

Size structure and pigment composition of phytoplankton communities in different hydrographic zones in Hong Kong's coastal seas

CHI HUNG TANG¹, CHONG KIM WONG¹, ALLE AN YING LIE² AND YING KIT YUNG³

¹School of Life Sciences, The Chinese University of Hong Kong, NT, Hong Kong SAR, China, ²Department of Biological Sciences, University of Southern California, 3616 Trousdale Parkway, Los Angeles, California 90089, USA, ³Water Policy and Planning Group, Hong Kong Government Environmental Protection Department, Hong Kong SAR, China

The abundance and community composition of phytoplankton are influenced by a suite of interacting environmental factors. Hong Kong's marine environment features a hydrographic gradient from an estuarine zone in the west to a transition zone in the middle and an oceanic zone in the east. Size fractionation combined with high performance liquid chromatography (HPLC) pigment analyses were used to investigate the phytoplankton communities in different hydrographic zones during summer (July–August 2009) and winter (December 2009–January 2010). Clear temporal and spatial variations in environmental parameters occurred among hydrographic zones. Results of principal component analysis (PCA) revealed that the major deviating factors among hydrographic zones were turbidity and salinity in summer and nitrate and phosphate in winter. Phytoplankton abundance showed significant temporal variations, but no zonal variations. Phytoplankton communities in all hydrographic zones were dominated by cells > 5 µm in both summer and winter. Chlorophyll a concentrations for most size fractions correlated significantly with temperature. The high concentration of fucoxanthin indicated that the phytoplankton community was dominated by diatoms in both summer and winter, while dinoflagellates, cryptophytes, cyanobacteria and other minor groups occurred sporadically in low abundance. The spatial pattern of phytoplankton in Hong Kong's coastal seas did not reflect the hydrographic zonation, but the phytoplankton in the semi-enclosed Tolo Harbour and Deep Bay were different from those in the other zones.

Keywords: phytoplankton, community composition, marker pigments, size-fractionated chlorophyll, HPLC, principal component analysis, multidimensional scaling, hydrographic zones, coastal waters, Hong Kong

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INTRODUCTION

The abundance and species composition of phytoplankton in marine environments are strongly influenced by a multitude of physiochemical (e.g. temperature, salinity and nutrient availability) and biological (e.g. grazing) factors (Officer & Ryther, 1980; Brand & Guillard, 1981; Burkill *et al.*, 1987; Doney, 2006; Moran *et al.*, 2010). How phytoplankton size structure and community composition shift with changes in environmental factors associated with the continuum from estuarine to coastal environments is not well understood.

Located near the Pearl River estuary in the southern coast of China, Hong Kong's coastal waters are characterized by a transition from an estuarine environment in the west to an oceanic condition in the east (Chau, 1958; Morton & Wu, 1975). Morton (1982) divided Hong Kong's coastal waters into three zones with different hydrographic characteristics

(Figure 1), but the boundaries of the hydrographic zones vary seasonally and have not been clearly defined. The estuarine zone in the western part of Hong Kong is strongly influenced by sediment-laden discharges from the Pearl River, especially during the rainy season (summer). The oceanic zone, which covers the entire eastern part of Hong Kong, is marked by marine waters with high salinity. The central region of Hong Kong, including Victoria Harbour and the southern part of Hong Kong Island, represents a zone of transition where the influence of estuarine waters from the west is strongest in summer and weakens in winter with reduced discharge from the Pearl River.

Phytoplankton monitoring in Hong Kong's coastal seas began in the 1980s. Data collected by the Hong Kong Environmental Protection Department (HKEPD) and other investigators have provided useful information on the relationships between hydrographic parameters and phytoplankton abundance (Yung *et al.*, 2001; Yin, 2002; Lie *et al.*, 2011; HKEPD, 2012). However, due to the lack of taxonomic expertise, past studies tended to focus on large diatoms and dinoflagellates which possess external morphological features for identification by light microscopy and are implicated in algal blooms. While the size

Corresponding author:
C.K. Wong
Email: chongkimwong@cuhk.edu.hk

structure of the phytoplankton community may impact the efficiency of energy transfer between trophic levels in the food web (Sheldon *et al.*, 1972; Barnes *et al.*, 2010), information on the abundance, distribution and taxonomic composition of small phytoplankton is limited. In recent years, chemotaxonomic marker pigments, separated by high performance liquid chromatography (HPLC), have been widely used to provide information on the pigment composition of phytoplankton communities and to identify small and fragile microalgae that are easily lost in microscopic analyses (Jeffrey *et al.*, 1997).

The objectives of this study are: (1) to use HPLC analyses of marker pigments to analyse the pigment compositions of phytoplankton of different size fractions in Hong Kong's coastal waters and (2) to study how phytoplankton communities change across the transition from estuarine to oceanic conditions in Hong Kong's marine environment. While several recent studies have investigated the relationship between macronutrient concentrations and phytoplankton abundance in the Pearl River estuary to the west of Hong Kong (Yin, 2002; Huang *et al.*, 2004; Ho *et al.*, 2010), few studies have focused on the community composition and size structure of the phytoplankton in marine areas within Hong Kong's territorial boundary. We hope to provide information to elucidate the linkage between environmental variables and phytoplankton community composition in coastal waters with different hydrographic conditions in an estuarine-coastal continuum.

MATERIALS AND METHODS

Field sampling

Phytoplankton sampling was conducted at 17 sampling stations in Hong Kong's coastal waters (Figure 1) in summer (July and August 2009) and winter (December 2009 and January 2010) aboard HKEPD's marine monitoring vessel 'Dr. Catherine Lam'. A rosette water sampler was used to collect water samples at 0.5 m below the surface. Water samples were immediately pre-filtered through a 200 μm mesh to remove debris and zooplankton larger than 200 μm , returned to the laboratory in two 2 l brown bottles, and stored at 4°C until processing. Environmental variables including temperature, salinity and turbidity were also recorded at 0.5 m below the surface using a conductivity-temperature-depth profiler (SEACAT 19+ CTD with OBS-3 turbidity sensor) connected to the water sampler and controlled by an on-board computer.

Samples and data analyses

Concentration of ammonium (NH_4^+), nitrate (NO_3^-), phosphate (PO_4^{3-}) and silica (SiO_2) were measured by HKEPD using methods described by the American Public Health Association (APHA, 1995). In our laboratory, water samples for pigment analysis were filtered through filters with different pore sizes to obtain phytoplankton of 20–200, 5–20, 2–5 μm

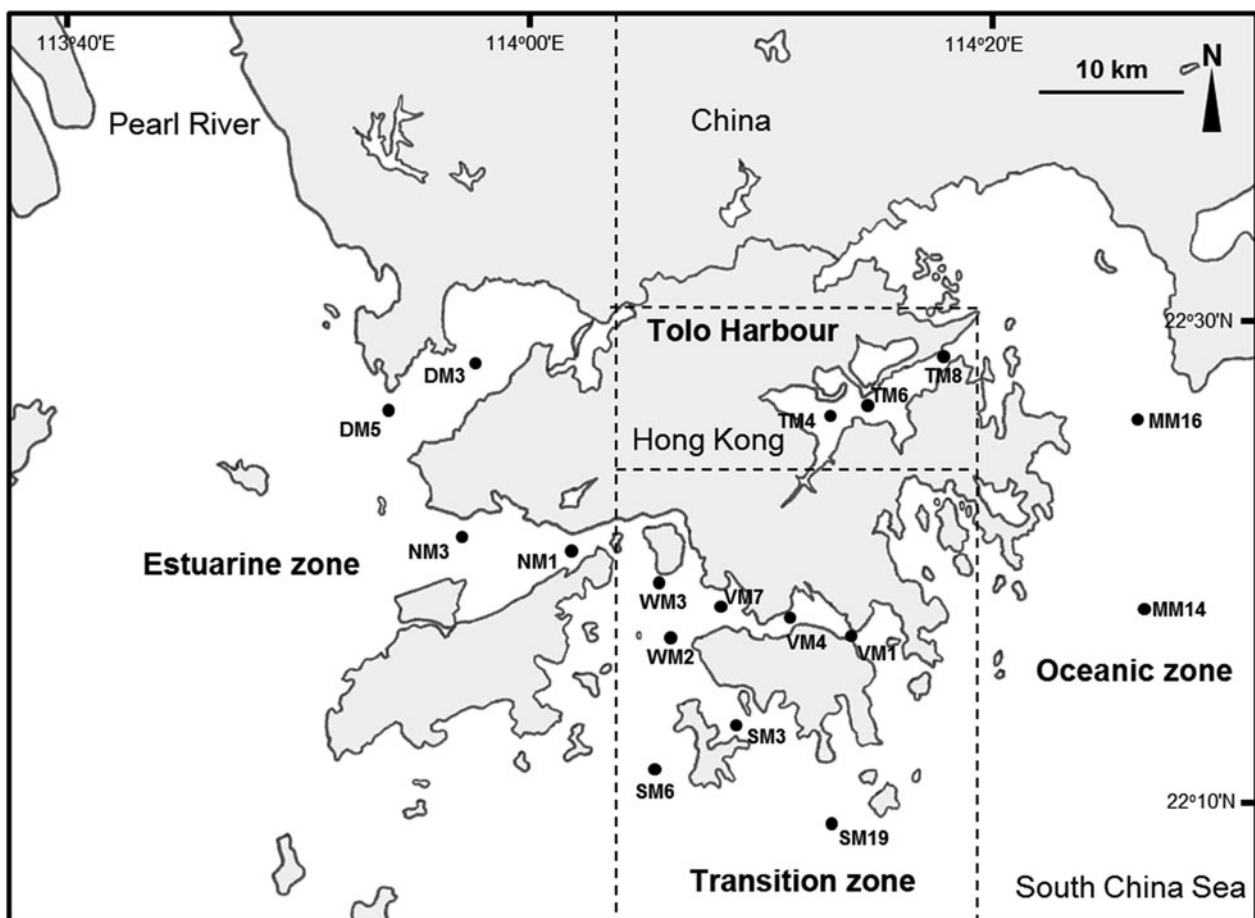


Fig. 1. Map of Hong Kong with locations of 17 sampling stations in the estuarine zone, transition zone, oceanic zone and Tolo Harbour.

(Poretics® polycarbonate membrane filters) and 0.7–2 µm (Whatman GF/F filters) size fractions. The filters were blotted dry, wrapped in aluminium foil, and stored at –80°C before pigment extraction. Pigments were extracted by cutting the filter papers into small pieces under dim light conditions and extracted in 90% acetone (HPLC grade) in darkness for 24 h. Extracts were centrifuged for 15 min at 4800 rpm at 4°C. The supernatant was collected with a syringe (Terumo) and filtered through a 0.2 µm PTFE filter membrane (Nalgene). A 20 µl aliquot of the filtered extract was injected into the HPLC machine (Hewlett Packard HP 1100 series) for pigment analyses according to the methods described in Wright *et al.* (1991) and Lie & Wong (2010).

Tolo Harbour, a semi-enclosed bay in the north-eastern corner of Hong Kong, was originally assigned to the transition zone by Morton (1982). In this study, Tolo Harbour was considered separately from the rest of the transition zone because of its landlocked topography, poor water circulation, and long history of eutrophication.

The software PRIMER 6 (PRIMER-E Ltd, Plymouth, UK) was used to perform principal component analysis (PCA) on the environmental variables and multidimensional scaling (MDS) analyses on the concentration of chlorophyll *a* (Chl *a*) and selected pigments. Environmental variables, including temperature, salinity, turbidity, NH₄⁺, NO₃⁻, PO₄³⁻ and SiO₂, were log (*x* + 1) transformed before conducting the PCA. Pigment concentrations were square-root transformed to construct a Bray–Curtis similarities matrix for the MDS analysis. Only MDS plots with stress values ≤ 0.05, which indicated excellent representation of the community composition on the ordination, were presented. Correlation analyses and two-way analyses of variance (two-way ANOVA) were carried out using Sigmaplot 12.0 (Systat Software Inc., USA) and SPSS 19 (IBM Corporation, USA).

RESULTS

Seasonal and spatial variations in environmental variables

SUMMER

Surface water temperature in Hong Kong ranged from 25.9 to 30.8°C in summer (Table 1). The estuarine zone was characterized by low salinity (14.1–17.6) and high turbidity (10.54–15.94 NTU). High concentrations of NH₄⁺ (7.21–22.74 µM), NO₃⁻ (12.90–13.23 µM), PO₄³⁻ (0.48–0.88 µM), and SiO₂ (71.57–79.89 µM) were recorded at DM3 and DM5 in the inner part of Deep Bay. Concentrations of NH₄⁺ and NO₃⁻ in the transition zone ranged from 1.22 to 5.05 µM and 1.61 to 5.65 µM, respectively. Concentrations of PO₄³⁻ averaged 0.16 µM at WM2, but samples with concentrations below the detection limit of 0.02 µM were also found in the same zone. The concentrations of SiO₂ were much higher in the estuarine zone (51.59–79.89 µM) than in the transition zone, the oceanic zone and Tolo Harbour. Nutrient concentrations at TM4, TM6 and TM8 in Tolo Harbour were generally low, with the concentrations of NH₄⁺ and SiO₂ ranging from 0.44 to 0.83 µM and 4.49 to 7.49 µM, respectively. The concentrations of NO₃⁻ and PO₄³⁻ were below the detection limits of 0.03 and 0.02 µM, respectively, for most samples collected from Tolo Harbour (Table 1).

WINTER

Surface water temperature dropped to 16.9–21.1°C in winter (Table 1). In the estuarine zone, a decrease in the discharge of fresh water from the Pearl River was marked by sharp increases in salinity (28.9–32.1) (Table 1). While turbidity at DM3 and DM5 remained high (14.33–29.58 NTU), a decrease in turbidity was recorded at NM1 and NM3 (0.24–0.25 NTU). As in summer, nutrient concentrations were generally much higher at DM3 and DM5 than in the other sampling stations. High concentrations of NH₄⁺ (10.54–54.35 µM), NO₃⁻ (4.19–6.29 µM) and PO₄³⁻ (0.38–1.47 µM) were recorded at DM3 and DM5. Surprisingly, the SiO₂ concentrations were lower at DM3 than at DM5, NM1 and NM3. Relatively high concentrations of NH₄⁺ (1.28–17.19 µM), PO₄³⁻ (0.09–0.43 µM) and SiO₂ (10.15–14.98 µM) were recorded in the transition zone. The oceanic zone was characterized by high concentrations of SiO₂ (11.48–14.81 µM) and low concentrations of PO₄³⁻ (0.08–0.25 µM). In general, nutrient concentrations were lower at TM4, TM6 and TM8 in Tolo Harbour than in other stations (Table 1).

ANALYSES OF ENVIRONMENTAL VARIABLES

Spatial and temporal variations in environmental variables were analysed by two-way ANOVA. Salinity varied both seasonally ($P < 0.001$) and spatially ($P < 0.001$), and there was significant interaction between season and zone ($P < 0.001$) (Table 2). According to simple-main-effects analysis, seasonal variations in salinity occurred in all zones except the oceanic zone ($F_{(1, 26)} = 1.159$, $P = 0.292$). Not unexpectedly, salinity varied among zones only in summer ($F_{(3, 26)} = 158.356$, $P < 0.001$), but not in winter. Significant variations among zones ($P < 0.001$) and significant interaction between season and zone ($P < 0.001$) were detected in SiO₂ concentrations (Table 2). According to simple-main-effects analysis, significant zonal variations in SiO₂ concentrations occurred in both summer ($F_{(3, 26)} = 21.281$, $P < 0.001$) and winter ($F_{(3, 26)} = 6.770$, $P = 0.002$). NO₃⁻ concentrations showed significant variation among zones and interaction between season and zone ($P < 0.001$, Table 2). Simple-main-effects analysis showed zonal variations in NO₃⁻ concentrations in both summer ($F_{(3, 26)} = 62.683$, $P < 0.001$) and winter ($F_{(3, 26)} = 13.345$, $P < 0.001$). Significant spatial variations were also found in NH₄⁺ ($P < 0.001$) and PO₄³⁻ ($P = 0.002$) concentrations.

According to PCA, principal components 1 (PC1) and 2 (PC2) explained collectively 85.2% of the total variations in environmental variables in summer (Figure 2A). The component PC1 gave relatively greater weights to turbidity (–0.427), salinity (0.422), and PO₄³⁻ (–0.417), while PC2 assigned significant weights to temperature (–0.895) and NH₄⁺ (0.343). Biplot of environmental variables in summer showed that PO₄³⁻, SiO₂ and turbidity were highly related, while NH₄⁺ was highly related to NO₃⁻. Hydrographic conditions of sampling stations were mainly distinguished by turbidity and salinity in summer.

In winter, PC1 and PC2 explained collectively 81.3% of the total variations in environmental variables (Figure 2B). The component PC1 assigned equal weights to NO₃⁻ (–0.461) and PO₄³⁻ (–0.461), while PC2 assigned significant weights to SiO₂ (0.622) and temperature (–0.598). Biplot of environmental variables in winter showed that NH₄⁺, PO₄³⁻ and

Table 1. Mean values of temperature, salinity, turbidity and nutrient concentrations at 17 sampling stations in Hong Kong's coastal waters in summer (July–August 2009) and winter (December–January, 2010).

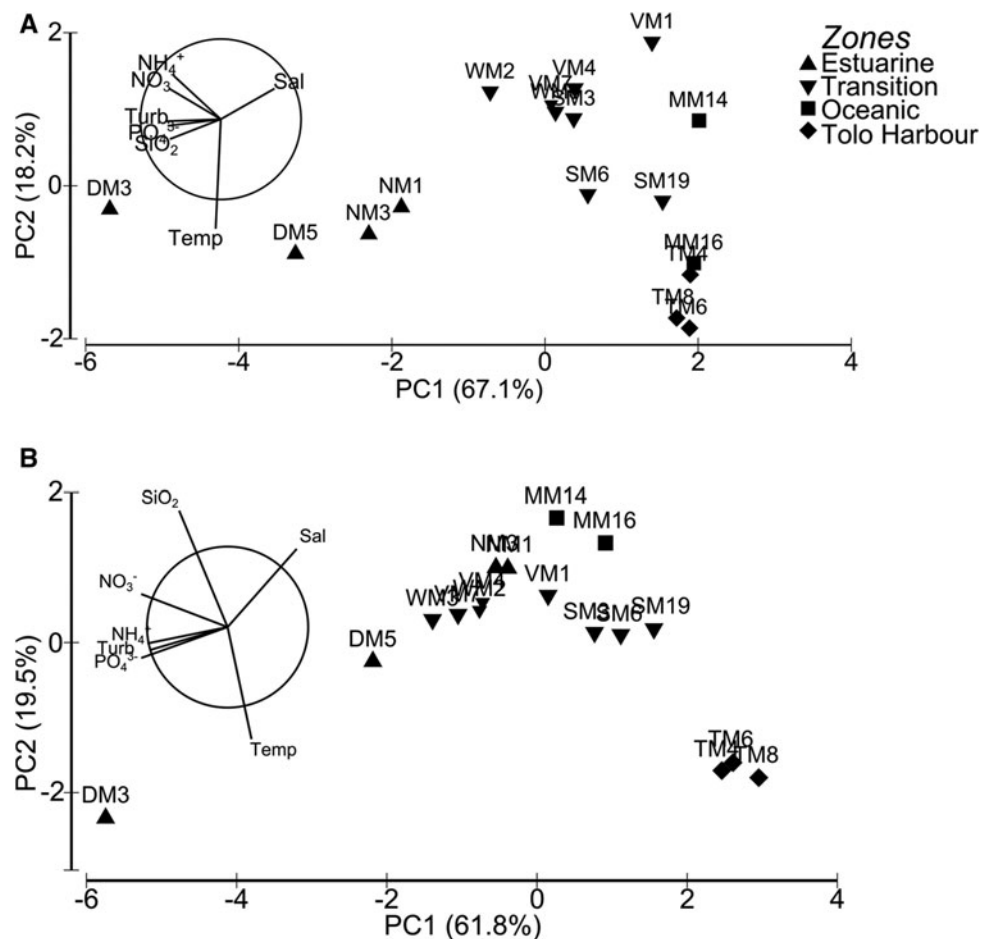
Zone	Station	Temp (°C)		Sal		Turb (NTU)		NH ₄ ⁺ (μM)		NO ₃ ⁻ (μM)		PO ₄ ³⁻ (μM)		SiO ₂ (μM)	
		Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
Estuarine	DM3	29.4	19.4	14.1	28.9	15.94	29.58	22.74	54.35	12.90	6.29	0.88	1.47	79.89	8.65
	DM5	29.9	20.9	15.6	31.6	10.54	14.33	7.21	10.54	13.23	4.19	0.48	0.38	71.57	19.97
	NM1	28.3	17.4	17.6	32.1	11.69	0.25	1.66	8.32	11.94	1.77	0.13	0.24	51.59	12.15
Transition	NM3	28.6	17.4	14.7	32.1	11.78	0.24	1.66	7.76	12.58	1.77	0.15	0.24	61.58	12.82
	SM19	28.8	20.5	26.9	33.0	8.99	1.76	1.22	1.28	1.61	0.60	≦0.02	0.09	0.83	11.98
	SM3	27.2	21.1	28.5	33.2	9.72	1.48	2.33	4.16	2.90	0.87	0.07	0.20	10.49	14.98
	SM6	29.0	20.8	26.4	33.2	10.68	1.38	2.88	1.50	1.94	0.58	≦0.02	0.13	2.00	13.31
	VM1	25.9	18.0	31.5	32.1	8.42	0.31	2.27	6.10	1.94	1.47	0.07	0.22	3.16	10.15
	VM4	27.1	18.1	30.1	31.9	9.48	0.23	3.83	11.09	3.39	1.94	0.12	0.39	4.99	11.98
	VM7	27.5	18.3	27.5	31.9	9.77	0.98	5.05	12.20	4.03	1.94	0.04	0.41	6.66	12.15
	WM2	26.8	18.4	27.3	32.1	10.27	0.93	3.55	12.76	5.65	1.77	0.16	0.36	28.29	12.32
	WM3	27.5	18.4	27.3	31.9	9.23	0.99	4.10	17.19	4.68	1.77	0.04	0.43	10.49	13.31
	Oceanic	MM14	27.0	16.9	30.7	32.3	8.92	0.27	1.61	0.61	0.05	1.94	0.03	0.25	1.33
MM16		29.4	17.1	30.5	32.3	8.36	0.24	0.50	0.89	≦0.03	1.15	0.02	0.08	8.49	11.48
Tolo Harbour	TM4	29.8	20.3	29.7	31.8	8.62	4.92	0.83	1.50	≦0.03	0.08	≦0.02	0.03	4.49	2.16
	TM6	30.8	20.5	28.3	32.2	8.52	4.07	0.50	1.22	≦0.03	0.06	≦0.02	0.04	5.33	2.33
	TM8	30.4	20.9	28.9	32.3	8.88	3.00	0.44	0.94	≦0.03	≦0.03	0.03	0.04	7.49	1.83

Temp, temperature; Sal, salinity; Turb, turbidity; NH₄⁺, ammonium; NO₃⁻, nitrate; PO₄³⁻, phosphate; SiO₂, silica.

Table 2. Result of two-way ANOVA on log ($x + 1$) transformed environmental variables and chlorophyll *a* concentrations. Bold values represent statistical significance at $P < 0.05$ level.

	Temp	Sal	Turb	NH ₄ ⁺	NO ₃ ⁻	PO ₄ ³⁻	SiO ₂	Chl <i>a</i>
Season	<0.001	<0.001	<0.001	0.100	0.061	0.096	0.510	0.004
Zones	0.003	<0.001	0.051	<0.001	<0.001	0.002	<0.001	0.554
Interaction	0.085	<0.001	0.163	0.604	<0.001	0.727	<0.001	<0.001

Temp, temperature; Sal, salinity; Turb, turbidity; NH₄⁺, ammonium; NO₃⁻, nitrate; PO₄³⁻, phosphate; SiO₂, silica; Chl *a*, chlorophyll *a* in the <200 μm size fraction.

**Fig. 2.** Principal component-analysis biplots based on temperature, salinity, turbidity and nutrient concentrations in 17 sampling stations: (A) summer; (B) winter.

turbidity were highly related, with NO₃⁻ and PO₄³⁻ being the major components distinguishing hydrographic conditions of stations.

Chl *a* and chemotaxonomic marker pigments

SPATIAL AND SEASONAL PATTERNS IN CHL *A* CONCENTRATIONS

Over the entire study area, total Chl *a* concentrations (<200 μm) averaged 6.93 μg l⁻¹ in summer and 1.44 μg l⁻¹ in winter. In summer, total Chl *a* concentrations ranged from 1.0 to 31.7 μg l⁻¹, while concentrations >10 μg l⁻¹ were recorded at SM3, SM6 and SM19 in the transition zone (Figure 3A). Over the entire study period, the 20–200 μm size fraction accounted for over 90% of the

total Chl *a* concentrations at most stations. Total Chl *a* concentrations ranged from 0.2 to 7.0 μg l⁻¹ in winter, with higher concentrations at TM4, TM6 and TM8 in Tolo Harbour (Figure 3B). On average, the 20–200 and 5–20 μm size fractions accounted for over 90% of total Chl *a* concentrations.

Results of two-way ANOVA showed significant seasonal variations ($P = 0.004$), but no zonal variations, for total Chl *a* concentrations (Table 2). In general, Chl *a* concentrations were higher in summer than in winter. Interaction between season and zone was also significant ($P < 0.001$). Analysis of simple main effects revealed that seasonal variations in total Chl *a* concentrations were significant only in the transition zone ($F_{(1, 26)} = 38.171$, $P < 0.001$). Positive correlations between Chl *a* concentrations of various size fractions (except the 2–5 μm size fraction) and surface water

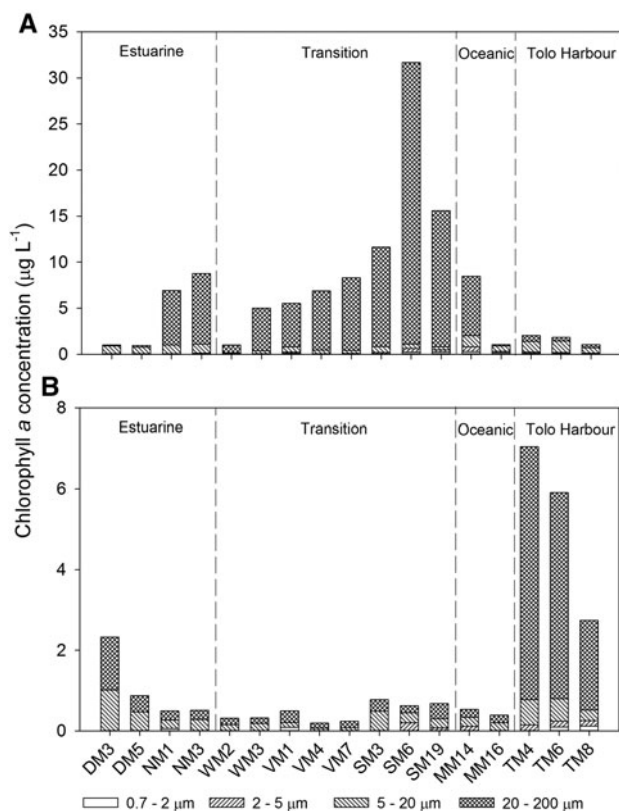


Fig. 3. Chlorophyll *a* concentrations in various size fractions in 17 sampling stations in Hong Kong's coastal waters: (A) summer; (B) winter.

temperature (Table 3) suggested that phytoplankton abundance increased with increasing temperature. In general, Chl *a* concentrations correlated poorly with nutrient (NH_4^+ , NO_3^- , PO_4^{3-} , and SiO_2) concentrations, although significant negative correlations were found between Chl *a* concentrations from the $<2 \mu\text{m}$ size fraction and the concentrations of NO_3^- , PO_4^{3-} and SiO_2 (Table 3).

SPATIAL AND SEASONAL PATTERNS IN MARKER PIGMENT CONCENTRATIONS

HPLC analyses of chemotaxonomic marker pigments provided information on the taxonomic composition of the phytoplankton community. Fucoxanthin, an indicator of diatoms, was the most abundant and ubiquitous accessory pigment at all stations during both summer and winter (Tables 4 and 5). The ratio of fucoxanthin concentration to Chl *a* concentration (Fuco:Chl *a* ratio, abbreviations for marker pigments listed in Tables 4 and 5) for the $>200 \mu\text{m}$

size fraction was >0.50 at most stations during both summer and winter. Peridinin, a signature pigment of dinoflagellates, was recorded at nine stations in summer and four stations in winter. An algal bloom occurred at SM6 during the summer when total Chl *a* concentration was $>30 \mu\text{g l}^{-1}$ and the phytoplankton assemblage was dominated by cells in the $20\text{--}200 \mu\text{m}$ size range. A Peri:Chl *a* ratio of 0.11 suggested that the algal bloom was caused by rapid growth of dinoflagellates. Surprisingly, Peri:Chl *a* ratios >0.15 were recorded at MM14 and MM16 in the oceanic zone in winter when total Chl *a* concentration was $<0.5 \mu\text{g l}^{-1}$.

The accessory pigment 19-hex-fucoxanthin was recorded at nine stations in summer and 10 stations in winter, but the 19 hex:Chl *a* ratios were <0.10 in all samples. Lutein and chlorophyll *b* are major pigments in green algae. Both pigments were common in the inner part of Deep Bay in the estuarine zone in summer. Alloxanthin, a marker pigment for cryptomonads, occurred at most stations during both summer and winter. Ratios of >0.20 of Allo:Chl *a* were recorded at DM3 in summer and WM2 in winter. Zeaxanthin, a marker pigment for cyanobacteria, was found only in summer. Zea:Chl *a* ratios >0.1 were detected at TM6 and TM8 in Tolo Harbour, and at MM16 in the oceanic zone.

Phytoplankton communities were separated into distinct clusters by MDS plots. In summer, a distinct cluster formed by DM3 and DM5 in Deep Bay in the estuarine zone was observed at the 65% similarity level for the $<200 \mu\text{m}$ size fraction (Figure 4A). Likewise, clustering of some stations from the estuarine and oceanic zones at the 65% similarity level suggested that these two zones were poorly separated based on phytoplankton in the $<200 \mu\text{m}$ size fraction. Clustering of stations from different zones suggested that zonal separation was poor based on phytoplankton in the $20\text{--}200 \mu\text{m}$ size fraction (Figure 4B). In winter, a distinct cluster formed by stations in Tolo Harbour (TM4, TM6 and TM8) was detected at the 65% similarity level for both the <200 and $20\text{--}200 \mu\text{m}$ size fractions (Figure 5A, B). However, no clear zonal separation was detected for phytoplankton $<5 \mu\text{m}$ in winter (Figure 5C).

DISCUSSION

Hydrographic conditions in coastal waters around Hong Kong

Hong Kong's coastal waters are influenced by water masses from the South China Sea throughout the year and by estuarine inputs from the Pearl River during the rainy season in summer.

Table 3. Pearson correlations between chlorophyll *a* concentrations in various size fractions and environmental variables. Bold values represent statistical significance at $P < 0.05$ level.

Size fractioned chlorophyll <i>a</i> (μm)	Temp	Sal	Turb	NH_4^+	NO_3^-	PO_4^{3-}	SiO_2
Total Chl <i>a</i> (<200)	+0.427	-0.227	+0.275	-0.193	+0.029	-0.293	-0.162
20-200	+0.390	-0.206	+0.243	-0.194	+0.022	-0.294	-0.171
5-20	+0.626	-0.504	+0.639	+0.102	+0.324	+0.108	+0.271
2-5	+0.313	+0.014	+0.073	-0.331	-0.233	-0.375	-0.257
<2	+0.463	+0.077	+0.110	-0.310	-0.389	-0.410	-0.413

Temp, temperature; Sal, salinity; Turb, turbidity; NH_4^+ , ammonium; NO_3^- , nitrate; PO_4^{3-} , phosphate; SiO_2 , silica.

Table 4. Pigment to total chlorophyll *a* ratios for phytoplankton (<200 μm) at 17 sampling stations in Hong Kong's coastal waters in summer (July–August 2009). ‘–’ represents cases where ratios were not calculated because pigment concentrations were below detection limit.

Pigments	Stations																
	DM3	DM5	NM1	NM3	WM2	WM3	VM1	VM4	VM7	SM3	SM6	SM19	MM14	MM16	TM4	TM6	TM8
Dv Chl <i>a</i>	–	–	0.00	–	–	–	–	–	–	–	0.01	–	–	0.15	–	0.04	–
Chl <i>b</i>	0.14	0.07	0.09	0.01	–	0.09	0.02	0.04	–	0.05	0.05	0.02	–	–	–	–	–
Allo	0.26	0.14	0.01	0.04	0.08	–	0.01	–	0.03	0.01	0.05	0.02	–	–	0.02	–	–
Fuco	0.10	0.16	0.69	0.57	0.58	0.43	0.68	0.68	0.76	0.51	0.67	0.78	0.77	0.77	0.74	0.65	0.72
19 hex	–	0.03	–	–	–	0.01	–	–	0.01	–	0.04	0.02	–	0.05	0.06	0.05	0.03
Lut	0.32	0.09	0.01	0.01	–	–	–	–	–	–	–	–	–	–	–	–	–
Peri	–	0.05	0.05	–	–	0.03	–	0.02	0.07	0.01	0.11	0.07	–	–	–	–	0.02
Viola	0.04	0.02	–	–	–	–	–	–	–	0.01	0.06	–	–	–	–	0.03	–
Zea	0.06	–	–	–	–	–	–	–	–	–	–	0.01	–	0.27	0.07	0.13	0.18

Dv Chl *a*, Divinyl chlorophyll *a*; Chl *b*, chlorophyll *b*; Allo, alloxanthin; Fuco, fucoxanthin; 19 hex, 19-hex-fucoxanthin; Lut, lutein; Peri, peridinin; Viola, violaxanthin; Zea, zeaxanthin.

Table 5. Pigment to total chlorophyll *a* ratios for phytoplankton (< 200 μm) at 17 sampling stations in Hong Kong's coastal waters in winter (December–January, 2010). ‘–’ represents cases where ratios were not calculated because pigment concentrations were below detection limit.

Pigments	Stations																
	DM3	DM5	NM1	NM3	WM2	WM3	VM1	VM4	VM7	SM3	SM6	SM19	MM14	MM16	TM4	TM6	TM8
Dv Chl <i>a</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Chl <i>b</i>	0.12	0.06	–	–	–	–	–	–	–	–	0.08	0.08	0.17	–	–	–	–
Allo	0.11	0.09	0.13	0.14	0.23	0.05	–	0.14	–	0.07	0.11	0.06	0.10	0.09	0.02	–	0.01
Fuco	0.58	0.56	0.62	0.68	0.59	0.57	0.71	0.89	0.69	0.60	0.56	0.73	0.49	0.47	0.60	0.62	0.64
19 hex	–	–	0.06	0.06	–	–	–	–	–	0.04	0.07	0.06	0.09	0.10	0.02	0.02	0.03
Lut	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Peri	–	–	0.05	–	–	–	–	–	–	–	–	–	0.19	0.15	0.01	–	–
Viola	0.04	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Zea	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Dv Chl *a*, Divinyl chlorophyll *a*; Chl *b*, chlorophyll *b*; Allo, alloxanthin; Fuco, fucoxanthin; 19 hex, 19-hex-fucoxanthin; Lut, lutein; Peri, peridinin; Viola, violaxanthin; Zea, zeaxanthin.

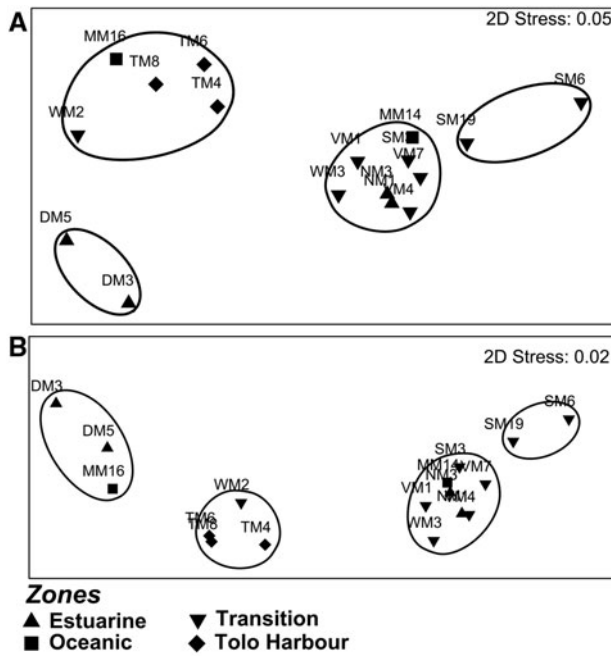


Fig. 4. Multidimensional-scaling-ordination plots based on Bray–Curtis similarity of pigment compositions of phytoplankton in summer of size fractions: (A) $< 200 \mu\text{m}$; (B) $20 - 200 \mu\text{m}$. Only plots with stress values ≤ 0.05 were shown and stations sharing $\geq 65\%$ similarity are encircled.

Impacted by discharges from the Pearl River, seawater in the estuarine zone to the west of Hong Kong is characterized by low salinity, high turbidity and high nutrient concentrations during the period from April to October (Broom & Ng, 1995; Zhang *et al.*, 1999). During the dry season, a decline in river flow is accompanied by an intrusion of seawater from the South China Sea into the Pearl River Estuary and marked increases in salinity in the entire estuarine zone.

Influenced by the influx of large quantities of suspended solids as well as pollutants in the form of agricultural and domestic wastes from Hong Kong and Shenzhen (Broom & Ng, 1995), turbidity at DM3 and DM5 in the inner part of Deep Bay remains high throughout the year. Light attenuation by suspended sediments may decrease phytoplankton productivity (Cloern, 1987). In this study, Chl *a* concentrations at DM3 and DM5 were low during the summer when turbidity was extremely high. However, it should be noted that data collected by HKEPD show that Chl *a* concentrations in the inner part of Deep Bay tend to be higher and more variable compared with other areas during the summer (HKEPD, 2012).

Marked increases in salinity, turbidity and nutrient concentrations allow the estuarine zone to be readily distinguished from the other hydrographic zones in summer. Deep Bay is considered to be highly eutrophic due to nutrient inputs from the Shenzhen River and the Yuen Long creeks (Qi & Zhang, 1995; HKEPD, 2012). Tolo Harbour can be distinguished from the other areas in winter. Tolo Harbour, a semi-enclosed bay in the north-eastern part of Hong Kong, is naturally eutrophic due to its landlocked topography and limited tidal exchange (Hodgkiss & Yim, 1995). Water residence time in the inner part of Tolo Harbour ranges from 16 to 42 days under normal conditions (Oakley & Cripps, 1972; Preston, 1975). Limited tidal exchange with Mirs Bay and a long flushing period allow nutrients, pollutants and phytoplankton to accumulate in Tolo Harbour.

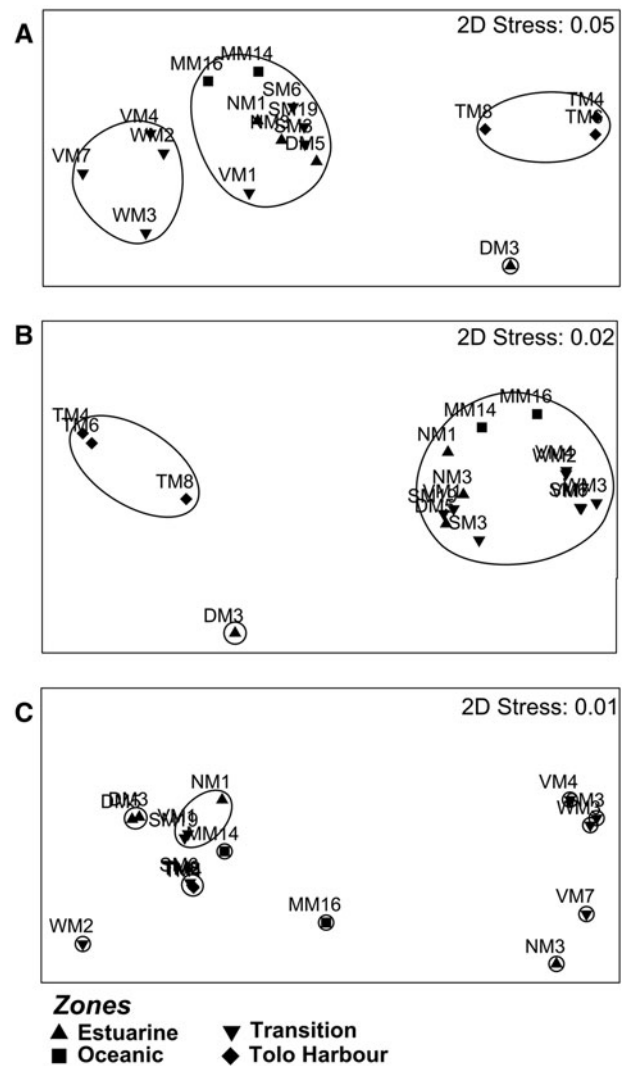


Fig. 5. Multidimensional-scaling-ordination plots based on Bray–Curtis similarity of pigment compositions of phytoplankton in winter of size fractions: (A) $< 200 \mu\text{m}$; (B) $20 - 200 \mu\text{m}$; (C) $< 5 \mu\text{m}$. Only plots with stress values ≤ 0.05 were shown and stations sharing $\geq 65\%$ similarity are encircled.

A decreasing trend in nutrient concentrations across Hong Kong’s coastal waters from the estuarine zone in the west to the oceanic zone in the east was observed in this study. This is in agreement with observations of decreases in nutrient concentrations along the estuarine-coastal continuum reported by other investigators (Yin *et al.*, 2000; Miao *et al.*, 2006). While it is commonly argued that many estuarine and coastal seas are nitrogen (N) limited (Nixon *et al.*, 1986; Howarth & Marino, 2006), phytoplankton growth in the western and southern waters of Hong Kong is believed to be limited mainly by phosphorus (Zhang *et al.*, 1999; Miao *et al.*, 2006). Reduction in N loading has been linked to increases in the abundance and diversity of N_2 -fixing cyanobacteria in the Neuse River Estuary in North Carolina (Piehler *et al.*, 2002). In this study, strong negative correlations between Chl *a* concentrations in the $< 2 \mu\text{m}$ size fraction and nutrient variables including NO_3^- , PO_4^{3-} and SiO_2 suggest that nutrients may not be the limiting factors for phytoplankton growth. An alternative explanation is that small phytoplankton are allowed to thrive

during periods when nutrient levels are too low to support the growth of larger cells. Since zeaxanthin, an indicator of cyanobacteria, was only detected in summer, it can be argued that the abundance of cyanobacteria was related to water temperature (Tang *et al.*, unpublished).

Seasonal and spatial patterns in phytoplankton communities

Our results reveal that Chl *a* concentrations differ between summer and winter, but do not display spatial patterns that reflect hydrographical zonation. Seasonal and spatial patterns in Chl *a* concentrations in Hong Kong's coastal waters have been documented by various investigators. According to Chiu *et al.* (1994), Chl *a* concentrations in Tai Tam Bay in the southern part of the transition zone were highest during the period from August to October. Miao *et al.* (2006) reported higher Chl *a* concentrations ($34.0 \mu\text{g l}^{-1}$) in the coastal seas to the south of Hong Kong Island in the transition zone than in Mirs Bay ($4.4 \mu\text{g l}^{-1}$) in the oceanic zone. However, studies of phytoplankton distribution over Hong Kong's entire coastal sea area are scarce.

Higher Chl *a* concentrations in the summer than the winter and positive correlation between Chl *a* concentrations and temperature suggest that phytoplankton growth in the subtropical waters of Hong Kong is triggered by increases in water temperature. The response of phytoplankton to increases in water temperature is probably species-specific (Huertas *et al.*, 2011). In Hong Kong's coastal seas, algal blooms formed by rapid growth of dinoflagellates are most common in the later winter and early spring when water temperature is increasing (Lam & Ho, 1989). In the temperate North Atlantic Ocean, contribution of small picophytoplankton ($<2 \mu\text{m}$) to the total phytoplankton biomass increases with temperature (Moran *et al.*, 2010).

Phytoplankton in the coastal seas of Hong Kong are dominated by cells $>2 \mu\text{m}$. Biomass of Chl *a* from the $<2 \mu\text{m}$ size fraction was low during both summer ($0.30 \mu\text{g l}^{-1}$) and winter ($0.12 \mu\text{g l}^{-1}$). This is in agreement with results from previous studies (Ho *et al.*, 2010; Lie *et al.*, 2013). Small phytoplankton are considered to have a competitive advantage in oligotrophic waters due to their higher surface area/volume ratios for more efficient acquisition of nutrients and light (Raven, 1986). It is commonly believed that the contribution to the total phytoplankton biomass by picophytoplankton tends to vary with the trophic status of the water body (Chisholm, 1992; Agawin *et al.*, 2000; Bell & Kalff, 2001). In this study, the low contribution to total Chl *a* by the $<2 \mu\text{m}$ size fraction suggests that Hong Kong's coastal waters are relatively eutrophic. Moreover, Chl *a* from the $<2 \mu\text{m}$ size fraction correlated negatively with concentrations of NO_3^- , PO_4^{3-} and SiO_2 , but positively with temperature. It can be argued that the abundance of small cells may be more strongly regulated by temperature rather than nutrient availability.

Ubiquity and high abundance of fucoxanthin suggest that phytoplankton communities in the coastal seas of Hong Kong are dominated by diatoms. Indeed, the widespread occurrence of diatoms in Hong Kong's coastal waters has been reported by various investigators (Yung *et al.*, 1997, 2001). Chiu *et al.* (1994) noted the prevalence of diatoms in terms of both species richness (70%) and cell density (97%)

at Tai Tam Bay in the southern part of the transition zone. Dickman *et al.* (2002) found a clear dominance of diatoms at Lamma and Port Shelter over dinoflagellates in the transition zone.

Dinoflagellates, indicated by the chemotaxonomic marker peridinin, occur sporadically in the estuarine zone, the southern part of the transition zone, and Tolo Harbour in summer, but are scarce in winter. Dinoflagellates are the major causative organisms of algal blooms in Hong Kong's coastal waters (Hodgkiss & Chan, 1987; Yung *et al.*, 1997; Huang *et al.*, 2004). Officer & Ryther (1980) argued that fluctuations in silicon (Si) availability may control the cycle of alternate dominance by diatoms and flagellates in phytoplankton-based ecosystems, and proposed that phytoplankton communities tend to be flagellate-dominated when Si is limited. Hodgkiss & Chan (1987) reported a progressive increase in the dominance of dinoflagellates during the 1980s when Tolo Harbour and Tolo Channel became increasingly eutrophic. Their observation was corroborated by that of Lam & Ho (1989) who reported a shift in the composition of the phytoplankton from diatoms to dinoflagellates in Tolo Harbour over the period from 1982 to 1985. However, several more recent studies found no evidence of a change in the composition of the phytoplankton community in Tolo Harbour (Yung *et al.*, 1997; Wong & Wong, 2004). Lie *et al.* (2011) suggested that shifts in N:Si ratios affected the abundance of diatoms in Tolo Harbour, but presented no evidence to show that the effect was strong enough to alter the dominance of diatoms.

Cryptophytes, indicated by the marker pigment alloxanthin, appear at low densities at most stations during both summer and winter. The presence of *Cryptomonas* spp. in Tolo Harbour has been confirmed previously by microscopic observations (Lam & Ho, 1989) and by the detection of alloxanthin (Yung *et al.*, 1997; Wong & Wong, 2004). Cryptomonads, in sizes ranging from 3 to 50 μm , have a tendency to become more abundant when the abundance of other species decreases (Klaveness, 1988). Yung *et al.* (1997) found a strong positive correlation between abundance of cryptomonads and nutrient concentrations in Tolo Harbour, which according to Sommer (1981) may reflect the ability of r-selected small cells to grow faster and take advantage of high nutrient availability. Strong grazing pressure imposed by zooplankton grazers may account for the low abundance of cryptophytes in Hong Kong's coastal waters. Cryptomonads are excellent food items for herbivorous zooplankton because of their unarmoured cells and high proportions of highly unsaturated fatty acids (Dunstan *et al.*, 2005; Graham *et al.*, 2009). Studies conducted in Tolo Harbour showed that cryptomonads were preferentially grazed by microzooplankton (Lie & Wong, 2010; Liu *et al.*, 2014) and the marine cladoceran *Penilia avirostris* (Wong *et al.*, 2006).

Cyanobacteria, indicated by the marker pigment zeaxanthin, appear in all zones during summer, and are particularly common in Tolo Harbour. Several studies have documented the presence of cyanobacteria in Tolo Harbour and other parts of Hong Kong's coastal seas (Hodgkiss & Chan, 1987; Huang *et al.*, 2004; Wong & Wong, 2004). Cyanobacteria can grow in nutrient-depleted waters by converting atmospheric nitrogen into ammonia for assimilation and by consuming suspended organic matter (Stanier & Cohen-Bazire, 1977; Stewart, 1980). The ability to adjust their buoyancy allows cyanobacteria to stay near the surface and maximize acquisition of light and nutrients in stratified waters (Paerl, 2002).

High surface-water temperature enhances stratification of the water column and provides conditions favourable to cyanobacteria (Paerl & Huisman, 2008). Certain strains of cyanobacteria can multiply rapidly when experiencing anthropogenic nutrient enrichment, and produce secondary compounds which are harmful to other organisms (Keating, 1978; Giussani & Bernardi, 1990; Jochimsen *et al.*, 1998). While Cyanophyceae accounted for only 2% of the total number of algal blooms recorded in Hong Kong (Lu & Hodgkiss, 2004), our findings suggest that they are widely distributed in Hong Kong's coastal waters and should be given more attention for better water-quality management.

Phytoplankton in different hydrographic zones

Except for the semi-enclosed Deep Bay and Tolo Harbour, phytoplankton communities in different hydrographic zones cannot be separated based on pigment composition. Specifically, it can be concluded that the phytoplankton communities in the oceanic zone, transition zone and at NM1 and NM3 of the estuarine zone are relatively similar in terms of their pigment composition. The spatial patterns of phytoplankton composition do not correspond with the delineation of the hydrographic zones. While PCA suggests that the principal factors distinguishing the hydrographic zones are turbidity and salinity in summer, and NO_3^- and PO_4^{3-} in winter, these factors correlate poorly with the size-fractionated Chl *a* concentrations. In fact, strong correlations between temperature and the Chl *a* concentrations from various size fractions point to the presence of the seasonality in phytoplankton abundance in Hong Kong's coastal waters.

The abundance and community composition of phytoplankton can be influenced by many factors. The absence of strong correlations between nutrient concentrations and phytoplankton abundance implies that nutrient availability is not the major factor affecting the distribution pattern of phytoplankton. Many studies have demonstrated that the grazing impact of herbivorous zooplankton can play a major role in shaping the community composition of phytoplankton (Burkill *et al.*, 1987; Strom & Welschmeyer, 1991). Chen *et al.* (2009) found that microzooplankton grazing rates in Hong Kong waters were higher in the west than in the east. They also reported that temperature was the pivotal factor affecting phytoplankton growth rates and microzooplankton grazing rates. Experiments conducted in Tolo Harbour revealed that both mesozooplankton and microzooplankton grazed selectively on particular taxa of phytoplankton (Wong *et al.*, 2006; Lie & Wong, 2010; Liu *et al.*, 2014), and reasoned that top-down control by planktonic herbivores may influence the community composition and size structure of the phytoplankton. Unfortunately, quantitative information on the grazing impacts of phytoplankton by zooplankton grazers in Hong Kong's coastal seas is still very limited.

Hong Kong's coastal waters are traditionally divided into three hydrographic zones. Our analyses on environmental variables from both summer and winter reveal clear differences between semi-enclosed bays and other water bodies, but provide no justification for separating the transition and oceanic zones. The absence of clear spatial patterns in the abundance and pigment composition of the phytoplankton suggests that the spatial variations in phytoplankton communities do not reflect the delineation of hydrographic zones. There is also no evidence to suggest that phytoplankton

abundance and pigment composition are determined by nutrient availability.

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Correspondence should be addressed to:

C.K. Wong
School of Life Sciences, The Chinese University of Hong Kong,
NT, Hong Kong SAR, China
email: chongkimwong@cuhk.edu.hk