal discharge waste from a petrochemical plant in western Taiwan. More than 234 species of phytoplankton were identified in 544 samples of the study. Significant seasonal variation was found in phytoplankton abundance peaking in spring and decreasing in winter. In 2001, two years after the industrial plant started operating, there was an abrupt drop in abundance of phytoplankton. The mean cell abundance of phytoplankton from 2002 to 2010 dropped to less than half that of the preceding years from 1994 to 2001. On average diatoms composed at least 92% of the phytoplankton, but species composition shifted dramatically over time. In the pre-2001 period, the dominant species were *Asterionella japonica, Biddulphia mobiliensis, Chaetoceros affinis, C. decipiens, Detonula pumila*, and *Pseudo-nitzschia delicatissima*; however, *Ditylum brightwellii* and *Proboscia alata* became dominant in the post-2001 period. Correlation analysis of phytoplankton abundance with various hydrographical parameters revealed phytoplankton abundance was positively correlated to pH. The yearly averaged pH values were 8.18 ± 0.06 in the pre-2001 period, and 7.94 ± 0.12 in the post-2001 period. This finding suggests that pH may be the key factor affecting the shift of diatom composition in this coastal area.

***Key words**: Phytoplankton, Diatom, Petrochemical plant, Hydrographical parameters, Acidification.*

1. Introduction

Phytoplankton are major primary producers and important constituent organisms in coastal ecosystems. Therefore, qualitative and quantitative changes in phytoplankton communities have significant implications for the coastal ecosystem. Phytoplankton takes up inorganic nutrients (e.g. nitrogen and phosphorus) and converts them to organic compounds, a process known as bottom-up control in ecosystems. In marine ecosystems, the standing stock of phytoplankton supports the entire food chain. Phytoplankton composition tends to correlate with environmental factors, such as water temperature, irradiance, and nutrient concentrations (Goldman, 1977; Nixon, 1981; Tilman et al., 1982; Correll, 1998, 1999; Carter et al., 2005; Howarth and Marino, 2006; Spatharis et al., 2007; Li et al., 2011).

Much industrial development has taken place in the coastal areas of western Taiwan over the past several decades starting from the 1970s. Electrical power plants were also built in a nearby industrial park to meet the electricity requirement. Coal fired power plants usually need to generate enormous amounts of cooling water for the combustion of coal. Thus ecological impact on the coastal environment caused by several kinds of industrial development became unavoidable. The main environmental impact has been the increasing water temperature and decreasing pH in affected coastal areas. In the past, thermal discharge has attracted most concern (Mercado and Gómez, 1999; Lo et al., 2004; Poornima et al., 2005; Chang et al., 2009). In comparison, studies seldom focus on pH decrease in petrochemical areas because seawater is believed to be an excellent buffer solution. In coal-burning power plants, about 95 percent or more of the sulfur produced

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when coal is burned is converted into sulfur dioxide (SO_2) (Smith et al., 2001; Kagel et al., 2007; Wu and Yu, 2006). Seawater flue gas desulfurization is a system for removing sulfur oxides and sulfur dioxides (SO_2) from the products of combustion or flue from coal-burning power plants located along coasts (Abrams et al., 1998; Lan et al., 2012). In order to remove sulfur oxides (SOx), the flue gas is brought into intensive contact with seawater in an absorption zone to ensure mass transfer from the gaseous into the liquid phase. Subsequently, the pH of the seawater used will decrease while its temperature will increase. To avoid acidification of seawater, NaOH is added before the water is discharged back into the environment. Unfortunately, a prior investigation documented that the pH may still decrease to 7.5 in this environment (Chu et al., 2008).

Unlike in open ocean, pH in coastal areas tends to fluctuate, and can be an important factor regulating phytoplankton abundance and composition (Higna, 1992; Hansen, 2002; Li et al., 2011). The fluctuation of pH reported in coastal waters could be up to 1 pH unit (e.g. in lower Narragansett Bay) (Higna, 1992). In addition to discharge of freshwater, there are many factors that can also cause pH fluctuation, including dissolved CO_2, alkalinity, discharge of organic matter from rivers, salinity, temperature, light, turbulence, diurnal effect in estuaries, and even seasonal variation of biological composition (such as the phytoplankton community) (Higna, 1992, 2002). A study by Berge et al. concluded that growth of up to 40% of 35 tested phytoplankton species would be affected when pH decreased to 7.6 (2010). However, studies on phytoplankton communities, have been limited and most pH studies do not address diatom species.

Yunlin County is located in central western Taiwan. On its coastline, the Yunlin Offshore Industrial Park occupies a region between two rivers (Jhuoshuei River and Sinhuwei River) about 32 km in length and 4 km in width. The industrial park is the site of the Sixth Naphtha Cracking Plant with its associated power plant, oil refinery, steel mill, industrial port and related downstream industries. In this report, we use long-term monitoring data to characterize seasonal and spatial changes in phytoplankton abundance and community structure in Yunling coastal waters over the past 17 years, a time span that covers the periods before and after the construction of the industrial park. Furthermore, major hydrographic parameters, including pH and temperature, were analyzed to reveal their impact on phytoplankton composition and abundance.

2. Materials and methods

2.1. Sampling

Phytoplankton and seawater samples were collected seasonally on board a rented fishing boat during the period from January 1994 to December 2010. Samples collected from January to March were regarded as winter samples. Similarly, samples collected from April to June, July to September, and October to December were regarded as spring, summer, and fall samples, respectively. Eight sampling stations were arranged in four transecting lines perpendicular to the coast of Yunlin County (Fig. 1). At each station, water samples were taken at 1-2 m depth with a metal-free Niskin bottle.

2.2. Hydrographic parameters

Immediately after collecting water samples, we measured them for temperature, salinity, and dissolved oxygen with a Sea-Bird 19 CTD (Conductivity-Temperature-Depth/Pressure) unit. Additional samples were collected from the Niskin bottles. Salinity in discrete samples was determined by measuring conductivity using an AUTOSAL salinometer 8400B calibrated with IAPSO standard seawater (batch no. P128) to a precision of 0.003. Dissolved oxygen in the discrete samples was measured by direct spectrophotometry (Pai et al., 1993), with a precision of about 0.32% at the 190 μmol/kg level. Water samples were then kept at 20°C in the dark for five days. We used the Winkle method to determine dissolved oxygen in the seawater before and after incubation. The difference between the initial dissolved oxygen and the final dissolved oxygen was the biochemical oxygen demand (BOD). pH was measured at 25 ± 0.05°C with

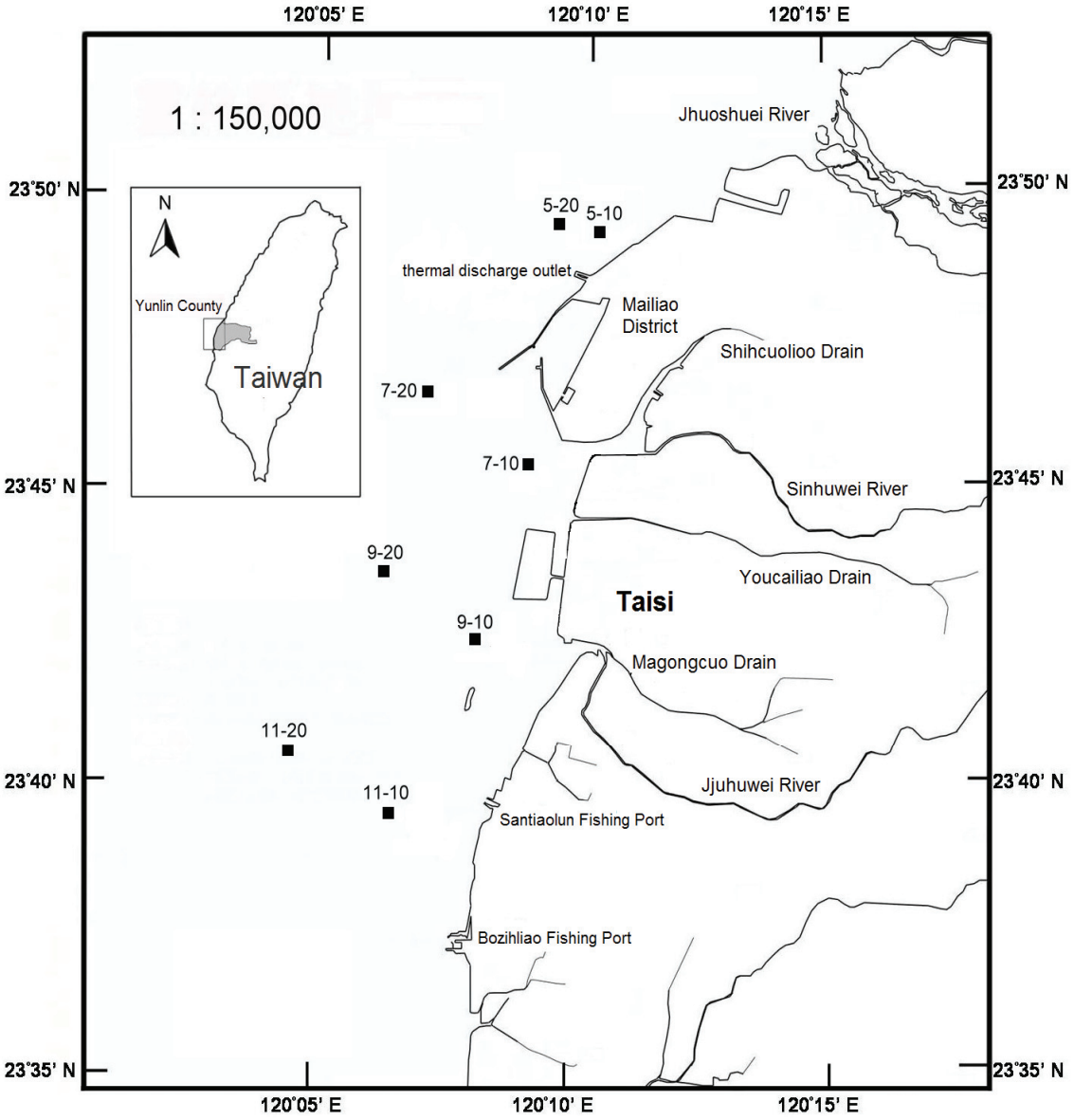

Fig. 1. Map of the study area and sampling site locations.

a Radiometer PHM-85 pH meter and a GK 2401C combination electrode. The electrode and the electrode shift were, respectively, calibrated using a TRIS seawater buffer and IAPSO standard seawater as the running standard. Precision was better than ±0.003 pH units. For determination of total suspended solids (SS), well-mixed water samples were filtered through a weighed 0.45 μm Millipore polycarbonate filter, and the residue retained on the filter was dried to a constant weight at 60°C. The increase in weight of the filter represented the total suspended solids. Measurement of transparency was conducted with a Secchi disk. For chlorophyll a (Chl. a) measurement, two drops of magnesium carbonate were added to a water sample, which was then prepared by filtering through a 0.45 μm pore-size Millipore polycarbonate filter. Filters were wrapped with aluminum foil and saved in a freezer during transportation. Once the filter was extracted by 90% of acetone in the dark (Strickland and Parsons, 1972), Chl. a was measured with a Turner Designs model 10-AU fluorometer.

2.3. Nutrients

Nitrate (NO_3^-) was measured by reducing nitrate to nitrite (NO_2^-) and then determining the nitrite with the pink azo dye method using a flow injection analyzer with an on-

line Cd coil. The precision of this method was about ±0.08 μM for NO_3^- and ±0.02 μM for NO_2^-. Ammonium (NH_4^+) concentration was determined according to the method described by Parson et al. (1984). The detection limit of NH_4^+ was 0.03 μM. Orthophosphate (PO_4^{3-}) was measured with the molybdenum blue method using a flow injection analyzer. Silica (SiO_2) was measured using the method of Fanning and Pilson (1973), also with a flow injection analyzer. The precision of the method was ±0.1 μmol/l. CSK standard in artificial seawater (Wako, Japan) was used for calibration (Pai et al., 1990).

2.4. Phytoplankton composition

The phytoplankton samples were concentrated from the Niskin-collected samples by filtering 20 L of seawater through a 45 μm filter to gather a final volume of 100 ml while on board. The concentrated phytoplankton samples were immediately preserved with Lugol's iodine solution (Schoen, 1988) and kept at 4°C in the dark until identification. The phytoplankton samples were subsampled using a graduated pipette to 1 ml in a Sedgwick-Rafter counting cell chamber. We let cells settle down by gravity, then identified them to either the species or the genus level under an inverted microscope (Leica, Leitz diaplan). The identification of species was mainly based on documentation by Cupp (1977), Tomas (1997), and Yamaji (1982). The species abundance was expressed as cells per liter.

2.5. Statistical analysis

We tested for differences in hydrographical variables and phytoplankton abundance with the ANOVA method, then if ANOVA was significant, stepwise for multiple comparisons (Sokal and Rohlf, 1980). Multiple regressions were used for evaluating the relative abundance of the six most dominant phytoplankton species and the hydrographical variables (Sokal and Rohlf, 1980).

3. Results

3.1. Hydrography and nutrients

The major hydrographic parameters in the seawater (temperature, salinity, pH, chlorophyll a, NO_3^-, NO_2^-, and SiO_2) all showed significant seasonal changes (two way ANOVA, $p < 0.001$) (Fig. 2). Spatial differences among stations were not observed for most parameters (two way ANOVA, $p > 0.5$), except for pH, NO_2^-, and PO_4^{3-}. Temperature ranged between 17.3 and 31.3°C, with an average value of 25.8°C during the 17 years of the study (Fig. 2A). Seasonal salinity ranged between 26.7 and 34.7‰ (Fig. 2B). Salinity also displayed obvious seasonal change, with an opposite trend to the temperature, but it showed no difference among stations (two way ANOVA, $p = 0.858$). Exceptionally large variations in temperature were found occasionally at stations close to the thermal discharge outlet of the power plant.

Chl. a content had an average value of 0.79 ± 0.67 μg/l (Fig. 2C). Over all, spring and summer were the two comparatively productive seasons with higher values of Chl. a, and the lower value was usually in winter. Normally, the seasonal peak was found in spring. An unusually high value of Chl. a was observed in the spring of 2009 caused by a phytoplankton bloom at two stations (St. 5-10 and St. 7-10) close to the shoreline. The average overall pH value was 8.02 ± 0.15 over the 17 sampled years. The ANOVA test showed significant differences in pH value among the sampled years ($p < 0.001$). The pH was never below 8 in all samples prior to 2000, in contrast, pH values were seldom higher than 8 after 2001. The seasonal maximum pH value was 8.34 ± 0.01 in the summer of 1999, compared to the minimum value 7.48 ± 0.20 in the fall of 2006. Furthermore, results clearly demonstrated that pH value dropped below 8.02 in most samplings after 2001. Thus, the average pH between 1994 and 1999 was 8.18 ± 0.06 and decreased to 7.94 ± 0.12 between 2000 and 2010 (t-test, $p < 0.001$) (Fig. 2D). Suspended solids (SS) and BOD displayed different patterns after 2000, and t-tests showed a significant difference between the two periods ($p < 0.001$). For SS, they were 15.88 ± 13.21 mg/l and 25.43 ± 22.16 mg/l, for 1994-1999 and 2000-2010, respectively. For BOD, the yearly average was 1.33 ± 0.40 mg/l and 0.64 ± 0.34 mg/l, respectively, for the pre-2000 and post-2000 periods, respectively.

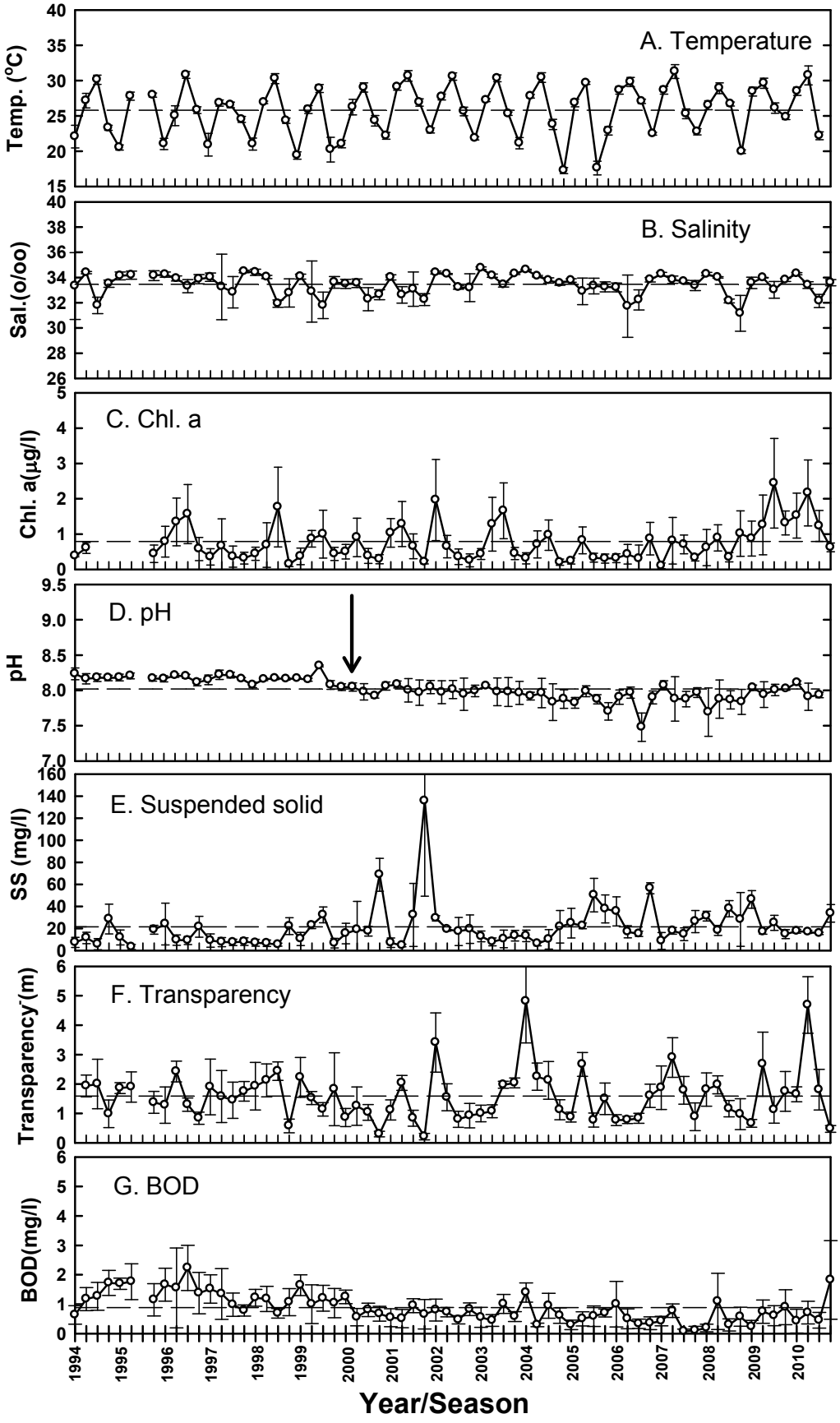

Fig. 2. Seasonal changes of major hydrological factors between 1994 and 2010; temperature (A), salinity (B), chlorophyll a (C), pH value (D), suspended solids (E), transparency (F), and BOD (G). The long dash line represents average number of the sampled data. Mean ± SD (n = 8).

Transparency showed no significant difference between the two periods (t-test, $p = 0.765$).

Nutrient concentrations (NO_3^-, NO_2^-, PO_4^{3-}, and SiO_2) all tended to have yearly maximum content in winter (Fig. 3). Nitrate (NO_3^-) represented the major inorganic nitrogen in seawater before 2000, while ammonium (NH_4^+) became the more predominant inorganic nitrogen source in most seasons after 2000. The average NH_4^+ before and after 2000 was 1.52 ± 0.91 μM and 4.56 ± 4.84 μM, respectively. The maximum $NO_3^- + NO_2^-$ concentration was 25.87 ± 36.76 μM in winter 1994, while the maximum NH_4^+ was 27.41 ± 46.28 μM in spring 2006. The total dissolved inorganic nitrogen (DIN) was significantly different pre-2000 and post-2000; namely, 4.84 ± 5.16 μM and 8.12 ± 5.99 μM (t-test, $p < 0.001$), respectively. Orthophosphate (PO_4^{3-}) concentrations produced higher values in the early 1990s (1994-1998). The average concentration of PO_4^{3-} was 0.45 ± 0.34 μM. Maximum value was 1.66 ± 1.15 μM (winter, 1994), while the minimum value was 0.09 ±

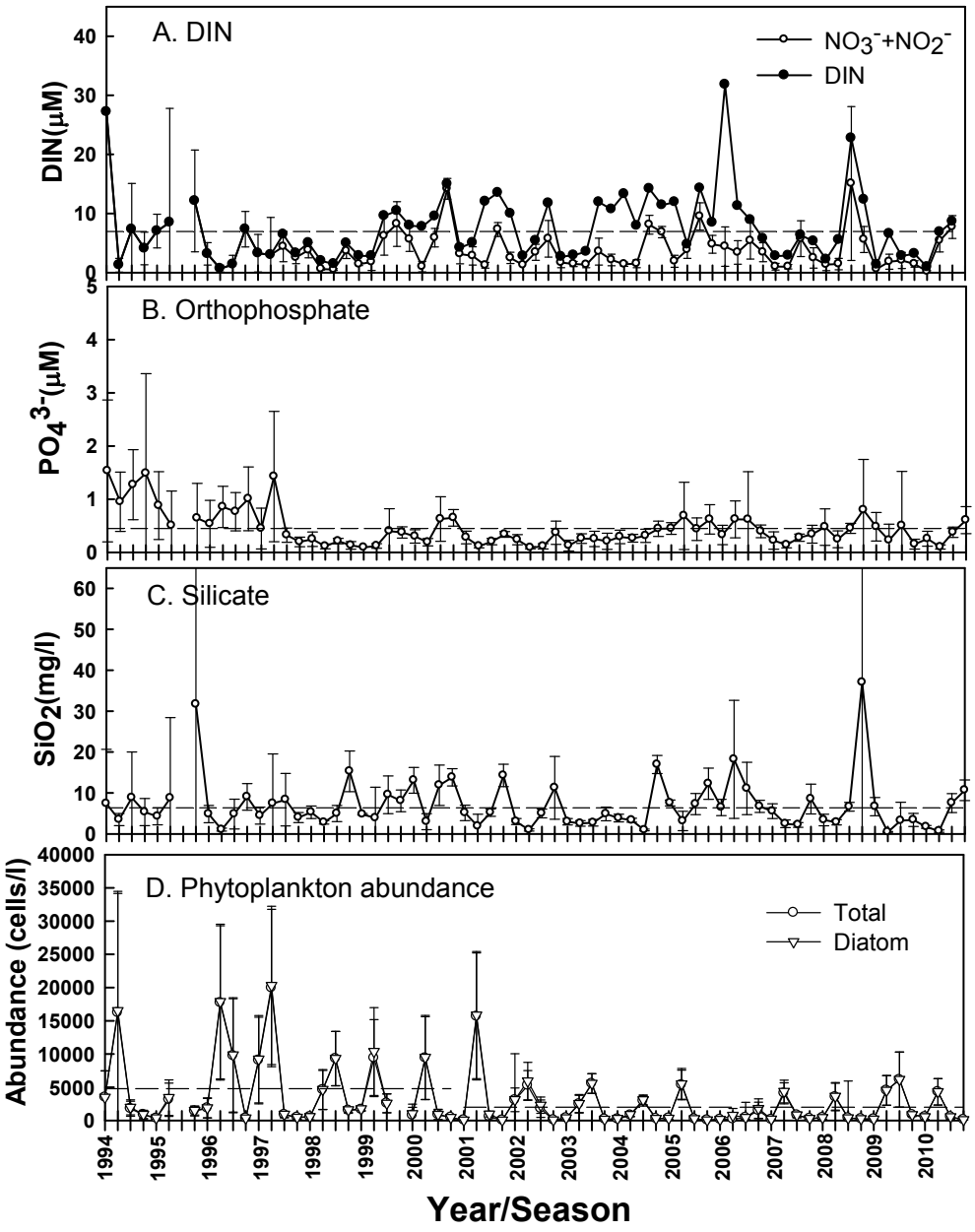

Fig. 3. Seasonal changes in nutrients; dissolved inorganic nitrogen ($NO_3^- + NO_2^- + NH_4^+$) and $NO_3^- + NO_2^-$ (A), orthophosphate (B), silica (C), and phytoplankton abundance (D). The long dash line represents average number of the sampled data. Mean ± SD (n = 8).

0.02 μM (spring, 2002). Yearly PO_4^{3-} content clearly dropped since the middle of 1997. The average prior to 2000 was 0.60 ± 0.44 μM, which was about twice the value (0.35 ± 0.18 μM) after 2000 (t-test, $p < 0.001$). The average SiO_2 concentrations for the two periods were 5.84 ± 4.09 μM and 6.66 ± 5.55 μM, respectively, indicating no significant difference (t-test, $p = 0.591$).

3.2. Phytoplankton abundance and community composition

We identified over 234 taxa in the 544 samplings. Diatoms displayed the greatest species richness in the coastal water, and made up at least 92.3% of phytoplankton (Fig. 3). The diatom assemblage was composed of 162 identified taxa. The remaining phytoplankton groups accounted for less than 10% of total phytoplankton and were composed of cyanobacteria (42 taxa), dinoflagellates (17 taxa), chlorophytes (12 taxa), euglenaphytes and other unidentified small unicellular species. The dominant diatoms were *Asterionella japonica*, *Biddulphia* spp., *Chaetoceros* spp., *Pseudo-nitzschia* spp., *Proboscia alata*, *Rhizosolenia* spp., and *Detonula pumila*. Cell counts of the phytoplankton abundance showed significant seasonal variation. The ANOVA test displayed significant differences across the four seasons ($p < 0.001$). The seasonal variation of phytoplankton showed a peak in spring, and was lowest in winter. Phytoplankton abundance reached its overall maximum value in spring 1997 (20,319 ± 11,910 cells/l), and its lowest point in the fall of 2001 (56 ± 48 cells/l). In addition, phytoplankton abundance displayed significant yearly differences ($p < 0.001$). Average abundance of the total phytoplankton community was 4,801 ± 7,741 cells/l between 1994 and 2001. That decreased to 1,725 ± 2,313 cells/l in the years sampled after 2001 (t-test, $p < 0.001$) (Fig. 4). The usual spring peak of phytoplankton also dropped dramatically after 2001.

A significant dominant diatom-shift was found in the two time periods. There were eight dominant species common to

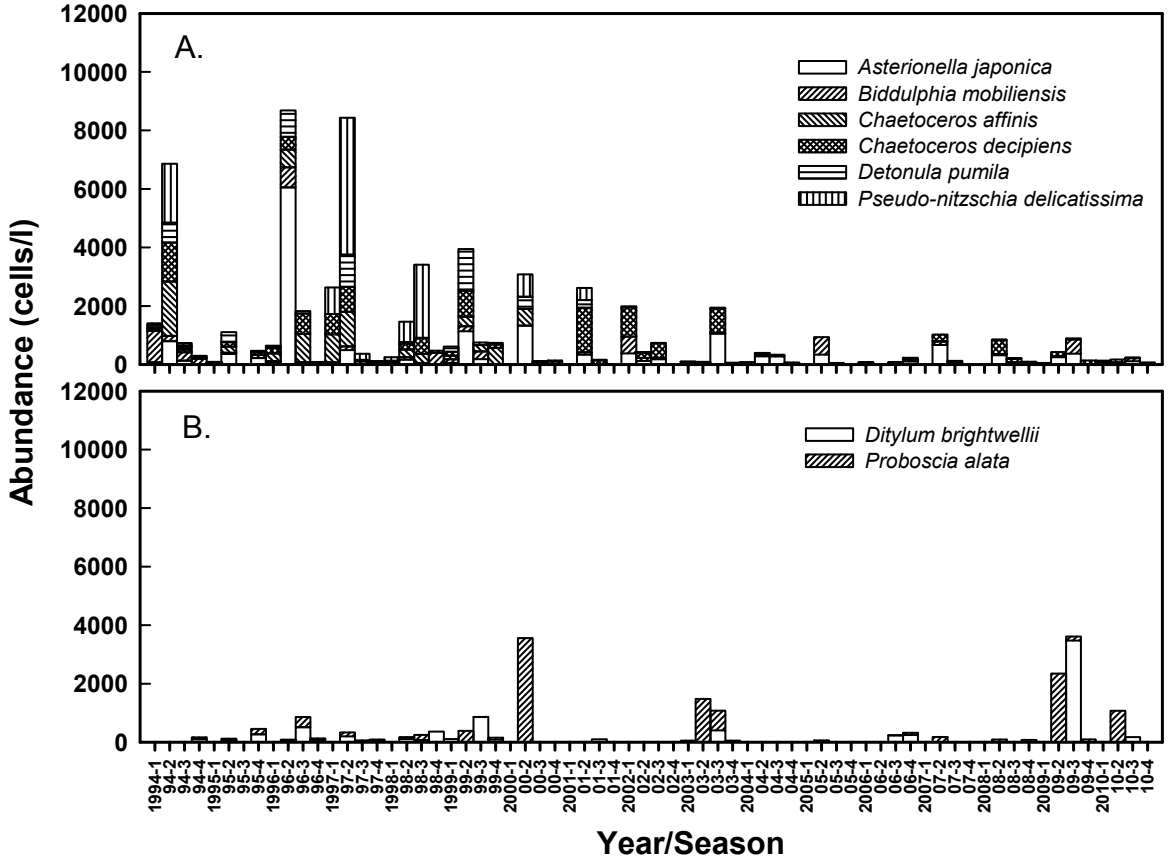

Fig. 4. Changes in seasonal abundance of dominant species. Mean ± SD (n = 8).

all the samples. All were diatoms, including *Asterionella japonica*, *Biddulphia mobiliensis*, *Chaetoceros affinis, C. decipiens, Ditylum brightwellii, Pseudo-nitzschia delicatissima, Proboscia alata*, and *Detonula pumila*. In the pre-2001 period, *A. japonica, B. mobiliensis, C. affinis, C. decipiens, P. delicatissima*, and *D. pumila* dominated, whereas *D. brightwellii* and *P. alata* become dominant in the post-2001 period (Fig. 5). The eight dominant species in descending order, *A. japonica, B. mobiliensis, C. affinis, C. decipiens, D. brightwellii, P. alata, P. delicatissima*, and *D. pumila*, reached their maximum percentage of total phytoplankton abundance as follows: 31.4% (fall, 2004), 38.7% (fall, 2010), 41.6% (fall, 2009), 32.0% (winter, 2002), 56.2% (summer, 2009), 57.8% (spring, 2003), 26.8% (summer, 1998), and 13.8% (spring, 1999), respectively. During the spring peak, the dominant species usually contributed less than 25% of the total phytoplankton.

3.3. Phytoplankton and environmental variables

Analysis of the correlation coefficient between phytoplankton abundance and major sea hydrographical parameters revealed that most factors, with the exception of salinity, were significantly correlated with phytoplankton abundance ($p < 0.001$, except NH_4^+ $p < 0.5$) (Table 1). Of these, temperature, pH, Chl. a, BOD and transparency were positively correlated with the abundance of phytoplankton, whereas the remaining variables were negatively correlated. Phytoplankton abundance and Chl. a were significant correlated ($p < 0.001$). Both methods of estimating phytoplankton biomass were clearly negatively correlated with nutrients (NO_3^-, NO_2^-, SiO_2) and transparency parameters ($p < 0.001$) (Table 1). Furthermore, cell count and Chl. a both showed significant positive relationship with pH ($p < 0.001$ and $p < 0.05$, respectively), temperature ($p < 0.001$), and transparency ($p < 0.001$). Chl. a showed no significant correlation with suspended solids (SS), while the cell count indicating phytoplankton abundance was significantly negatively correlated with SS (Table 1). The Spearman correlation coefficients between the dominant species and hydrographic parameters are summarized in Table 2. All tested species exhibited significant correlation to major hydrographic parameters.

4. Discussion

The phytoplankton composition was dominated by diatoms that are similar to various coastal waters worldwide. We found 162 identified diatom taxa, which accounted more than 92% of the phytoplankton abundance (Fig. 3). These characteristics agreed with previous studies done in adjacent coastal waters of Taiwan, such as north Taiwan (Lo et al., 2004; Wu and Chou, 2004), southwestern Taiwan (Su et al., 2004), the East China Sea (Huang, et al., 2012), and even with coastal waters elsewhere: e.g., the Gulf of Mexico (Schaeffer et al., 2012) and the Portugal coastal upwelling region (Silva et al., 2009). Diatoms are believed to contribute more than 40% of primary production in the ocean, and they are the most species-rich and predominant group of phytoplankton in eutrophic coastal waters, since they generally have rapid growth rates (Furnas, 1990; Mann, 1999; Sarthou et al., 2005).

The Yunlin coastal waters are a nitrogen rich ecosystem with DIN almost always larger than 1 μM. In contrast, the low level of dissolved PO_4^{3-} tended to become the limiting factor for the growth of phytoplankton. The DIN values were 4.84 ± 5.16 μM and 8.12 ± 5.99 μM pre-2000 and post-2000, respectively, whereas the average concentration of PO_4^{3-} was 0.45 ± 0.34 μM, with a maximum value of 1.66 ± 1.15 μM (winter, 1994) and a minimum value of 0.09 ± 0.02 μM (Spring, 2002). The annual PO_4^{3-} concentration dropped sharply since the middle of 1997 (Fig. 2), coinciding with an outbreak of foot-and-mouth disease (FMD) in the pig farming industry in early 1997, when over 3.8 million swine were culled in Taiwan. That meant about 40% of the pig population was lost due to the disease, and it destroyed the pork export market (Chang et al., 1997; Shieh, 1997). Since then, the Taiwanese pig farming industry in central Taiwan has not recovered to its previous extent. This might account for the lower levels of nutrient discharge carried by rivers into the coastal waters, especially the inorganic form of

Table 1. Spearman correlation coefficients between phytoplankton abundance (logPA) and hydrographic parameters. Measured hydrographic parameters include salinity (Sal), dissolved oxygen (DO), pH, chlorophyll a (Chl.a), NH_4^+, NO_3^-, PO_4^{2-}, SiO_2, BOD, suspended solids (SS), and transparency (Trans).

	Temp	Sal	DO	pH	Chl.a	NH4+	NO3-	NO2-	PO43-	SiO2	BOD	SS	Trans
Sal	-0.424***												
DO	-0.789***	0.348***											
pH	0.036 NS	0.185***	0.104*										
Chl.a	0.299***	-0.051NS	-0.277***	0.089*									
NH_4^+	0.028 NS	-0.190***	0.031 NS	-0.366***	-0.003 NS								
NO_3^-	-0.267***	-0.388***	0.195***	-0.325***	-0.245***	0.232***							
NO_2^-	-0.268***	-0.223***	0.182***	-0.164***	-0.268***	0.246***	0.680***						
PO_4^{2-}	-0.073 NS	-0.251***	0.007 NS	-0.048 NS	-0.001 NS	0.397***	0.533***	0.423***					
SiO_2	-0.194***	-0.482***	0.136***	-0.288***	-0.397***	0.220***	0.762***	0.705***	0.460***				
BOD	-0.128**	0.076 NS	0.211***	0.480***	0.046 NS	-0.104*	0.028 NS	0.050 NS	0.164***	0.020 NS			
SS	-0.103*	-0.274***	0.021 NS	-0.414***	-0.077 NS	0.129*	0.336***	0.292***	0.134**	0.378***	-0.153***		
Trans	0.072 NS	0.377***	0.014 NS	0.224***	0.290***	-0.108***	-0.506***	-0.484***	-0.275***	-0.604***	-0.020 NS	-0.509***	
logPA	0.360***	0.032NS	-0.201***	0.436***	0.473***	-0.135*	-0.467***	-0.430***	-0.150***	-0.519***	0.201***	-0.333***	0.411***

NS: $P > 0.05$, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

Table 2. Spearman correlation coefficients among major hydrographic parameters and eight dominant species of phytoplankton. Measured hydrographic parameters include temperature (Temp), salinity (Sal), pH, chlorophyll a (Chl.a), NH_4^+, NO_3^-, PO_4^{3-}, SiO_2, suspended solids (SS), and transparency (Trans).

	Temp	Sal	pH	Chl.a	NH_4^+	NO_3^-	NO_2^-	PO_4^{3-}	SiO_2	SS	Trans
A. japonica	0.323***	-0.027NS	0.012 NS	0.394***	0.167***	-0.237***	-0.254***	-0.035NS	-0.337***	-0.071 NS	0.290***
B. mobiliensis	0.201***	-0.170***	0.132**	0.218***	0.047NS	-0.010 NS	0.005 NS	0.129**	-0.014NS	0.090*	-0.018***
C. affinis	-0.061NS	0.218***	0.570***	0.045 NS	-0.154**	-0.169***	-0.103*	0.146***	-0.130**	-0.435***	0.212***
C. decipiens	0.198***	0.112**	0.361***	0.312***	-0.336***	-0.368***	-0.334***	-0.170***	-0.404***	-0.245***	0.358***
D. brightwellii	0.317***	-0.310***	0.073 NS	0.108*	-0.016 NS	0.054 NS	0.134**	0.003 NS	0.178***	0.094*	-0.117**
D. pumila	-0.097*	0.149***	0.405***	0.188***	-0.063NS	-0.168***	-0.166***	0.099*	-0.244***	-0.227***	0.203***
P. alata	0.184***	0.227***	0.271***	0.358***	-0.135**	-0.426***	-0.480***	-0.058 NS	-0.518***	-0.304***	0.426***
P. delicatissima	0.082 NS	-0.017 NS	0.535***	0.068 NS	-0.114*	-0.185***	-0.127**	0.153***	-0.143***	-0.337***	0.144***

NS: $P > 0.05$, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

PO_4^{3-}. The nutrient data in this study basically agreed with the nutrient range released by Taiwan's Environmental Protection Agency (Taiwan EPA, 2012).

Phytoplankton growth tends to be limited by nutrients, such as nitrogen (N) and phosphate (P) (Nixon, 1981; Tilman et al., 1982; Hecky and Kilham, 1988; Correll, 1998 and 1999; Carter et al., 2005; Howarth and Marino, 2006; Spatharis et al., 2007). For coastal and estuary environments, phytoplankton tends to be N limited, while it is P limited in freshwater (Carpenter and Capone, 1983; Hecky and Kilham, 1988). Addition of N (NO_3^- in most experiments) is used to stimulate growth and biomass of phytoplankton in many enrichment experiments. For diatoms, silicate is believed to be another limiting factor for growth as it is essential for forming silicate shell (Wassmann et al., 1999; Sarthou et al., 2005). Enrichment with silicate enhanced diatom biomass in some studies (Turner et al., 1998; Wassmann et al., 1999; Wu and Chou, 2003; Gilpin et al., 2004). It is believed that ratios of elements play an important role in growth of phytoplankton (Redfield, 1958). The Redfield ratio of N:Si:P is 16:16:1 in marine diatom biomass. Therefore, the Redfield ratio of marine phytoplankton and the ratio of nutrients in water are often measured and used as an indicator of phytoplankton abundance and productivity potential. Turner and colleagues (1998) concluded that diatom growth will be limited once the Si:DIN ratio drops below 1:1 base on the Redfield ratio. Furthermore, some researchers have demonstrated that the limiting concentration of silicate for diatoms was 2 μM (Egge and Aksnes, 1992; Wassmann et al, 1999).

Thereafter, it seems unlikely phytoplankton was limited by nutrients in Yunlin coastal water. The average concentrations of DIN, PO_4^{3-}, and silicate in the post-2000 period were 8.12±5.99 μM, 0.35 ± 0.18 μM, and 6.66 ± 5.55 μM, respectively. The total DIN in the environment increased slightly in the post-2000 period. Increasing nitrogen might originate from greater amounts of NH_4^+ brought by rivers discharging into seawater. The average NH_4^+ before and after 2000 was 1.52 ± 0.91 μM and 4.56 ± 4.84 μM, respectively. The study area is along the coast of Yunlin County, which is basically an agricultural and aquaculture area. Nutrients may originate from fertilizers used in agriculture and discharge from aquaculture farms. Increase of DIN did not stimulate blooms of common eutropical diatom species (e.g. *Chaetoceros* spp and *Pseudo-nitzschia delicatium*) in this study. Thus, it looks like nutrients (N, P, and silicate) are not the controlling factors driving the successive changes in phytoplankton composition after 2001. In contrast, the increase of DIN in the post-2000 might also indicate an overall increasing DIN as a result of reduced phytoplankton consumption, since the amount of phytoplankton had decreased.

Estimating phytoplankton biomass by Chl. a may result in bias due to hydrological effect. Although phytoplankton cell count significantly correlated with Chl. a content, these two methods for phytoplankton biomass measurement displayed varying ranges. Cell counts significantly decreased after 2001, but Chl. a content somehow increased slightly (Fig. 2). The average Chl. a was 0.65 ± 0.44 and 0.86 ± 0.77 prior to 2001 and 2001, afterwards. However, phytoplankton abundance clearly dropped during that period, from 4,801 ± 7,741 cells/l (1994-2001) to 1,725 ± 2,313 cells/l (2002-2010) (Fig. 3). The increase of Chl. a might in fact be caused by increasing suspended solids (SS) in the seawater (from 15.88 ± 13.21 mg/l to 25.43 ± 22.16 mg/l). Measurement of Chl. a is used as an index of phytoplankton biomass by many researchers. However, Chl. a is probably a poor measure of phytoplankton biomass, since Chl. a represents only 1% of the dry weight of a phytoplankton cells (Cullen, 1982). Furthermore, pigment concentration in cells may vary with irradiance (Everitt et al., 1990; Henriksen et al., 2002; Veldhuis and Kraay, 1990). Pigment content decreases with increasing irradiance (Geider et al., 1997; Falkowski and Raven, 1997; Verity, 1981). Reduction of pigment content under high irradiance allows algae to reduce the rate of energy cost in light harvesting (Geider et al., 1997). Falkowski and Raven (1997) concluded that phytoplankton which acclimated to high irradiance levels generally have relatively increased carotenoid: Chl. a ratios. Schagerl and Müller (2006) also demonstrated that under high irradiance, some freshwater blue green algae would exhibit enhanced cellular carotenoid content

and carotenoid to Chl. a ratios, with carotenoid produced as a photoprotective compound. In addition, physiological conditions, like nutrient-limitation and growth phase, both affect the pigment content of phytoplankton cells (Geider et al., 1997; Henriksen et al., 2002). Chl. a levels can differ from two to ten fold under such circumstances (Geider et al., 1997; Sarthou et al., 2005). Another possible explanation for Chl. a increasing faster than cell count in the post-2000 period may be the contribution from small flagellated algae and picoplankton. However, sedimentation of water samples to concentrate phytoplankton did not confirm this. The contribution to total abundance of small flagellated species was little (data not shown).

Both Chl. a and cell count to indicate phytoplankton abundance exhibited a unimodal seasonal pattern, with significantly greater values in spring and the lowest values in winter. The typical one-peak cycle in phytoplankton abundance was slightly postponed in the summers of 1998, 2003, 2004, and 2009. This seasonal pattern was in contrast to nutrient concentrations in the environment, and correlated to temperature. The average abundance of the total phytoplankton community was 4,801 ± 7,741 cells/l between 1994 and 2001, and decreased to 1,725 ± 2,313 cells/l in the following years. Abundance of *C. affinis* and *D. pumila* were negatively correlated with temperature, while the remaining species had a positive relationship with temperature. This supports the idea that different species have various optimal temperature ranges (Goldman, 1977). Therefore, various species tended to dominate in different seasons (Marshall, 1976). In some other studies, the proportion and peak time of the dominant species varied with different N/P treatments (Huang et al., 2012). Many studies have shown that diatoms such as *Skeletonema costatum* have faster growth rates and are so-called r-strategists (Carter et al., 2005; Huang et al., 2012). Our hydrographical data showed abrupt change in the environment in early 2000 (Fig. 2) that turned out to affect the abundance of phytoplankton since 2001.

The phytoplankton abundance peak was more obvious between 1994 and 2001 than in the following decade. Therefore, it suggests the spring peak of the phytoplankton community dropped for some reason. The phenomenon of lower phytoplankton abundance coincided with the decrease of pH values after 2001. The analysis of correlation between phytoplankton abundance and pH confirmed that pH was a significant factor affecting the abundance of the phytoplankton community. The long period of low pH in our study area strongly suggested pH drop derived from the use of the FDG method in the power plant had caused the decrease of phytoplankton density. Studies dealing with the possible effect of pH on the growth and succession of marine phytoplankton are sparse. A few reports have been carried out indicating that pH may indeed drive species succession (Berge et al., 2010; Hinga, 1992). The change of pH parameters had been documented to affect intracellular pH, membrane potential, energy partitioning, and enzyme activity (Giordano et al., 2005). Therefore, lower pH in the environment may reduce the growth rate of phytoplankton. Changes in environmental pH will affect not only the growth rate of phytoplankton but also biochemical composition of the cells, including extracellular production. The pH affects carbohydrate production and free amino acid content of *Skeletonema costatum* (Taraldsvik and Myklestad, 2000). Low pH had the effect of causing *Chaetoceros mulleri* to store less carbohydrate in cells, while exuding more dissolved carbohydrate into the surrounding medium in batch cultures (Thornton, 2009).

Our findings suggest that a pH value of 8.02 may be the key point that controls phytoplankton abundance. Hinga (1992) has demonstrated that diatoms generally exhibited optimal growth near pH 8.1, and the abundance decreased with decreasing pH. Studies of the effect of pH on the growth and succession of phytoplankton have almost all been designed for addressing the issue of global warming. Global warming is known to result in increasing of atmospheric CO2 concentration and consequently also the total inorganic carbon content of the surface ocean. The associated drop in the average surface seawater pH from 8.2 to 7.8 indicates that progressive ocean acidification can be predicted to occur by the end of this century (Berge et al., 2010). While global warming was not evident, regional ocean acidification was discovered in the study area. The

pH fluctuation already discovered in the Yunlin coastal area could be due to many reasons, including dissolved CO_2, alkalinity, river discharge of organic matter, salinity, temperature, light, turbulence, the diurnal effects in the estuary, and even seasonal variation of biological composition (such as the phytoplankton community) (Higna, 1992, 2002).

Our long-term hydrological tracking since 1994 revealed the average pH value dropped from 8.18 ± 0.06 to 7.94 ± 0.12 after 2001 (Fig. 2). The extreme low pH values were always found in two stations (i.e. 5-10 and 7-10), which were close to the thermal discharge outlet of the power plant. In addition, low pH values close to 7.5 were detected occasionally at inshore stations near the Yunlin Offshore Industrial Park after 2001. In fact, the pH values were never lower than 8 in the 1990s, whereas in contrast, the pH values were seldom higher than 8 after 2001. This suggests that the activity of a coal fired power plant and the petrochemical industry caused the decrease of pH in adjacent coastal seawater. Ocean acidification may potentially both stimulate and reduce primary production by marine phytoplankton. It showed phytoplankton community structure was significantly affected by pH after 2001. Neither *D. brightwellii* nor *P. alata* were the dominant species until the year 2001 (Fig. 4). Although, acidification in Yunlin coastal water is not a result of global warming, the findings from this study will help predict the effects of acidification in natural coastal water. Our results showed occasional blooms of some phytoplankton species, i.e., *D. brightwellii* and *P. alata*, which are consistent with other reports of phytoplankton seasonal change in the coastal waters off northern Taiwan (Lo et al., 2004), but those involved different phytoplankton species. The variation in the phytoplankton community was more obvious seasonally than spatially within a single season, since there was no obvious algal composition difference among sampling sites in years prior to 2001. The results of the Spearman correlation analysis showed that, with the exception of *A. japonica* and *D. brightwellii*, the other dominant species were all positively correlated with pH (Table 2). This can be explained that *A. japonica* and *D. brightwellii* can both be found in natural environments within a pH range from 7.5 to 8.2. Even diatom species are usually most dominant in coastal waters; most studies relating to pH have not dealt with diatom species. *In vitro* experiments have previously indicated that *Ceratium limeatum, Heterocapsa triquetra*, and *Prorocentrum minimum* all grew best at pH 7.5 to 8.0 (Hansen, 2002). The growth of the common diatom *Skeletonema costatum* would not be affected at pH 6.5 (Taraldsvik and Myklestad, 2000). Berge and colleagues had a overall study on growth of 35 phytoplankton species, and they concluded that growth of up to 40% of those tested species would be affected when pH decreased to 7.6 (2010). They also summarized that growth of *P. alata* and *A. japonica* both would be affected when pH is lower than 7.5.

In the past, most studies were *in vitro* experiments, conducted to test growth substance uptake or final population abundance under a series of pH gradients. Few studies have been carried out in marine enclosures and field populations. The effect of pH on the other dominant diatom species (i.e. *B. mobiliensis, C. affinis, C. decipiens, D. brightwellii, P. delicatissima*, and *D. pumila*) has never been studied. Further study on single species and community composition must be conducted in order to understand the effect of pH effect on phytoplankton growth and succession. In conclusion, pH is one of the most important factors similar to temperature and nutrients that affect phytoplankton community structure and abundance in Yunlin coastal waters. Once phytoplankton abundance in seawater drops, it may impact the energy flow of the overall coastal ecosystem and change the food chain structure.

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台灣亞熱帶沿岸17年間浮游藻群落中矽藻組成變化探討

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本研究追蹤台灣西部沿岸長達17年間，石化工業電廠排放廢水對當地浮游藻類群落組成之影響。研究期間由544次的採樣中共鑑定得234種浮游藻。藻類組成顯示具有明顯季節變化，春天浮游藻達豐度高峰而冬天時最低。在2001年工業電廠開始運作的兩年後，浮游藻豐度明顯下降。2002-2010年間浮游藻平均豐度下降到僅為1994-2001年間的一半不到。矽藻佔浮游藻平均組成的92%，然優勢藻在兩個時間段落間出現明顯變化。在1994-2001年間優勢藻為*Asterionella japonica*, *Biddulphia mobiliensis*, *Chaetoceros affinis*, *C. decipiens*, *Detonula pumila*, and *Pseudo-nitzschia delicatissima*，2002-2010年間優勢藻則轉變為*Ditylum brightwellii* and *Proboscia alata*。浮游藻組成與水質因子的相關性分析顯示pH值與浮游藻組成呈現正相關。1994-2001年間pH平均值為8.18 ± 0.06，2002-2010年間pH平均值下降為7.94 ± 0.12。此研究結果顯示，pH值可能是造成西部沿岸浮游藻組成改變的主要因素。

關鍵字：浮游植物，矽藻，石化工廠，水質因子，酸化。

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