# Phytoplankton Pigments in Maryland Coastal Bay Sediments as Biomarkers of Sources of Organic Matter to Benthic Community

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# Phytoplankton Pigments in Maryland Coastal Bay Sediments as Biomarkers of Sources of Organic Matter to Benthic Community

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**ABSTRACT**

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In this study, the sources of pelagic organic matter and their transport to the surface sediment in the Maryland Coastal Bays (MCB), part of the U.S. mid-Atlantic coastal lagoon system, were examined. Photosynthetic pigments (chlorophylls *a* and *b*), accessory pigments (peridinin, fucoxanthin, zeaxanthin, alloxanthin, *etc.*), and chlorophyll *a* decomposition products (chlorophyllide *a*, pheophorbide *a*, pheophytin *a*, and pyropheophytin *a*) from surface sediments collected at 13 sites in the MCB in March 2013 were analyzed using high performance liquid chromatography. The spatial distributions of diagnostic pigments in surface sediments indicated that organic matter was mainly derived from nano- and picophytoplankton (*i.e.* cyanobacteria, cryptophytes, and chlorophytes) at sites characterized by high nutrient input, although diatoms dominated the standing phytoplankton biomass throughout the MCB except at one site. We attribute this phenomenon to selective microzooplankton grazing on nano- and picophytoplankton in the cold season and low grazing pressure on large phytoplankton species of diatoms and dinoflagellates resulting in the diatom bloom in early spring. At sites characterized by strong tidal current and mixing of bay waters and ocean waters, information from pigment data supports the deposition of dead/senescent diatoms. Results from this study indicate that nano- and picophytoplankton may play a crucial role in supplying organic matter to the benthic community, although their standing stock is low in the cold season.

**ADDITIONAL INDEX WORDS:** *Spring diatom bloom, chlorophyll* a *decomposition products*, *selective grazing*.

## INTRODUCTION

Coastal lagoons are among the most productive environments worldwide, representing important feeding and nursery grounds for invertebrates, fish, and birds (Vizzini and Mazzola, 2008). High primary production is supported by several factors, such as a large number of primary producers, high nutrient concentrations, retention, and recycling of both internal nutrients and those from adjacent habitats (terrestrial, wetland, and open sea). An important feature of the coastal marine ecosystem is the interaction between pelagic and benthic communities. Pelagic primary production may often be the major source of organic material to benthic communities (Bianchi, Findlay, and Dawson 1993; Nowicki and Nixon, 1985; Welschmeyer and Lorenzen, 1985).

The flux of organic matter from the water column to benthic sediment in shallow coastal lagoons is expected to be high due to enhanced primary productivity throughout the water column and high deposition rates of particulate matters in shallow coastal lagoons (Clavier, Chardy, and Chevillon, 1995). The mechanisms for such a flux include direct deposition of dead phytoplankton cells and fecal pellets from zooplankton grazing. Chlorophyll *a* (Chl-*a*) is commonly present in phytoplankton (Bidigare *et al*., 1986; Millie, Paerl, and Hurley, 1993), and its degradation products are diagnostic indicators of the physiological condition and grazing processes affecting phytoplankton (Lorenzen, 1967; Welschmeyer *et al*., 1984). The most important degradation products of Chl-*a* found in the marine environment are chlorophyllide *a* (Chlide-*a*), pheophorbide *a* (Phide-*a*), pheophytin *a* (Phtin-*a*), and pyropheophytin *a* (pPhtin-*a*). These derivatives are formed during the breakdown of Chl-*a* by herbivorous zooplankton and senescent algae cells (Bianchi and Findlay, 1991; Chen, Bianchi, and Bland, 2003; Louda *et al*., 1998; Louda *et al*., 2000; Roy, 1989; Welschmeyer *et al*., 1984).

Chl-*a* decomposition products have been used as biomarkers of organic matter derived from phytoplankton in the water column and surface benthic sediments in various environments, from oligotrophic open oceans to eutrophic inland lakes (Bianchi, Dibb, and Findlay, 1993; Bianchi, Findlay, and Dawson, 1993; Chen, Bianchi, and Bland, 2003; Mantoura and Llewellyn, 1983; Villanueva and Hastings, 2000). In shallow coastal lagoons, the application of Chl-*a* decomposition products as biomarkers of phytoplankton source material is complicated by the fact that there are multiple sources contributing to overall Chl-*a* and its decomposition products (*i.e.* terrestrial input, benthic micro- and macroalgae, and seagrasses). In addition, benthic grazing also contributes to the decomposition of Chl-*a* and further breakdown of its decomposition products deposited from the water column to benthic

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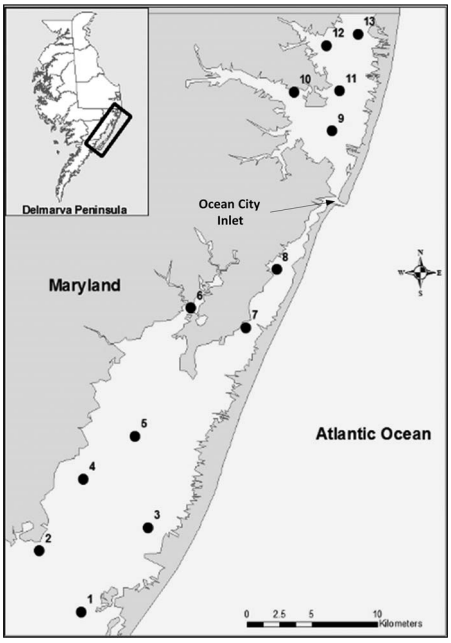


Figure 1. Map of sampling sites in the MCB. Sites 1–7 are located in the southern bays, while sites 8–13 are located in the northern bays. Mixing is greatest at sites 8 and 9, due to their proximity to the Ocean City inlet.

sediments (Cartaxana, Jesus, and Brotas, 2003). Furthermore, the mechanisms controlling transformation of Chl-*a* in aquatic environments are still a subject of controversy. While numerous studies have addressed this problem, many studies give contradicting results. For example, Phide-*a* has been considered a biomarker of zooplankton grazing (Bianchi, Findlay, and Dawson, 1993; Carpenter *et al*., 1988; Welschmeyer and Lorenzen 1985), while other studies show that this pigment is the main transformation product resulting from cell senescence of some diatom species (Head, Hurgruve, and Subba Rao, 1994).

Other factors contributing to variability in decomposition of Chl-*a* are the various types of phytoplankton and zooplankton grazing. Nano- and picophytoplankton cells sink very slowly when they die and are more likely to be recycled in the water column before reaching the bottom, whereas large phytoplankton cells sink faster. For example, diatoms have siliceous walls and thus sink very fast when they die. This is responsible for the large quantity of diatom deposits on the surface of sediments following a spring diatom bloom. Microzooplankton (20∼200 μm) are able to graze on small cells (<20 μm) such as pico- and nanoplankton, and they channel carbon from the smallest primary producers to the pelagic food web (Stelfox *et al*., 1999). Larger grazers (*e.g*., copepods) can only graze on large phytoplankton species such as diatoms and dinoflagellates (Sherr, Sherr, and Paffenhfer, 1986; Stelfox *et al*., 1999). In addition to the size, the sinking velocity of fecal material is also determined by the shape and density of the pellets (Fowler and Small, 1972). All these further complicate the application of Chl-*a* decomposition products as biomarkers of source organic matter transported from the water column to the benthic sediments.

In this study, we collected surface sediments following a spring diatom bloom at various sites in the Maryland Coastal Bays (MCB). Photosynthetic pigments and Chl-*a* decomposition products were analyzed using high performance liquid chromatography (HPLC) for each site. The main objective of this study was to examine the source(s) of organic matter deposited on surface sediments and the relative importance of grazing and senescence in the transportation of phytoplankton-derived material from the water column to the benthic sediments in a shallow coastal lagoon.

## METHODS

Water and surface sediment samples were collected at 13 sites in the MCB in March 2013. The study area and the procedures of sample collection, processing, and analysis are described below.

### Study Sites

The Maryland Coastal Bays, part of the mid-Atlantic coastal lagoon system in the United States, are characterized by shallow waters (<3 m) and relatively long water residence times (Wazniak, Wells, and Hall, 2004) due to limited exchange with the open Atlantic Ocean through small channels (Figure 1). Circulation within the bays is controlled by wind and tides. The river inputs are fairly low due to the area's flat landscape and sandy soils, and groundwater is a major pathway for the introduction of freshwater and nutrients to the bays (Wazniak, Wells, and Hall, 2004). Water and surface sediment samples were collected at 13 preselected sites with variable characteristics (Table 1). Site 8, which is near the Ocean City inlet, showed higher mixing of bay water and seawater than other sites, as indicated by high bottom stress and low clay/silt content in sediments (type I). Sites 4, 6, 10, 12, and 13 are located near stream/river mouths, where nutrient input is usually stronger, and sediments at these sites are muddier than at other sites (type III). Other sites (type II) are generally considered open bay sites with characteristics between type I and type III sites.

### Sample Collection/Processing

Approximately 1 L of water was collected from 1.0 m below the surface at each site using a peristaltic pump and was stored on ice in a cooler. In the laboratory, the water samples were filtered through a Whatman GF/F 47 mm glass fiber membrane (GE Healthcare Bio-Sciences, Pittsburgh, Pennsylvania, U.S.A.) with a Fisherbrand vacuum microfiltration system (Fisher Scientific, Pittsburgh, Pennsylvania, U.S.A.). The filtrates were used for nutrient analysis. The filters were then placed in 5–10 mL of 100% acetone in a 15 mL polypropylene centrifuge tube, sonicated, and extracted in darkness at 4°C overnight. The pigment extracts were then centrifuged and the supernatants collected and dried by exposing to a nitrogen stream. To redissolve the residual, 200 μL methanol/water (9:1 v/v) was added and 20 μL solution was injected to the HPLC.

Surface sediment samples were collected with a bottom grab sampler, stored in glass bottles, and stored on ice in a box. In the laboratory, about 100 g sediment were freeze dried, and approximately 1 g freeze-dried sediment was weighed into a 10

Table 1. *Salinity, mud content, sediment texture, and nutrients of the sampling sites.*

| Site | Salinity[a] | % Clay + Silt[b] | Site Characteristics | Nutrient Condition[c] | TDN (mg N/L) | DRP (mg P/L) | DSi (mg $SiO_2$/L) | Type[d] |
|---|---|---|---|---|---|---|---|---|
| 1 | 30.9 ± 4.2 | 5.7 | open bay | low | 0.25 | 0.08 | 0.05 | II |
| 2 | 30.1 ± 4.1 | 14.4 | open bay | low | 0.21 | 0.06 | 0.08 | II |
| 3 | 30.5 ± 4.1 | 6.3 | open bay | low | 0.24 | 0.08 | 0.05 | II |
| 4 | 30.2 ± 4.1 | 37.1 | open bay | low | 0.19 | 0.08 | 0.06 | III |
| 5 | 30.0 ± 3.7 | 2.5 | open bay | low | 0.22 | 0.07 | 0.10 | II |
| 6 | 25.7 ± 3.8 | 23.7 | river mouth | high | 0.27 | 0.14 | 0.57 | III |
| 7 | 31.2 ± 4.4 | 1.9 | narrow bay | low | 0.15 | 0.12 | 0.12 | I |
| 8 | 32.6 ± 3.2 | 0.2 | narrow bay/ near inlet | low | 0.18 | 0.06 | 0.10 | I |
| 9 | 32.1 ± 2.8 | 20.0 | open bay | low | 0.18 | 0.05 | 0.23 | II |
| 10 | 29.0 ± 3.1 | 34.2 | near shore/ river mouth | high | 0.70 | 0.10 | 1.03 | III |
| 11 | 30.2 ± 3.6 | 18.2 | open bay | low | 0.18 | 0.05 | 0.20 | II |
| 12 | 28.1 ± 3.7 | 48.3 | river mouth | high | 0.28 | 0.06 | 0.13 | III |
| 13 | 27.5 ± 4.1 | 23.8 | river mouth | high | 0.32 | 0.06 | 0.08 | III |

[a] Average of salinities measured from June 2011 to July 2013.
[b] Sediment size distribution of dried sediment was measured with a set of six sieves; the mud (silt + clay) content is the fraction which passes the 0.039 mm sieve.
[c] Nutrient condition refers to nutrient input from stream.
[d] Type 1 = Low clay/silt content and high bottom stress, Type 2 = Medium clay/silt content, Type 3 = High clay/silt content.

mL test tube. Pigments were extracted with 5 mL acetone, mixed thoroughly with a vortex mixer, sonicated, and allowed to stand in the dark at 4°C overnight. Test tubes were placed in a centrifuge at 2500 rpm for 5 minutes, and 3 mL extract was filtered with a syringe filter (0.2 μm). The extract was then dried with a nitrogen stream. To the residual, 200 μL methanol/water (9:1 v/v) was added and 20 μL solution was injected into HPLC.

### Nutrient Analysis

Total dissolved nitrogen (TDN) in the filtrate was analyzed by high-temperature combustion method using a TNM-1 total nitrogen analyzer module (Shimadzu Scientific Instruments, Columbia, Maryland, U.S.A.) coupled to a TOC-$V_{CSH}$ total organic carbon analyzer (Schimadzu Scientific Instruments) (Álvarez-Salgado and Miller, 1998). Standard calibration for TDN was achieved using potassium nitrate. A DR4000 spectrophotometer (Hach Company, Loveland, Colorado, U.S.A.) was used to measure dissolved reactive phosphorus (DRP; Hach method 8048) and dissolved silica (DSi, Hach method 8185). TDN was chosen to represent the availability of nitrogen nutrients since concentration of nitrates is usually very low year-round and represents only a minor contribution to the total dissolved nitrogen pool (Glibert *et al*., 2014).

### HPLC Analysis of Pigments

The separation of pigments was conducted on an 1100 Series model G1322A HPLC with a binary pump (Agilent Technologies, Santa Clara, California, U.S.A.) following the method described in Zapata, Rodríguez, and Garrido (2000). The two eluents used were methanol/acetonitrile/0.25 M aqueous pyridine (50:25:25 v/v/v) and acetonitrile/acetone (80:20 v/v). All organic solvents were HPLC grade and prepared as described in Zapata, Rodríguez, and Garrido (2000). Analytical separations were performed using a Symmetry $C_8$ column (150 × 4.6 mm, 3.5 μm particle size, 100 Å pore size; Waters Corporation, Milford, Massachusetts, U.S.A.). The gradient was a version modified from Zapata, Rodríguez, and Garrido (2000) that achieves partial separation between zeaxanthin (Zeax) and lutein (Lut).

Briefly, after injection of 20 μL of sample, a gradient program began with 100% A, which ramped to 40% B in 18 minutes, then ramped to 100% B in 4 minutes, remained isocratic for 13 minutes, and then returned to 100% A in 3 minutes. The flow rate remained constant at 1 mL/min. An Agilent model G1316A diode array detector (DAD, Agilent Technologies) and model G1321A fluorescence detector (FLD, Agilent Technologies) were used to detect pigments. Chromatograms at 432 nm and 666 nm were recorded for quantitative analysis of carotenoids (Lut, Zeax, *etc*.) and pheopigments (Phide-*a*, Phtin-*a*, and pPhtin-*a*), respectively. Chromatograms from FLD (excitation = 440 nm, emission = 660 nm) were used for quantification of Chl-*a* and chlorophyll *b* (Chl-*b*). ultraviolet/visible spectra from 350 to 700 nm were also recorded by DAD for qualitative analysis. Pigments were identified based on the retention time and ultraviolet/visible spectra characteristics (Table 2). Chl-*a*, Chl-*b*, and β-carotene (β-Car) standards were purchased from Sigma-Aldrich (St. Louis, Missouri, U.S.A.), while other carotenoids were purchased from DHI (Hrsholm, Denmark). The Phtin-*a* standard was prepared by acidifying the Chl-*a* standard. The responding factor of Phtin-*a* was used for quantification of other pheopigments (Phide-*a* and pPhtin-*a*).

## RESULTS

In this section we exhibit relevant data obtained from pigment analysis, describing the community characteristics, spatial variation of Chl-*a*, accessory pigments, and Chl-*a* decomposition products in both the water column and surface sediments of the MCB.

### Phytoplankton Community Characteristics in the MCB

In March 2013, fucoxanthin (Fuco) was the dominant accessory pigment at all sites except site 10, where peridinin (Peri, 3.90 nMol/L) was higher than Fuco (Figure 2). The highest concentration of Fuco (7.49 nMol/L) was detected at site 6, where relatively high concentrations of TDN, DRP, and DSi were observed. The unusual nutrient condition at site 10, with

Table 2. *Spectral and chromatographic information of chlorophylls, accessory pigments, and Chl-*a *decomposition products.*

| No. | Pigment | Abbreviation | Retention Time (min) | $\lambda_{max}$ (nm) | Marker for |
|---|---|---|---|---|---|
| 1 | peridinin | Peri | 12.2 | 474 | dinoflagellates |
| 2 | chlorophyllide *a* | Chlide-*a* | 12.5 | 432, 663 | grazing |
| 3 | chlorophyllide *a* epimer | Chlide-*a*′ | 12.8 | 432, 663 | grazing |
| 4 | pheophorbide *a* | Phide-*a* | 14.6 | 410, 665 | grazing |
| 5 | pheophorbide *a* epimer | Phide-*a*′ | 14.9 | 410, 665 | grazing |
| 6 | fucoxanthin | Fuco | 16.3 | 450, 471 | diatoms |
| 7 | alloxanthin | Allox | 21.7 | 454, 483 | cryptophytes |
| 8 | zeaxanthin | Zeax | 22.6 | 454, 481 | cyanobacteria |
| 9 | lutein | Lut | 22.8 | 448, 476 | chlorophytes |
| 10 | chlorophyll *b* | Chl-*b* | 25.9 | 456, 645 | chlorophytes |
| 11 | chlorophyll *a* allomer | O-Chl-*a* | 26.8 | 431, 663 | not specific |
| 12 | chlorophyll *a* | Chl-*a* | 27.2 | 431, 663 | not specific |
| 13 | chlorophyll *a* epimer | Chl-*a*′ | 27.7 | 431, 663 | not specific |
| 14 | pheophytin *a* | Phtin-*a* | 29.2 | 411, 665 | senescence/grazing |
| 15 | pheophytin *a* epimer | Phtin-*a*′ | 29.5 | 411, 665 | senescence/grazing |
| 16 | β-carotene | β-Car | 30.2 | 454, 481 | not specific |
| 17 | pyropheophytin *a* | pPhtin-*a* | 30.5 | 410, 666 | grazing |

the highest TDN and DSi among all sites (Table 1), may explain the high abundance of dinoflagellates, indicated by high concentration of Peri at this nearshore site. Excluding sites 6 and 10, Chl-*a* had a moderate negative relationship with both TDN ($R = -0.64$, $p < 0.05$) and DRP ($R = -0.73$, $p < 0.05$) and a positive relationship with DSi ($R = 0.50$, $p < 0.05$), indicating that the primary production was likely limited by DSi throughout the bay except at sites where nutrients were supplied by streams.

The average concentration of Fuco (2.32 ±0.27 nMol/L) was higher than the other diagnostic pigments by approximately one order of magnitude. In addition, the average Fuco concentration in March 2013 was highest among all sampling periods since 2012, when we started monitoring the photosynthetic pigments in the MCB; this is consistent with a moderate spring diatom bloom. Another diagnostic carotenoid detected in water samples at all sites was alloxanthin (Allox), a biomarker for cryptophytes; at most sites, Allox was the second-highest accessory pigment (Figure 2). Peri, Zeax, Lut, and Chl-*b* were only detected in the water column at a few sites (6, 10, 12, 13) where higher nutrients from riverine inputs were expected (Glibert *et al.*, 2014).

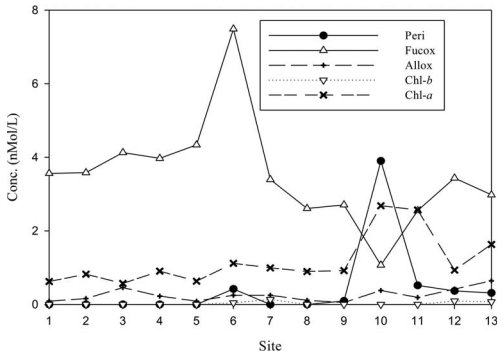


Figure 2. Photosynthetic pigments in the water column in March 2013 showing spatial variations of Chl-*a* (sum of normal and epimer of Chl-*a*) and major accessary pigments.

## Spatial Variation of Chl-*a* and Accessory Pigments in Surface Sediments

Strong spatial variation in Chl-*a* and accessory pigments was observed in surface sediments (Figure 3). The average concentration of fresh Chl-*a* (0.065 ±0.043 nMol/g) in sediments is generally low throughout the coastal bays. The highest concentration of Chl-*a* was observed at site 10 (0.12 nMol/g) followed by Sites 2, 7, 8, and 9 (Figure 4). Fuco, Zeax, Lut, Allox, Peri, and Chl-*b* were detected in surface sediment at all sites, and prasinoxanthin (Pras) was detected at most sites. Neoxanthin, violaxanthin, 19′-butanoyloxy-fucoxanthin, and 19′-hexanoyloxy-fucoxanthin were only detected at a few sites, and their concentrations were also much lower than the other pigments. Average concentration of Zeax (0.13 ±0.15 nMol/g) was the highest among accessory pigments, followed by Fuco (0.080 ±0.062 nMol/g), Lut (0.045 ±0.048 nMol/g), Allox (0.044 ±0.054 nMol/g), Peri (0.027 ±0.018 nMol/g), β-Car (0.023 ±0.027 nMol/g), and Pras (0.017 ±0.022 nMol/g). This suggested that the main sources of phytoplankton-derived organic matter included cyanobacteria, diatoms, cryptophytes, dinoflagellates, and prasinophytes. However, caution must be used when interpreting photosynthetic pigments as biomarkers of sources of phytoplanktonic material, since stability

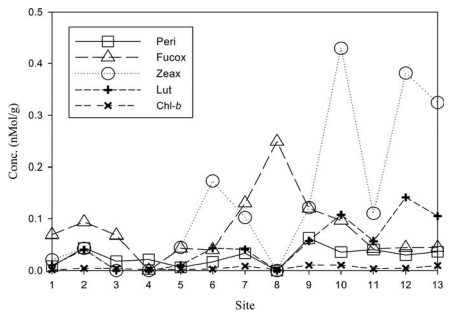


Figure 3. Spatial distribution of major accessory pigments in surface sediments in the MCB. Pigment concentrations were higher in the northern bays than in the southern bays due to higher nutrient input from the surrounding creeks.

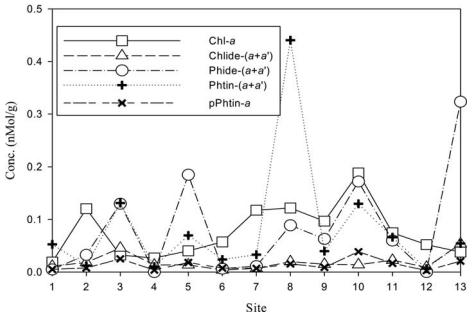


Figure 4. Spatial distribution of Chl-*a* and its decomposition products in surface sediments of the MCB, indicative of the source of organic matter either through senescence or zooplankton grazing. Chl-*a*, Chlide-*a*, Phide-,*a* and Phtin-*a* in this figure represent the sum of normal chloropigments and their epimers, respectively.

varies substantially among pigments. For example, Fuco is much more labile than Zeax (Abele-Oeschger, 1991; Burford, Long, and Rothlisberg, 1994; Louda *et al.*, 2004; Repeta and Gagosian, 1982). Therefore, the abundance of accessory pigment in sediments cannot be directly used to evaluate the relative magnitude of contribution of phytoplankton groups represented by these pigments in sediments.

The spatial distributions were very similar for most accessory pigments, with the exception of Fuco and Peri. For other accessory pigments, relatively high concentrations were observed in the northern bays (sites 10, 12, and 13). The similar spatial distribution for most accessory pigments except Fuco and Peri was also evidenced by the strong linear relationships between Allox, Zeax, Lut, and β-Car (Table 3), indicating that cyanobacteria, cryptophytes, and some chlorophytes shared similar niches where nutrient inputs were usually high. Spatial variation of Peri was not as remarkable as the other accessory pigments, indicating that dinoflagellates were more evenly distributed throughout the bays.

Spatial variation of Fuco in sediments seems to follow an opposite trend when compared with other accessory pigments. The highest concentration of Fuco was observed at site 8 (0.25 nMol/g), where other accessory pigments were very low or not detected. Relatively high concentrations of Fuco were also detected at sites 7 and 9, the two sites closest to site 8. This distribution is different from the water column Fuco distribution, which actually peaked at site 6 and remained relatively high throughout the bays (Figure 3). We speculate that the high Fuco concentration in surface sediment at site 8 might be caused by sinking of diatoms at locations with relatively stronger mixing of bay water and seawater. In addition, the bottom stress at site 8 was also the highest, as indicated by very low clay/silt contents (Table 1). High bottom stress may also prevent deposition and burial of organic matter derived from other phytoplankton groups due to lack of silicate shells such as diatoms.

## Spatial Variation of Chl-*a* Decomposition Products in Surface Sediments

The major Chl-*a* decomposition products in surface sediments are Phtin-*a* (mean 0.083 ±0.114 nMol/g) and Phide-*a* (mean 0.083 ±0.097 nMol/g) at all sites (Figure 4). There were strong spatial variations in Chl-*a* decomposition products. Phtin-*a* showed a very different pattern than Chlide-*a*, Phide-*a*, and pPhtin-*a*. The highest concentration of Phtin-*a* was observed at site 8 (0.44 nMol/g), where the highest concentration of Fuco was observed. The highest concentration of Phide-*a* (0.17 nMol/g) was observed at site 13. Chlide-*a* and pPhtin-*a* generally followed the pattern of Phide-*a*, and this is further supported by regression analysis (Table 3). In this table, the concentrations of Chlide-*a*, Phide-*a*, and Phtin-*a* include their respective epimers.

## Statistical Analysis

Pearson correlation analysis was conducted for sedimentary photosynthetic pigments and Chl-*a* decomposition products with SPSS Statistics 20 (IBM Corporation, Armonk, New York, U.S.A.) to provide information on sources of phytoplankton-derived organic matter in benthic sediments and Chl-*a* decomposition pathways in the MCB (Table 3). We observed strong correlations between Fuco and Phtin-*a* ($R = 0.81$, $p < 0.05$), some pheopigments (Chlide-*a*, Phide-*a* and pPhtin-*a*), and also some carotenoids (Allox, Zeax, Lut, and β-Car).

Table 3. *Correlations (*R*) between pigments (n = 13) from Pearson correlation analysis. Significant relationships (*p < 0.05*) are given in bold. Abbreviations of pigments are listed in Table 2.*

| Pigment | Per | Chlide-*a* | Chlide-*a*′ | Phide-*a* | Phide-*a*′ | Fuco | Allox | Zeax | Lut | Chl-*b* | O-Chl-*a* | Chl-*a* | Chl-*a*′ | Phtin-*a* | Phtin-*a*′ | β-Car |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chlide-*a* | −0.02 | | **0.93** | **0.79** | **0.61** | 0.03 | −0.08 | 0.09 | 0.09 | 0.05 | −0.30 | 0.20 | −0.14 | 0.30 | 0.17 | −0.16 |
| Chlide-*a*′ | 0.14 | **0.93** | | **0.71** | **0.65** | −0.13 | −0.17 | 0.01 | 0.01 | 0.08 | −0.25 | −0.26 | −0.15 | 0.08 | 0.12 | v0.21 |
| Phide-*a* | −0.02 | **0.79** | **0.71** | | **0.89** | 0.04 | 0.19 | 0.34 | 0.25 | −0.06 | −0.10 | 0.05 | 0.18 | 0.28 | 0.23 | 0.08 |
| Phide-*a*′ | 0.16 | **0.61** | **0.65** | **0.89** | | −0.18 | 0.24 | 0.36 | 0.25 | −0.12 | 0.03 | 0.00 | 0.26 | −0.07 | 0.07 | 0.16 |
| Fuco | −0.11 | 0.03 | −0.13 | 0.04 | −0.18 | | −0.11 | −0.18 | −0.20 | −0.18 | 0.15 | **0.68** | −0.03 | **0.81** | 0.33 | −0.15 |
| Allox | 0.21 | −-0.08 | −0.17 | 0.19 | 0.24 | −0.11 | | **0.94** | **0.87** | −0.25 | 0.03 | 0.32 | **0.71** | −0.21 | −0.01 | **0.92** |
| Zeax | 0.35 | 0.09 | 0.01 | 0.34 | 0.36 | −0.18 | **0.94** | | **0.96** | −0.04 | −0.13 | 0.30 | **0.69** | −0.22 | 0.03 | **0.91** |
| Lut | 0.44 | 0.09 | 0.01 | 0.25 | 0.25 | −0.20 | **0.87** | **0.96** | | 0.08 | −0.11 | 0.19 | **0.61** | −0.28 | −0.06 | **0.85** |
| Chl-*b* | 0.23 | 0.05 | 0.08 | −0.06 | −0.12 | −0.18 | −0.25 | −0.04 | 0.08 | | −0.12 | v0.03 | 0.24 | −0.09 | 0.38 | 0.10 |
| O-Chl-*a* | −0.05 | −0.30 | −0.25 | −0.10 | 0.03 | 0.15 | 0.03 | −0.13 | −0.11 | −0.12 | | 0.09 | 0.16 | −0.10 | −0.39 | −0.03 |
| Chl-*a* | 0.30 | −0.20 | −0.26 | 0.05 | 0.00 | **0.68** | 0.32 | 0.30 | 0.19 | −0.03 | 0.09 | | **0.50** | 0.44 | 0.41 | 0.40 |
| Chl-*a* | 0.21 | −0.14 | −0.15 | 0.18 | 0.26 | −0.03 | **0.71** | **0.69** | **0.61** | 0.24 | 0.16 | **0.50** | | −0.11 | 0.42 | **0.85** |
| Phtin-*a* | −0.49 | 0.30 | 0.08 | 0.28 | −0.07 | **0.81** | −0.21 | −0.22 | −0.28 | −0.09 | −0.10 | 0.44 | −0.11 | | 0.45 | −0.23 |
| Phtin-*a*′ | −0.17 | 0.17 | 0.12 | 0.23 | 0.07 | 0.33 | −0.01 | 0.03 | −0.06 | 0.38 | −0.39 | 0.41 | 0.42 | 0.45 | | 0.18 |
| β-Car | 0.27 | −0.16 | −0.21 | 0.08 | 0.16 | −0.15 | **0.92** | **0.91** | **0.85** | 0.10 | −0.03 | 0.40 | **0.85** | −0.23 | 0.18 | |
| pPhtin-*a* | 0.00 | 0.44 | 0.47 | **0.72** | **0.68** | 0.12 | 0.30 | 0.37 | 0.20 | 0.09 | −0.12 | 0.44 | **0.56** | 0.31 | **0.64** | 0.36 |

## DISCUSSION

In this section, we describe in detail the source of photosynthetic pigments, selective zooplankton grazing, and the pathway of Chl-*a* decomposition in surface sediments of the MCB.

### Sources of Photosynthetic Pigments in Surface Sediments

Photosynthetic pigments in MCB waters have been monitored monthly since February 2012. No strong spring blooms have been observed. In both 2012 and 2013, average Chl-*a* concentrations increased moderately from February (<1 nMol/L) to March (1–2 nMol/L) and quickly decreased to very low levels (<0.2 nMol/L; O. Oseji, unpublished data) in April. The highest average Chl-*a* concentration, which may reach >5 nMol/L at certain sites in some years, usually occurs in June or July and is caused by a "brown tide" (Trice *et al*., 2004). Diatoms are dominant species year-round, and their dominance is more remarkable in winter and early spring. Chl-*a* concentration remains relatively high in summer and decreases gradually in fall and winter.

The main pathway for pelagic diatoms to reach surface sediments is through zooplankton grazing and direct deposition of dead/senescent diatom cells (Itoh *et al*., 2007; Roy, 1989). There is strong evidence that diatoms were deposited directly at site 8 near the Ocean City inlet, where stronger mixing of coastal bay water with seawater occurred. First, Phtin-*a* and Fuco dominated Chl-*a* decomposition products and accessory pigments at site 8, indicating that the most likely source of Phtin-*a* at this site was the deposition of dead/senescent diatoms. This trend is further evidenced by the strong linear relationship between Fuco and Phtin-*a* ($r^2 = 0.81$, $p < 0.01$; Table 3). The concentrations of other accessory pigments in surface sediment were very low at site 8, probably due to low grazing activity or high bottom stress, which prevents the deposition and burial of organic matter derived from phytoplankton. Furthermore, correlation analyses also suggested that the main source of Chlide-*a*, Phide-*a*, and pPhtin-*a* was similar—most likely grazing, as indicated by strong linear relationships between these biomarkers (Table 3). However, no accessory pigments showed any clear linear relationship with Chlide-*a*, Phide-*a*, and pPhtin-*a*, likely due to spatial variation in the composition of phytoplankton.

Another source of photosynthetic pigments and decomposition products is the phytobenthos. Wazniak (2004) measured Chl-*a* of viable algal cells in surface sediments and found that its concentration varied remarkably (from 8.5 to 259 mg/m$^2$). Wazniak (2004) suggested that the phytobenthos may represent an important primary producer within the system. The pigments in our samples included both pelagic and benthic sources, and the benthic contribution to photosynthetic pigments may vary significantly. For example, at site 8, a remarkably high concentration of Fuco coincided with the highest concentration of Phtin-*a* among all sites. Chl-*a* was not higher at site 8 than the adjacent sites (7 and 9), indicating the pigments at this site were mainly derived from deposition of senescence/death of pelagic diatoms. In contrast, at the four sites located near the river mouths with high concentrations of nutrients and finer sediments (sites 6, 10, 12, and 13), Zeax concentrations were overwhelmingly higher than other carotenoids, while Zeax concentration in the water column was very low, suggesting that benthic cyanobacteria may contribute remarkably to the overall pigment budget (Chl-*a* and Zeax). However, the relatively low concentration of fresh Chl-*a* and high concentrations of Chl-*a* decomposition products at these sites indicated that viable phytobenthos was not likely the major source of photosynthetic pigments.

### Selective Grazing on Phytoplankton in the Water Column

Selective grazing of zooplankton on phytoplankton has been observed in many previous studies (Burkill *et al*., 1987; Granéli and Turner, 2002; Huang *et al*., 2008; Liu *et al*., 2010; Stoecker, Guillard, and Kavee, 1981). A recent study on the grazing impact of microzooplankton on phytoplankton in Xiamen Bay, China, revealed that microzooplankton preferentially grazed on nano- and picophytoplankton and avoided harvesting large phytoplankton species such as diatoms in the cold season (Huang *et al*., 2008). The peak grazing pressures for dinoflagellates and chlorophytes occurred in early spring, and for cryptophytes and cyanobacteria in winter (December). In contrast, peak grazing pressures for diatoms occurred in summer, presumably due to the larger zooplankton species such as copepods. If the seasonal variations of zooplankton grazing in the MCB follow the same pattern, the relatively low standing biomass of nano- and picophytoplankton (prasinophyte, chlorophyte, cyanobacteria, and cryptophyte) in winter can be interpreted as preferential grazing by microzooplankton, and the remarkable dominance of standing biomass of diatoms in the cold season can be attributed to the lack of grazing pressure.

High concentrations of Zeax, Lut, and Allox coupled with high concentrations of Chlide-*a*, Phide-*a*, and pPhtin-*a* in sediments at sites 6, 10, 12, and 13 with high nutrients in the water column are consistent with selective grazing at these sites. The strong linear relationships among Zeax, Allox, and Lut, and the linear relationships among Chlide-*a*, Phide-*a*, and pPhtin-*a* further support this view (Table 3), indicating that the main source of phytoplankton-derived organic matter at these sites were from fecal materials from microzooplankton grazing. Phytobenthos may contribute to the total chlorophyll and carotenoids, and benthic grazers and suspension feeders may contribute to the production of Chl-*a* decomposition products. However, since the diets of benthic grazers and suspension feeders are not selective or are less selective compared with zooplankton/protozoa, the inference about selective grazing based on the pigment compositions in the water column and sediments holds true. In addition, the low temperature during the sampling period may subdue the feeding activities of benthic grazers and suspension feeders.

### Chl-*a* Decomposition Pathway

The results from this study also shed light on the Chl-*a* decomposition pathway in the MCB. Two main pathways can be proposed based on the current results. The presence of maximum concentrations of both Fuco and Phtin-*a* at site 8 and the strong linear relationship between Fuco and Phtin-*a* support the production of Phtin-*a* through the senescence of diatoms at that site. The low concentrations of Chlide-*a* and

pPhtin-*a*, and the lack of linear relationship between dephytylized derivatives (Chlide-*a* and Phide-*a*) and both Phtin-*a* and Fuco, indicate that the autolytic decomposition of Chl-*a* in the dead/senescent diatoms catalyzed by the enzyme chlorophyllase (Jeffrey, 1974; Jeffrey and Hallegraeff, 1987) was, at least, not significant at this site. However, this does not rule out the production of Phide-*a* in dead/senescent cells through enzymatic dephytylization at this site. As a matter of fact, a moderately high concentration of Phide-*a* (0.09 nMol/L), lower only than Phtin-*a* (0.44 nMol/L), was observed at site 8 (Figure 4), indicating that production of Phide-*a* through enzymatic autolytic decomposition of Phtin-*a* or demetalization of Chlide-*a* is also an important pathway in cell senescence.

Furthermore, strong evidences support another decomposition pathway of Chl-*a* leading to production of Chlide-*a*, Phide-*a*, and pPhtin-*a* through microphytoplankton grazing on nano- and picophytoplankton and feeding of benthic grazers and suspension feeders. (1) High concentrations of Chlide-*a*, Phide-*a*, and pPhtin-*a* occurred at sites with high abundance of nano- and picophytoplankton due to high nutrient concentrations. As mentioned earlier, high concentrations of Allox, Zeax, Lut, and β-Car in surface sediments at the same sites were most likely caused by selective grazing of nano- and picophytoplankton. (2) A strong linear relationship between Chlide-*a* and Phide-*a* and between Phide-*a* and pPhtin-*a* indicated that they were derived from the same processes, most likely microzooplankton grazing on nano- and picophytoplankton and benthic grazing and suspension filter feeding. (3) The lack of linear relationship between Phtin-*a* and Chlide-*a*/Phide-*a*/pPhtin-*a* indicated that Chlide-*a*/Phide-*a* /pPhtin-*a* were produced through a distinctly different pathway than Phtin-*a*. The presence of significant amounts of Phide-*a* is usually considered an indication of a high contribution of zooplankton fecal pellets in sinking particles (Lorenzen, 1967). The high abundance of Phide-*a* (comparable to Phtin-*a*) in this study is consistent with the selective grazing of nano- and picophytoplankton species mentioned earlier. Therefore, the dominant decomposition pathway of Chl-*a* at most sites (*e.g.*, sites 6, 10, 12, and 13) was the formation of Chlide-*a*, Phide-*a*, and pPhtin-*a* through microzooplankton grazing on nano- and picophytoplankton species and feeding of benthic grazers and suspension feeders.

## CONCLUSIONS

In this study, we examined accessory pigments and Chl-*a* decomposition products in surface sediments at various locations in the MCB in March 2013. At sites characterized by strong mixing of coastal bay water and seawater, deposition of dead/senescent diatoms dominated transport of organic matter from the water column to surface sediments; in contrast, at sites characterized by high nutrient input, deposition of organic matter derived from selective grazing of microzooplankton on nano- and picophytoplankton species (cyanobacteria, cryptophytes, prasinophytes, and chlorophytes) dominated. Lack of grazing pressure may also explain the relatively high standing stock of large phytoplankton species (diatoms and dinoflagellates) in the cold season in the MCB.

There were two dominant pathways of Chl-*a* decomposition. One was the transformation of Chl-*a* to Phtin-*a* and formation of Phide-*a* through chlorophyllase-catalyzed autolytic decomposition of Chl-*a* during diatom senescence, a process limited to sites with strong mixing of bay water and seawater. Another pathway is formation of Chlide-*a*, Phide-*a*, and pPhtin-*a* through selective grazing of microzooplankton on nano- and picophytoplankton and/or benthic grazing and suspension filter feeding.

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