Effect of accessory pigment composition on the absorption characteristics of a dinoflagellate bloom in a coastal embayment

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Abstract

The light absorption properties of a dinoflagellate (*Noctiluca scintillans* Macartney) bloom in Manila Bay were analysed during the onset of a whole-bay-scale bloom in March 2004. The chlorophyll *a* concentrations varied over a very wide range from 1.4 μg l^{-1} to extremely high values of 521 μg l^{-1}. The chlorophyll specific absorption coefficients of phytoplankton ($a_{ph}^*(\lambda)$) varied significantly in shape and magnitude. The spectrally averaged values of $a_{ph}^*(\lambda)$ varied by two orders of magnitude

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within and outside the bloom patch. The total suspended solid concentration was high in the middle of the bay (≥ 4 mg l^{-1}). The non-photosynthetic pigment (NPP) index was ~ 0.6 at most of the stations, mainly due to the presence of photoprotective pigments like zeaxanthin, lutein and neoxanthin, which led to variations in the blue absorption maxima of the chlorophyll-specific absorption coefficients. The absorption properties of the accessory pigments were masked owing to the presence of overlapping pigment absorption bands. The fourth derivative of the absorption spectra was able to resolve these overlapping features and enhance the absorption characteristics of prominent accessory pigments.

1. Introduction

Coastal oceanic environments are sites of dynamic physical and biogeochemical processes. Over the last few decades, eutrophication-related algal bloom events have been on the rise in coastal areas. Such events alter the colour of the water as a result of the transient proliferation of phytoplankton. The absorption of light by phytoplankton is a major factor contributing to the optical variability of waters both in coastal regions and the open ocean. The shape and magnitude of the phytoplankton absorption spectrum reflect the pigment composition and its concentration in the water. Factors contributing to the variability in $a_{ph}^*(\lambda)$ include pigment packaging (Duysens 1956) and concentrations of non-photosynthetic pigments (Allali 1997, Vijayan et al. 2009). The latter contribute significantly to absorption in the 460–640 nm region of the photosynthetically active radiation (Bidigare 1989b), particularly in coastal waters (Bricaud et al. 1995, Cleveland 1995).

The study area, Manila Bay, is a highly eutrophic coastal water body located between latitudes 14°23′–14°87′N and longitudes 120°53′–121°03′E and is reported to be a pollution hot spot in East Asia (Maria et al. 2009). There have been many reports of the repeated occurrence of algal bloom events caused by *Pyrodinium* in the 1980s and 1990s (Gonzales 1989, Furio & Gonzales 2002); more recently, the blooming species changed to green *Noctiluca* (Furuya et al. 2006). The bay is subject to multifarious biogeophysical conditions, which have created a complex bio-optical environment within the bay. Most of the studies conducted in Manila Bay have focused on the physico-chemical parameters (Prudente et al. 1994, Velasquez & Jacinto 1995, Velasquez et al. 1997, Jacinto et al. 2011) and taxonomic aspects of phytoplankton (Azanza & Miranda 2001, Siringan et al. 2008), algal photophysiology (Hansen et al. 2004), modelling the physical characteristics of the environment (De las Alas & Sodusta 1985, Fuji-ie et al. 2002), heavy metal pollution (Hosono et al. 2010 and references therein) and the bloom dynamics of *Pyrodinium* (Villanoy et al. 1996, 2006). The bio-optical properties of seawater in Manila Bay are poorly documented. In an attempt to redress the paucity of bio-optical data and

to elucidate the mechanism of large-scale blooms of green *Noctiluca*, a survey was conducted in 2004 during which a whole-bay-scale bloom was observed. The environmental conditions, pigment characteristics, growth activity etc., relating to the bloom are described in detail elsewhere (Furuya et al. 2006).

The primary objective of this work is to describe the phytoplankton-specific absorption characteristics of the bay during the bloom. Secondly, an attempt is made to identify the pigments responsible for the major absorption peaks by resolving the overlapping features in the absorption spectra through derivative analysis.

2. Material and methods

Samples were collected during fieldwork carried out in Manila Bay from 19 to 23 March 2004. The stations were distributed along two transects: an east-west (EW) transect between Manila and Limay (stn. MB7–13) and a north-south (NS) transect from the mouth of the bay to Pampanga (stn. MB1–5, MB10 & 11) (Figure 1). Physical parameters like temperature,
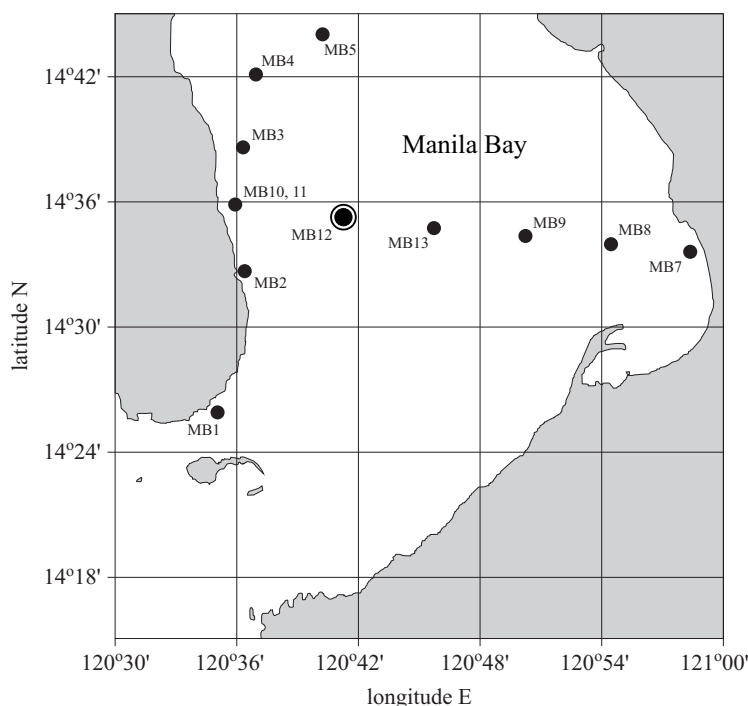

Figure 1. Map of Manila Bay showing the sampling locations. The stations are distributed along two transects – an east-west (EW) transect from Limay to Manila and a north-south (NS) transect from the mouth of the bay to Pampanga

salinity and conductivity were obtained using a portable CTD profiler. Samples for phytoplankton composition based on HPLC and phytoplankton spectral absorption were collected from different depths down to 23 m using a Nansen sampler; surface (~ 5 cm) sampling was done using a bucket.

2.1. Spectral absorption coefficient of phytoplankton

Seawater samples (0.5–1 litres) were filtered onto 25 mm GF/F glass fibre filters under low vacuum pressure (< 25 hPa). The absorption spectra of total particulate matter was recorded in the wavelength range 350–750 nm at a resolution of 1 nm with a double-beam spectrophotometer (Shimadsu MPS-2400) following the guidelines of Mitchell (1990). For each of the measured spectra, the optical density obtained at 750 nm was subtracted from all other wavelengths. The optical density of the total suspended matter was corrected for the path length amplification (β effect) according to Cleveland & Weidemann (1993). The optical density of detritus particles was measured following the pigment extraction method of Kishino et al. (1985). The chlorophyll-specific absorption coefficients of phytoplankton ($a_{ph}^*(\lambda)$) were obtained by dividing the absorption coefficient of phytoplankton ($a_{ph}(\lambda)$) by the total Chl a (TChl a) concentration. TChl a and TChl b includes both mono and divinyl forms.

2.2. Phytoplankton pigment composition by high performance liquid chromatography (HPLC)

Biomarker pigments were separated and quantified using reverse-phase gradient elution HPLC following Zapata et al. (2000). Seawater was filtered under a gentle vacuum (< 100 mm Hg) onto 25 mm glass fibre filters (Whatman GF/F) and stored immediately in liquid nitrogen. Pigments were extracted using methanol (95%), and the extract was mixed with 1 M ammonium acetate as the ion pairing reagent. It was then filtered through 0.2 μm PTFE filter (Whatman) and mixed with milli-Q water (5:1 v:v); thereafter 500 μl was injected into the HPLC system (Shimadzu) equipped with a Symmetry C8 column (Waters). The peaks of the following pigments were identified based on their retention time compared with those of pure standards: chlorophyll a, chlorophyll b including their divinyl forms; peridinin, 19-butanoyloxyfucoxanthin, fucoxanthin, neoxanthin, prasinoxanthin, violaxanthin, 19-hexanoyloxyfucoxanthin, diadinoxanthin, antheraxanthin, alloxanthin, diatoxanthin, zeaxanthin, lutein and β-carotene.

The NPP index was calculated as the weight:weight ratio of non-photosynthetic pigments, i.e. zeaxanthin, diatoxanthin, diadinoxanthin and

β-carotene, to total pigment concentration, i.e. photosynthetic and non-photosynthetic carotenoids and chlorophylls, following Babin et al. 1996.

2.3. Derivative analysis

The derivative analysis was carried out using Microcal Origin 8.0 Scientific Analysis Software. To calculate the fourth derivative of the $a_{ph}^*(\lambda)$ curves, 41 point fourth degree polynomial smoothing was applied, followed by differentiation using the Savitzky-Golay method (Savitzky & Golay 1964). The polynomial smoothing was applied to reduce the effects of high frequency noise in the spectra (Gómez et al. 2001).

The first and the n-th derivative are obtained using eqations (1) and (2) respectively

$$\frac{d_s}{d_{\lambda_i}} \approx \frac{s(\lambda_i) - s(\lambda_i)}{\Delta\lambda}, \tag{1}$$

$$\frac{d^n s}{d\lambda_j^n} \approx \frac{d}{d\lambda}\left(\frac{d^{(n-1)}s}{d\lambda^{(n-1)}}\right), \tag{2}$$

where s – spectrum, $s(\lambda_i)$ – the spectral value at wavelength λ_i, and $s(\lambda_j)$ – the spectral value at λ_j. Also, $\Delta\lambda = \lambda_j - \lambda_i$, where $\lambda_j > \lambda_i$.

Peaks in the fourth derivative curves were selected using the peak finder tool found in Origin 8.0. The qualitative information regarding pigment composition was obtained on the basis of the wavelength position of absorption features in the derivative spectra, compared with various published data (Bidigare et al. 1989a, Moore et al. 1995, Millie et al. 1995, Gómez et al. 2001). In this procedure the positive peaks in the fourth derivative represent accessory pigment absorption maxima. This approach has the advantage that a maximum in the original spectrum corresponds to a maximum in the derivative spectrum (Lange & Balny 2002). Moreover, the fourth derivatives are more selective for narrow bands compared to second derivatives.

3. Results

3.1. Hydrographic parameters and total suspended solids

The vertical temperature distribution across the two transects exhibited very weak thermal stratification (Figure 2). The highest temperature of 29.25°C coincided with the peak Chl a concentration at the surface of stn. MB9. The lowest temperature was observed at 20 m of stn. MB12 (25.68°C). Surface salinities were high towards the mouth and also in the western parts of the Bay and ranged from 33.48 to 33.56 PSU. The increase in salinity level at the mouth of the Bay could be an indication of the
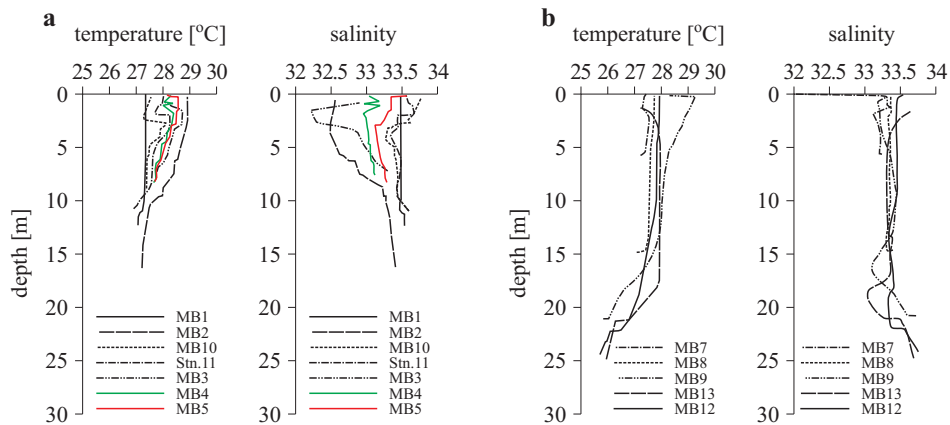
Figure 2. Distribution of temperature and salinity along the NS transect (a), the EW transect (b)
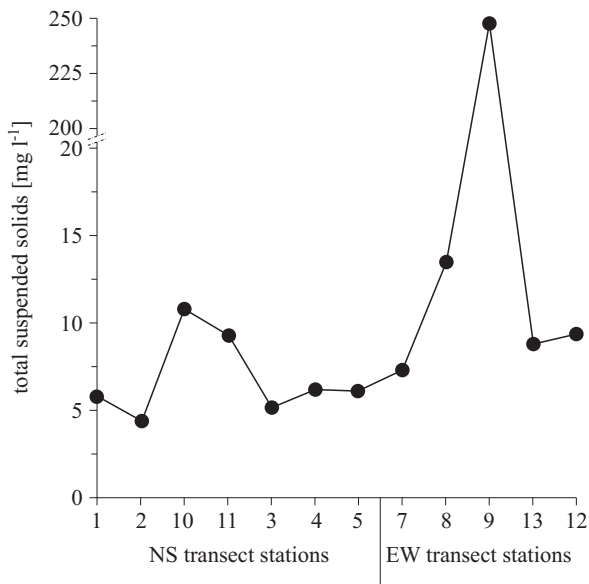

Figure 3. Distribution of total suspended solids (TSS) at the surface of the bay

influx of sea water from the South China Sea. Surface salinity values were relatively low in the north-western part of the bay. This can definitely be attributed to the influx from the major river systems in Pampanga and Bulacan. The lowest salinity was recorded at stn. MB7, located near the channel of the River Pasig. At this station, temperature was also low owing to the possible effect of anthropogenic inputs from metropolitan Manila. Total suspended solids (TSS) were > 8 mg l^{-1} towards the eastern part of

the bay. The highest value of TSS was 247 mg l^{-1} recorded at stn. MB9, which was located at the centre of the bloom patch (Figure 3).

3.2. Taxonomic marker pigments and NPP index

The surface chlorophyll a concentration varied widely, between 1.4 μg l^{-1} at stn. MB1 near the bay mouth and 521 μg l^{-1} at stn. MB9 in the middle of the bloom patch (Figure 4). The surface Chl a values of EW transect stations were high (> 3 μg l^{-1}). Similarly, the northern part of the bay (stn. MB3) also had comparatively high levels of TChl a at the surface. Accessory pigments like peridinin, fucoxanthin, zeaxanthin, lutein, violaxanthin, neoxanthin and antheraxanthin, when normalised to TChl a, displayed considerable variation among stations as a function of depth.
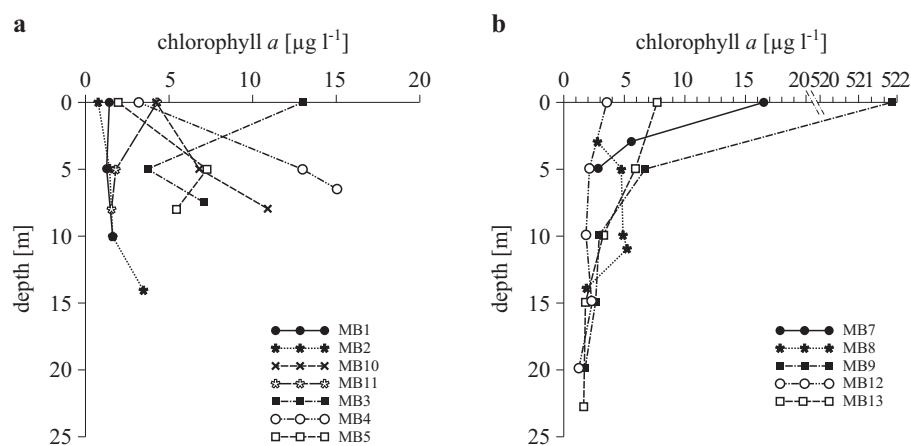

Figure 4. Vertical profile of Chl a along the NS transect (a), the EW transect (b)

The TChl b/TChl a ratios were high (81%) towards the northern part of the bay (stn. MB5) – an indication of a high chlorophyte abundance (Figure 5). The photosynthetic pigment peridinin/TChl a and fucoxanthin/TChl a ratios were also higher in the mid part of the bay, with mean values of 0.05 and 0.13 μg l^{-1} respectively. Zeaxanthin and lutein were the most dominant accessory non-photosynthetic pigments. The peridinin/TChl a ratio was exceptionally high (60%) in the surface waters at stn. MB7 and outweighed all other pigments, showing a clear dominance of dinoflagellates. On the EW transect at stns. MB12, MB13 and MB9 the fucoxanthin/TChl a ratio increased markedly below 15 m, owing to the aggregation of the diatoms *Haslea gigantea* and *Chaetoceros* spp. (unpublished data). Lutein, a marker pigment for chlorophytes and prasinophytes, was also ascribed to chlorophytes since microscopic observations revealed
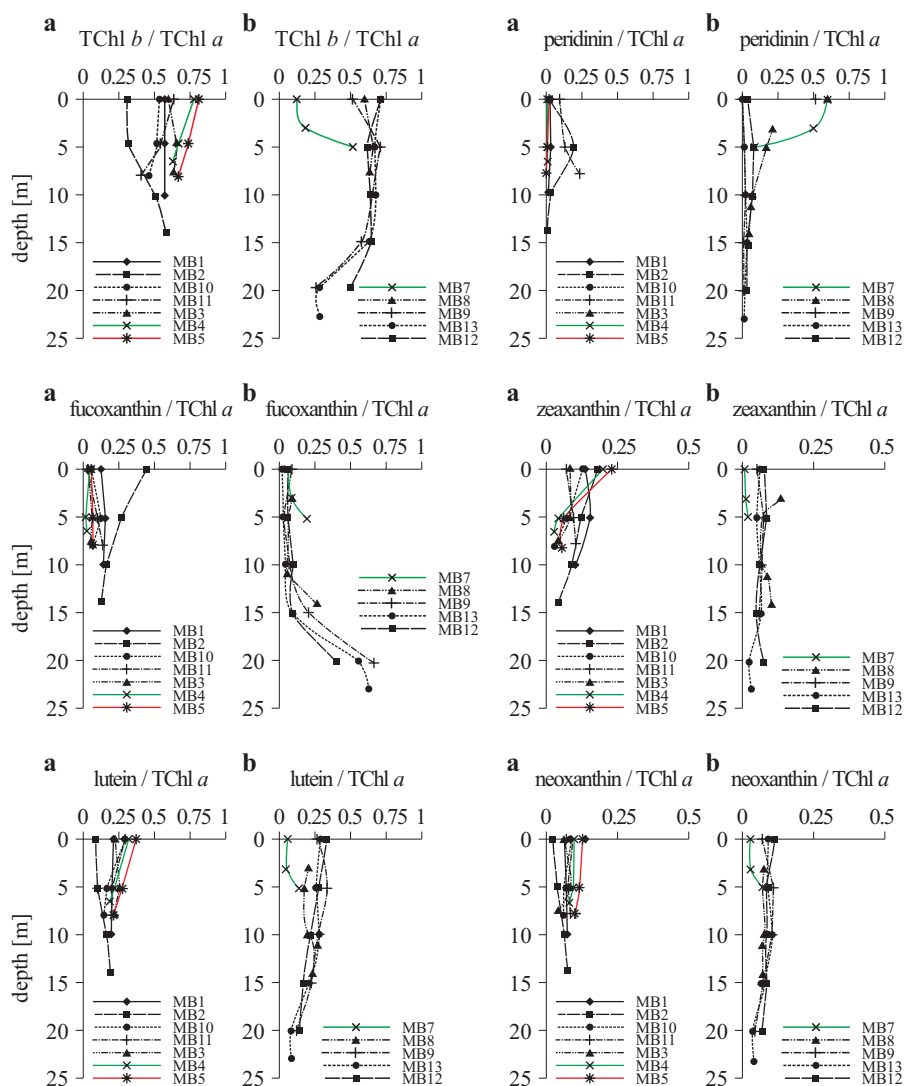
Figure 5. Ratio of selected diagnostic marker pigments to TChl a along the NS transect (a), the EW transect (b)

the absence of prasinophytes in the samples (see Furuya et al. 2006). On the NS transect at stns. MB2, 4 and 5 the high zeaxanthin/TChl a ratios of > 0.18 μg l^{-1} coincided with comparatively high temperatures ($> 28.1°$C). The NPP index, a measure of the relative importance of non-photosynthetic pigments with respect to total pigment concentration, showed high values at the surface (> 0.6) at most of the stations (Figure 6). On the EW transect NPP values ranged from 0.54 to 0.68, whereas on the NS transect NPP
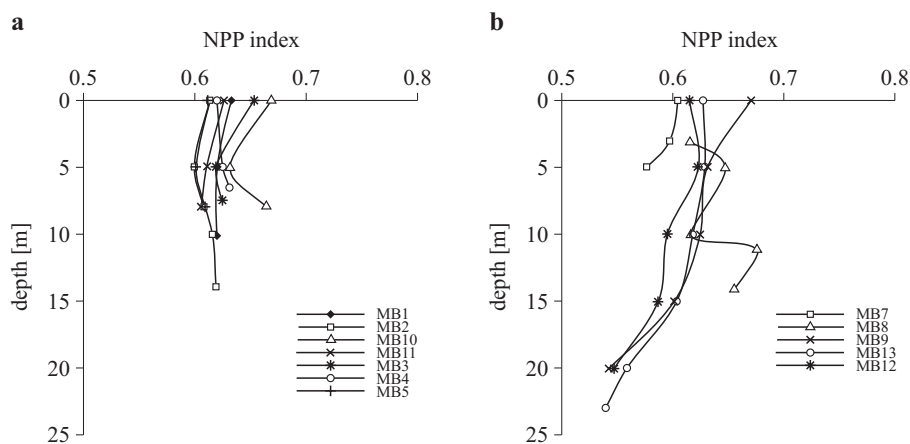
Figure 6. Vertical profile of the NPP index along the NS transect (a), the EW transect (b)

ranged from 0.60 to 0.67. Surface NPP values also varied considerably on the EW transect: 0.67 at stn. MB9 and 0.63 at the nearby stn. MB13.

3.3. Variations in the phytoplankton absorption coefficients

The chlorophyll specific absorption coefficients varied widely in the bay, within and outside the bloom patch. The lower $a_{ph}^*(\lambda)$ values recorded in this study are typical of eutrophic waters containing larger phytoplankton species, thus demonstrating greater pigment packaging. The spectrally averaged chlorophyll-specific absorption coefficients $(\bar{a}_{ph}^*(\lambda))$
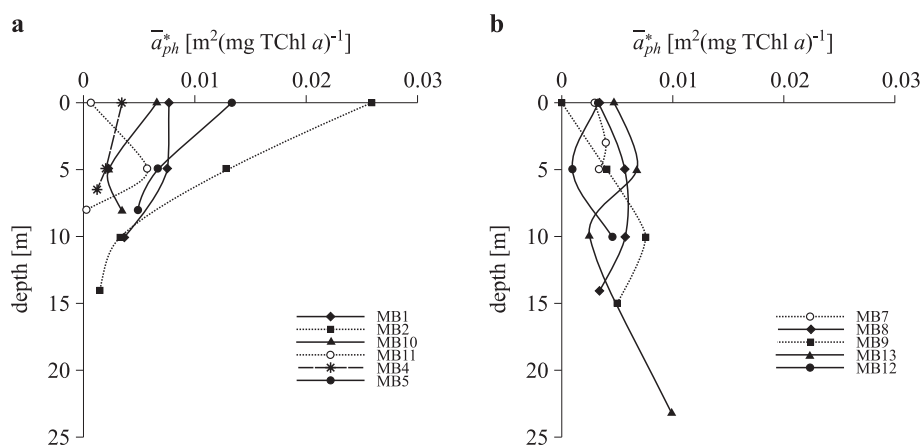

Figure 7. Depth profile of the spectrally averaged absorption coefficient of phytoplankton $a_{ph}^*(\lambda)$ along the NS transect (a), the EW transect (b)
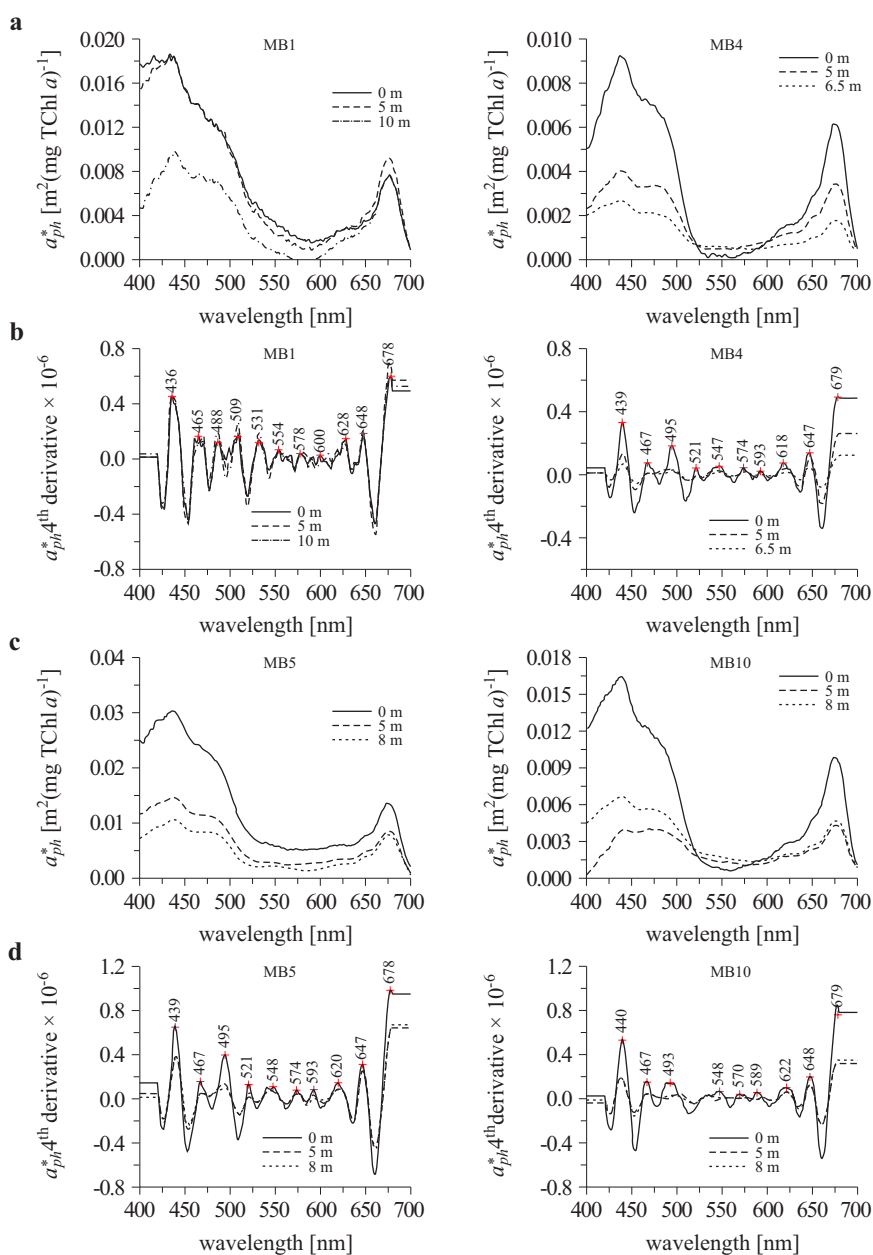
Figure 8. Absorption spectrum at stns. MB1, MB4, MB5 and MB10 (upper – a and c); absorption peaks determined by 4th derivative analysis (lower – b and d)

showed a decreasing trend with depth. Apart from the major absorption peaks (blue absorption maximum at 440 nm and red absorption maximum near 676 nm), marked absorption peaks at 475 nm and 653 nm were seen

at almost all stations. Along the EW transect, which passed through the centre of the bloom patch, where extremely high values of Chl a were recorded (521 μg l^{-1} at stn. MB9), the $a_{ph}^*(\lambda)$ spectrum of the surface water was flat in the blue-green region. The surface Chl a concentrations on the NS transect were generally lower (< 5 μg l^{-1}) and $a_{ph}^*(\lambda)$ values were high (≥ 0.003 m^2(mg TChl a)$^{-1}$) at most of the stations (Figure 7). At the stations where the marker pigment for diatoms was high (> 0.5 μg l^{-1}), very low $\bar{a}_{ph}^*(\lambda)$ values (≥ 0.003 m^2(mg TChl a)$^{-1}$) were recorded.

The blue-red peak ratios ($a_{ph}^*(440) : a_{ph}^*(675)$), a reflection of accessory pigment absorption as well as of pigment packaging, showed a value of 1.45 ± 0.56 (mean $\pm$ SD) on the NS transect and 1.56 ± 0.38 on the EW transect. Comparatively low ratios (< 1.2) of $a_{ph}^*(440) : a_{ph}^*(675)$ at stn. MB12 could be associated with the combined effect of packaging and relatively elevated ratios of accessory pigments like fucoxanthin, peridinin and diadinoxanthin. The marked absorption peaks at 455 nm and 653 nm at almost all stations can also be attributed to a high ratio of Chl b to TChl a.

3.4. Derivative analysis of the absorption spectra

It was observed that the fourth derivative of the absorption spectra was useful for identifying pigment peaks (Figure 8). At most stations Chl a absorption maxima were found around wavelengths 440 and 675 nm, while accessory pigments displayed their absorption peaks in the 490–550 nm regions. The stations on the north-south transect showed small peaks in the 560–618 nm region, which could be accounted for by degradation products and Chl c. Fucoxanthin peaks could be identified in the 521–530 nm region in the stations towards the northern part of the bay. The diadinoxanthin peak was detectable at 425–500 nm at most stations. Phycoerythrobilin was suggested for the peak at 548 nm. Smaller peaks were observed in the 589–594 nm region and also at 627 and 647 nm. The regression of the chlorophyll absorption maxima at the red region with the chlorophyll a concentration showed a good correlation for chlorophyll a ($r^2 = 0.71$, $n = 39$).

4. Discussion

The a_{ph}^* values recorded in the present study are typical of eutrophic waters, and such values are similar for a diatom dominated condition (Prézelin & Boczar 1986). The inverse correlation observed in this study between chlorophyll-specific absorption and Chl a concentration ($y = -291.65 + 8.5873$; $r^2 = 0.268$, $n = 29$) is well documented in many previous studies (Prieur & Sathyendranath 1981, Bricaud et al. 1995, 2004, Cleveland 1995, Ciotti et al. 1999, Sathyendranath et al. 2001). There

was a pronounced variation in the values of $a_{ph}^*(440)$ at the centre of the bloom patch and beyond it. At stn. MB9, where the highest chlorophyll concentration was observed at the surface, the value of $a_{ph}^*(\lambda)$ was very low, varying by two orders of magnitude with respect to the nearby stations (stn. MB7, MB8 and MB13) (see Figure 7). In Manila Bay the inverse relationship between $a_{ph}^*(440)$ and Chl a values may primarily have been due to the package effect caused by the dominance of larger green *Noctiluca* (up to 2000 μm; Fukuyo et al. 1990) cells and diatoms with higher intracellular pigment concentrations owing to nutrient enrichment. Another reason could be the relative contribution of non-photosynthetic pigments to total absorption (Bricaud et al. 1995, Ciotti et al. 1999, Vijayan et al. 2009). These observations are supported by reports that nutrient enrichment leads to an increased dominance of large phytoplankton (Chisholm 1992) and that the increase in cellular Chl a concentration with high nutrient availability can lead to a decrease in $a_{ph}^*(\lambda)$ (Sosik & Mitchell 1995).

The green *Noctiluca* bloom causes a greenish discolouration as it harbours a green, flagellated endosymbiont *Pedinomonas noctilucae* (Subramanian) Sweeny (Ostroumoff 1924, Sweeney 1971). Apart from Chl a, the major pigments of *P. noctilucae* are neoxanthin, violaxanthin, zeaxanthin, antheraxanthin, lutein and Chl b (Furuya & Lirdwitayaprasit 2000). The inverse relation between $a_{ph}^*(440)$ and Chl a can also be attributed to the higher ratios of non-photosynthetic pigments like neoxanthin, zeaxanthin and lutein to TChl a. Compared to the EW transect, the surface Chl a concentrations of the NS transect were generally lower ($< 5\mu$g l^{-1}) and $a_{ph}^*(\lambda)$ values were high (≥ 0.003 m^2(mg TChl a)$^{-1}$) for most of the stations. The NS transect stations had high ratios of zeaxanthin/TChl a, suggestive of a high contribution of smaller algal groups like Cyanophyceae, which absorb mainly in the blue region (Bidigare et al. 1989b). The prominent secondary peak observed at 480 nm at the surface at stns. MB4 and MB5 (Figure 8) was due primarily to zeaxanthin (Moore et al. 1995). In the EW transect there was a predominance of dinoflagellates and diatoms, as evidenced by the HPLC pigment signatures. There were prominent absorption peaks and shoulders due to Chl a (672 and 438 nm), Chl c (630–462 nm), peridinin (535–540 nm) and diadinoxanthin (495 nm) (Halldal 1970, Prézelin et al. 1976, Yentsch 1980). Similar characteristic peaks of absorption spectra had been reported earlier by Balch & Haxo (1984) for *Noctiluca miliaris* Suriray during bloom conditions. The zeaxanthin pigment, which has a high absorption between 454 and 480 nm, had a linear relation with $a_{ph}^*(440)$. The secondary peak in the blue and red region may be due to the enhanced contribution of Chl b (Bidigare et al. 1990), which in the present study is ascribed to the abundance of chlorophytes. As the numerical abundance

of chlorophytes was low, based on the pigment signatures of *P. noctilucae* (Furuya & Lirdwitayaprasit 2000), the Chl *a* allocated to chlorophytes were ascribed to *P. noctilucae* (Furuya et al. 2006). A small peak found at 462 nm at stn. MB9 is ascribable to Chl *c* (Barlow & Lamont 2012). At stns. MB5 and MB12 the surface NPP index (≥ 0.6) is the cumulative contribution of high ratios of photoprotective pigments like zeaxanthin, lutein and neoxanthin to TChl *a*.

The predominant role of river inputs in the modulation of dissolved and particulate matter is a general pattern in coastal waters (Kowalczuk 1999). The $a_{ph}^*(\lambda)$ spectra lacked sharply defined peaks or shoulders at stations where suspended solids were high, because detrital matter is generally present in an oxidised state and lacks resonance (Kiefer & SooHoo 1982). The weak stratification shows that mixing was prominent as a result of physical forcing, which might also contribute to sediment resuspension. Particles suspended in the water column diminish PAR availability in the subsurface waters by absorbing and reflecting light, which alters phytoplankton photosynthesis and biomass production.

Furuya et al. (2006) reported that streak-shaped red tides are common in the case of *N. scintillans*. Le Févre & Grall (1970) observed that the mechanical convergence of *N. scintillans* helps to maintain a dense condition. Nutrient availability can also induce modifications in light absorption (Babin et al. 1996), but nutrients were not exhausted in Manila Bay at the time of this survey (Furuya et al. 2006). The high temperatures recorded at stations with high TChl *a* concentrations provide evidence for enhanced absorption by the algal biomass (Lewis et al. 1983).

In summary, the bloom in Manila Bay was dominated by *Noctiluca scintillans* Macartney. Extremely high TChl *a* and elevated levels of peridinin, fucoxanthin and TChl *b* were also recorded. Since this was a highly polluted coastal environment, the absorption features of the accessory pigments were masked, due partly to the elevated contribution of detrital matter and partly to the presence of overlapping pigment absorption bands. Derivative analysis effectively resolved the overlapping features and enhanced the absorption characteristics of the accessory pigments. We conclude that a high intracellular accessory pigment concentration along with the large size of *Noctiluca* contributed significantly to the variability in the $a_{ph}^*(\lambda)$ spectrum in Manila Bay. Even though Chl *a* took a major share of the total light absorption, photosynthetic pigments like Chl *b*, peridinin and fucoxanthin also made a significant contribution. The general trend of non-photosynthetic carotenoid absorption decreasing with depth, especially at the NS transect stations, points towards lower photoprotection due to increased turbidity.

Acknowledgements

References

Allali K., Bricaud A., Claustre H., 1997, *Spatial variation in the chlorophyll-specific absorption coefficients of phytoplankton and photosynthetically active pigments in Equatorial Pacific*, J. Geophys. Res., 102 (C6), 12413–12423, http://dx.doi.org/10.1029/97JC00380.

Azanza R. V., Miranda L. N., 2001, *Phytoplankton composition and Pyrodinium bahamense toxic blooms in Manila Bay, Philippines*, J. Shellfish Res., 20, 1251 –1255.

Babin M., Morel A., Falkowski P. G., Claustre H., Bricaud A., Kobler Z., 1996, *Nutrient and light-dependent variations of the maximum quantum yield of carbon fixation in eutrophic, mesotrophic and oligotrophic systems*, Deep Sea Res. Pt. 1, 43 (8), 1241–1272, http://dx.doi.org/10.1016/0967-0637(96) 00058-1.

Balch W. M., Haxo F. T., 1984, *Spectral properties of Noctiluca miliaris Suriray, a heterotrophic dinoflagellate*, J. Plankton Res., 6 (3), 515–525, http://dx.doi. org/10.1093/plankt/6.3.515.

Barlow R., Lamont T., 2012, *Phytoplankton absorption and pigment adaptation of a red tide in the Benguela ecosystem*, Afr. J. Marine Sci., 34 (2), 241–248, http://dx.doi.org/10.2989/1814232X.2012.709968.

Bidigare R. R., Morrow J. H., Kiefer D. A., 1989a, *Derivative analysis of spectral absorption by photosynthetic pigments in the western Sargasso Sea*, J. Mar. Res., 47 (2), 323–341, http://dx.doi.org/10.1357/002224089785076325.

Bidigare R. R., Ondrusek M. E., Morrow J. H., Kiefer D. A., 1990, *In vivo absorption properties of algal pigments*, Proc. SPIE, 1302, 290–302, http: //dx.doi.org/10.1117/12.21451.

Bidigare R. R., Schofield O., Prezelin B. B., 1989b, *Influence of zeaxanthin on quantum yield of photosynthesis of Synecococcus clone WH7803 (DC2)*, Mar. Ecol.-Prog. Ser., 56, 177–188, http://dx.doi.org/10.3354/meps056177.

Bricaud A., Babin M., Morel A., Claustre H., 1995, *Variability in the chlorophyll-specific absorption coefficient of natural phytoplankton: analysis and parameterization*, J. Geophys. Res., 100 (C7), 13 321–13 332, http://dx. doi.org/10.1029/95JC00463.

Bricaud A., Claustre H., Ras J., Oubelkheir K., 2004, *Natural variability of phytoplanktonic absorption in oceanic waters: Influence of size structure of*

algal populations, J. Geophys. Res., 109, C11010, http://dx.doi.org/10.1029/ 2004JC002419.

Chisholm S. W., 1992, *Primary productivity and bio-geochemical cycles in the sea*, Plenum Press, New York, 213–237.

Cleveland J. S., Weidemann A. D., 1993, *Quantifying absorption by aquatic particles: A multiple scattering correction for glass-fiber filters*, Limnol. Oceanogr., 38 (6), 1321–1327, http://dx.doi.org/10.4319/lo.1993.38.6.1321.

Cleveland J. S., 1995, *Regional models for phytoplankton absorption of chlorophyll a concentration*, J. Geophys. Res., 100 (C7), 13333–13344, http://dx.doi.org/ 10.1029/95JC00532.

Ciotti A. M., Cullen J. J., Lewis M. R., 1999, *A semi-analytical model of the influence of phytoplankton community structure on the relationship between light attenuation and ocean color*, J. Geophys. Res., 104 (C1), 1559–1578, http://dx.doi.org/10.1029/1998JC900021.

De las Alas J. G., Sodusta J. A., 1985, *A model for the wind driven circulation of Manila Bay*, Nat. Appl. Sci. Bull., 37 (2), 159–170.

Duysens L. M. N., 1956, *The flattening effect of the absorption spectra of suspension as compared to that of solutions*, Biochim. Biophys. Acta, 19, 1–12, http: //dx.doi.org/10.1016/0006-3002(56)90380-8.

Fuji-ie W., Yanagi T., Siringan F., 2002, *Tide, tidal current and sediment transport in Manila bay*, La Mer, 40, 137–145.

Fukuyo Y., Takano H., Chihara M., Matsuoka K., 1990, *Red tide organisms in Japan. An illustrated taxonomic guide*, Uchida Rokakuho Co., Ltd., Tokyo, 407 pp.

Furuya K., Lirdwitayaprasit T., 2000, *Pigment composition of Pedinomonas noctilucae (Pedinophyceae), an endosymbiont of green Noctiluca (Dinophyceae)*, La Mer, 38, 95–97.

Furuya K., Saito H., Sriwoon R., Vijayan A. K., Omura T., Furio E. E., Borja V. M., Boonyapiwat S., Lirdwitayaprasit T., 2006, *Persistent whole-bay red tide of Noctiluca scintillans in Manila Bay, Philippines*, Coast. Mar. Sci., 30 (1), 74 –79.

Furio E. F., Gonzales C. L., 2002, *Practical guide on paralytic shellfish poisoning monitoring in the Philippines*, BFAR, Manila and JICA, Tokyo, 3–27.

Gonzales C. L., 1989, *Biology, epidemiology and management of Pyrodinium red tides*, ICLARM Conf. Proc., Fisheries Dept., Ministry of Development, Brunei Darussalam and Int. Center Living Aquat. Resourc. Manage., Manila, Philippines., 21, 39–47.

Gómez R. A., Weeks A. R., Boxall S. R., 2001, *The identification of phytoplankton pigments from absorption spectra*, Int. J. Remote Sens., 22, 315–338, http: //dx.doi.org/10.1080/014311601449952.

Halldal P., 1970, *Photobiology of microorganisms*, Wiley-Interscience, London, 17 –55.

Hansen P. J., Miranda L., Azanza R., 2004, *Green Noctiluca scintillans: a dinoflagellate with its own greenhouse*, Mar. Ecol.-Prog. Ser., 275, 79–87, http://dx.doi.org/10.3354/meps275079.

Hosono T., Su C.-C., Siringan F., Amano A., Onodera S.-I., 2010, *Effects of environmental regulations on heavy metal pollution decline in core sediments from Manila Bay*, Mar. Pollut. Bull., 60, 780–785, http://dx.doi.org/10.1016/j.marpolbul.2010.03.005.

Jacinto G. S., Sotto L. P. A., Senal M. I. S., Diego-McGlone M. L. S., Escobar M. T. L., Amano A., Miller T. W., 2011, *Hypoxia in Manila Bay, Philippines during the northeast monsoon*, Mar. Pollut. Bull., 63 (5–12), 243–248, http://dx.doi.org/10.1016/j.marpolbul.2011.02.026

Kiefer D. A., SooHoo J. B., 1982, *Spectral absorption by marine particles of coastal waters of Baja California*, Limnol. Oceanogr., 27 (3), 492–499, http://dx.doi.org/10.4319/lo.1982.27.3.0492.

Kishino M., Takahashi M., Okami N., Ichimura S., 1985, *Estimation of the spectral absorption coefficients of phytoplankton in the sea*, Bull. Mar. Sci., 37, 634–642.

Kowalczuk P., 1999, *Seasonal variability of yellow substance absorption in the surface layers of Baltic Sea*, J. Geophys. Res., 104, 30047–3005, http://dx.doi.org/10.1029/1999JC900198.

Lange R., Balny C., 2002, *UV-visible derivative spectroscopy under high pressure*, Biochim. Biophys. Acta, 1595, 80–93, http://dx.doi.org/10.1016/S0167-4838(01)00336-3.

Le Févre J., Grall J. R., 1970, *On the relationship of Noctiluca swarming off the western coast of Brittany with hydrological features and plankton characteristics of the environment*, J. Exp. Mar. Biol. Ecol., 4 (3), 287–306, http://dx.doi.org/10.1016/0022-0981(70)90040-7.

Lewis M. R., Cullen J. J., Platt T., 1983, *Phytoplankton and thermal structure in the upper ocean: consequence of non-uniformity in chlorophyll profile*, J. Geophys. Res., 88, 2565–2570, http://dx.doi.org/10.1029/JC088iC04p02565.

Maria E. J. S., Siringan F. P., Bulos A. D., Sombrito E. Z., 2009, *Estimating sediment accumulation rates in Manila Bay, a marine pollution hot spot in the seas of East Asia*, Mar. Pollut. Bull., 59 (4–7), 164–174, http://dx.doi.org/10.1016/j.marpolbul.2009.04.017.

Millie D. F., Kirkpatrick G. J., Vinyard B. T., 1995, *Relating photosynthetic pigments and in vivo optical density spectra to irradiance for the Florida red-tide dinoflagellate Gymnodinium breve*, Mar. Ecol.-Prog. Ser., 120, 65–75, http://dx.doi.org/10.3354/meps120065.

Mitchell B. G., 1990, *Algorithms for determining the absorption coefficient of aquatic particulates using the quantitative filter technique (QFT)*, Soc. Photo-Opt. Instr. Eng., Ocean Opt., X, 137–148, http://dx.doi.org/10.1117/12.21440.

Moore L. R., Goericke R., Chisholm S. W., 1995, *Comparative physiology of Synecococcus and Prochlorococcus: influence of light and temperature on*

growth, pigments, fluorescence and absorptive properties, Mar. Ecol.-Prog. Ser., 116, 259–275, http://dx.doi.org/10.3354/meps116259.

Ostroumoff A., 1924, *Noctiluca miliaris in Symbiose mit grünen Algen*, Zool. Anz., 58, 162 pp.

Prézelin B. B., Ley A. C., Haxo F. T., 1976, *Effects of growth irradiance on the photosynthetic action spectra of the marine dinoflagellate, Glenodinium sp.*, Planta, 130, 251–256, http://dx.doi.org/10.1007/BF00387829.

Prézelin B. B., Boczar B. A., 1986, *Progress in physiological research*, Biopress, Bristol, 349–464.

Prieur L., Sathyendranath S., 1981, *An optical classification of coastal and oceanic waters based on the specific spectral absorption curves of phytoplankton pigments, dissolved organic matter and other particulate material*, Limnol. Oceanogr., 26 (4), 671–689, http://dx.doi.org/10.4319/lo.1981.26.4.0671.

Prudente M. S., Ichihashi H., Tatsukawa R., 1994, *Heavy metal concentrations in sediments from Manila Bay, Philippines, and inflowing rivers*, Environ. Pollut., 86 (1), 83–88, http://dx.doi.org/10.1016/0269-7491(94)90009-4.

Sathyendranath S., Cota G., Stuart V., Maas H., Platt T., 2001, *Remote sensing of phytoplankton pigments: A comparison of empirical and theoretical approaches*, Int. J. Remote Sens., 22(2–3), 249–273, http://dx.doi.org/10.1080/014311601449925.

Savitzky A., Golay M. J. E., 1964, *Smoothing and differentiation of data by simplified least squares procedures*, Anal. Chem., 36 (8), 1627–1639, http://dx.doi.org/10.1021/ac60214a047.

Siringan F. P., Azanza R. V., Macalalad N. J. H., Zamora P. B., Sta. Maria M. Y. Y., 2008, *Temporal changes in the cyst densities of Pyrodinium bahamense var. compressum and other dinoflagellates in Manila Bay, Philippines*, Harmful Algae, 7, 523–531, http://dx.doi.org/10.1016/j.hal.2007.11.003.

Sosik H. M., Mitchell B. G., 1995, *Light absorption by phytoplankton, photosynthetic pigments and detritus in the California Current System*, Deep-Sea Res. Pt. I, 42 (10), 1717–1748, http://dx.doi.org/10.1016/0967-0637(95)00081-G.

Sweeney B. M., 1971, *Laboratory studies of a green Noctiluca from New Guinea*, J. Phycol., 7, 53–58.

Velasquez I. B., Jacinto G. S., 1995, *ASEAN criteria and monitoring: advances in marine environmental management and human health protection*, Proc. ASEAN-Canada Midterm Rev. Conf. Marine Sci., Singapore, 278–284.

Velasquez I. B., Jacinto G. S., Narcise C. I. S., Cuaresma N. T., 1997, *ASEAN marine environment management: Quality criteria and monitoring for aquatic life and human health protection*, Proc. ASEAN-Canada Tech. Conf. Mar. Sci., Penang, Malaysia, III-67–III-78.

Vijayan A. K., Yoshikawa T., Watanabe S., Sasaki H., Matsumoto K., Saito S.-I., Takeda S., Furuya K., 2009, *Influence of non-photosynthetic pigments on light absorption and quantum yield of photosynthesis in the western equatorial Pacific and the subarctic North Pacific*, J. Oceanogr., 65 (2), 245–258, http://dx.doi.org/10.1007/s10872-009-0023-y.

Villanoy C., Corrales R. A., Jacinto G. S., Cuaresma N., Crisostomo R., 1996, *Harmful toxic algal blooms*, IOC of UNESCO, Paris, 189–192.

Villanoy C., Azanza R. V., Altemerano A., Casil A. L., 2006, *Attempts to model the bloom dynamics of Pyrodinium, a tropical toxic dinoflagellate*, Harmful Algae, 5 (2), 156–183, http://dx.doi.org/10.1016/j.hal.2005.07.001.

Yentsch C. S., 1980, *The physiological ecology of phytoplankton*, Univ. California Press, Berkeley, 95–128.

Zapata M., Rodriguez F., Garrido J. L., 2000, *Separation of chlorophylls and carotenoids from marine phytoplankton: a new HPLC method using reverse phase C8 column and pyridine-containing mobile phases*, Mar. Ecol.-Prog. Ser., 195, 29–45, http://dx.doi.org/10.3354/meps195029.