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Seasonal Study of Phytoplankton Pigments and Species at a Coastal Station off Sydney: Importance of Diatoms and the Nanoplankton

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Abstract

Phytoplankton pigments and species were studied at a coastal station off Sydney (New South Wales, Australia) over one annual cycle. Sudden increases in chlorophyll *a* (up to 280 mg m^{-2}), due to short-lived diatom blooms, were found in May, July, September, January and February. These were superimposed upon background levels of chlorophyll *a* (20 to 50 mg m^{-2}), due mostly to nanoplankton flagellates, which occurred throughout the year. The nanoplankton (< 15 μm) accounted for 50 to 80% of the total phytoplankton chlorophyll, except when the diatom peaks occurred (10 to 20%). The annual cycle of populations of 16 dominant species-groups was followed. Possible explanations as to alternation of diatom-dominated and nanoplankton-dominated floras are discussed. Thin-layer chromatography of phytoplankton pigments was used to determine the distribution of algal types, grazing activity, and phytoplankton senescence in the water column. Chlorophyll *c* and fucoxanthin (diatoms and coccolithophorids) and chlorophyll *b* (green flagellates) were the major accessory pigments throughout the year, with peridinin (photosynthetic dinoflagellates) being less important. Grazing activity by salps and copepods was apparent from the abundance of the chlorophyll degradation products pheophytin *a* (20 to 45% of the total chlorophyll *a*) and pheophorbide *a* (10 to 30%). Chlorophyllide *a* (20 to 45%) was associated with blooms of *Skeletonema costatum* and *Chaetoceros* spp. Small amounts of other unidentified chlorophyll *a* derivatives (5 to 20%) were frequently observed.

Introduction

Phytoplankton species and pigments in East Australian coastal waters have been investigated spasmodically since the early 1930's. Dakin and Colefax (1940) presented the first comprehensive phytoplankton species calendar for the region. Later, Humphrey (1960, 1963) studied three annual cycles of chlorophylls *a*, *b* and *c* at the Port Hacking 100 m station by trichromatic spectrophotometry. He observed chlorophyll peaks in August, September, October (early spring), and December, January, February (summer), and these were correlated with intrusions of nutrient-rich slope water onto the continental shelf (Humphrey, 1963; Newell, 1966). Species occurrence was investigated at the same station by Grant and Kerr (1970) and Jeffrey and Carpenter (1974). The latter authors recorded succession patterns of diatoms and dinoflagellates similar to those described by Dakin and Colefax (1940) and, by examining phytoplankton alive, fragile flagellates normally destroyed by preservatives were also observed. In another study, sensitive thin-layer chromatography was used to investigate a spring diatom bloom (Jeffrey, 1974), which demonstrated for the first time the usefulness of this methodology for seawater samples. For example, the pigments chlorophyll *b* (green algae), fucoxanthin (diatoms + golden-brown flagellates), and peridinin (dinoflagellates) marked the presence of major algal types in the water column. The occurrence of chlorophyll degradation products which could be separated chromatographically from the parent compounds gave clues to the physiological state of the phytoplankton and grazing activity by zooplankton.

The present study describes the results of spectrophotometric and thin-layer chromatographic analyses of phytoplankton pigments over one annual cycle (April 1978 to April 1979) at the Port Hacking 100 m station. The importance of the nanoplankton fraction is evaluated, and species counts on live phytoplankton and hydrological measurements (temperature, salinity, nutrients) are also included.

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Materials and Methods

Collection of Samples

The Port Hacking 100 m station (Latitude 34°05′30″S; Longitude 151°15′30″E) is situated 20 miles south of Sydney and 8 miles offshore, where the depth is about 110 m. This station was sampled at weekly intervals from April 1978 to April 1979, weather permitting. Water samples, each 24 l, were collected from 0, 10, 20, 30, 40, 50, 75 and 100 m depth by making duplicate casts with a twin 6 l water sampler (Jitts, 1964). The samples were brought back to the laboratory within 2 h of collection.

Hydrological Measurements

At each station temperature, salinity and nitrate were measured through the water column according to methods described by Major *et al.* (1972). Density (σ_t) was calculated from temperature and salinity data.

Spectrophotometric Pigment Analysis

Ten litres of seawater were filtered under slight negative pressure (10 to 15 in. Hg) on to Whatman GF/C filters. $MgCO_3$ was omitted from the filtration procedure, following recommendations by Holm-Hansen and Riemann (1978) (control tests with cultured algae showed absence or only a few percent formation of pheophytin *a*). Pigments were extracted overnight in the dark (5 °C) in centrifuge tubes with 5 ml 100% acetone. Absolute acetone (not 90%) was used to inactivate the chlorophyllase found in some unicellular algae (Barrett and Jeffrey, 1964, 1971; Jeffrey and Hallegraeff, in preparation). The filters were then mechanically ground in a glass homogenizer (1 to 2 min), filter fibres and cell debris were removed by centrifugation, and when necessary further extracted with small volumes of acetone. Water was added to adjust the concentration of acetone to 90% and the extinctions read immediately at 664 nm in a Unicam SP 500 spectrophotometer. Chlorophyll *a* concentration was calculated using an extinction coefficient of 87.67 l g^{-1} cm^{-1} (Jeffrey and Humphrey, 1975). The results obtained were within 2 to 5% of those calculated with the trichromatic equations by these authors, which correct for interference from chlorophylls *b* and *c*.

Size-Fractionation of Phytoplankton

The proportion of nanoplankton was estimated on a further 5 l of seawater from the same sample. This was gently drawn through 10 μm nylon plankton gauze (Henry Simon and Co., Sydney) mounted on top of the receiver flask of the filter holder. The resulting filtrate was filtered on to Whatman GF/C filters and extracted for pigments as described above.

Tests of the 10 μm plankton gauze in fractionating various size-ranges of cultured algae are given in Table 1. The cultures (70 ml) were diluted with 10 l of filtered seawater to give chlorophyll concentrations of 1 to 3 μg l^{-1}. Two separate portions (4 l) were filtered through plankton gauze, and chlorophyll content of the filtrates compared with that of a remaining unfiltered portion (2 l). The diatoms *Schroederella delicatula* and *Chaetoceros affinis* were completely retained, and the small flagellates *Cricosphaera carterae*, Cryptomonad STX-157, and *Isochrysis galbana* completely passed through the gauze. Difficulties occurred with the dinoflagellate *Amphidinium carterae* (20% retained) and the fragile chain-forming diatom *Skeletonema costatum* (40% retained). Because of these difficulties, samples filtered through 10 μm plankton gauze were arbitrarily designated the < 15 μm fraction.

Thin-Layer Chromatography

After spectrophotometric analysis, the pigments were transferred from acetone to diethyl ether for thin-layer chromatography (Jeffrey, 1968, 1974). Both powdered sucrose (Sunny Cane brand, Industrial Sugar Mills, Sydney) and cellulose (Macherey Nagel, MN-300) were used as adsorbents. The best resolution was obtained with cellulose which was purified with chloroform/light petroleum mixtures (Jeffrey, 1981) before use. Small (10.5 x 8 cm) chromatographic plates were used, which allowed for separation of small quantities of pigments

Table 1. Tests of 10 μm plankton gauze in fractionating various size-ranges of cultured algae

Organism	Culture Code No.	Cell dimensions	% chlorophyll *a* passing screen (2 experiments)
Schroederella delicatula	CS 72	10–15 × 25–40 μm; chains 120–500 μm length	0%; 0%
Chaetoceros affinis	CS 78	20–25 × 23–25 μm; chains 100–500 μm length	0%; 0%
Skeletonema costatum	CS 76	5–7 × 10–15 μm; chains 30–150 μm length	57%; 64%
Amphidinium carterae	CS 21	8–12 × 14–17 μm	80%; 86%
Cricosphaera carterae	CS 40	7–12 μm	100%; 100%
Cryptomonad STX-157	CS 48	4 × 8 μm	100%; 100%
Isochrysis galbana	CS 22	4–7 μm	100%; 100%

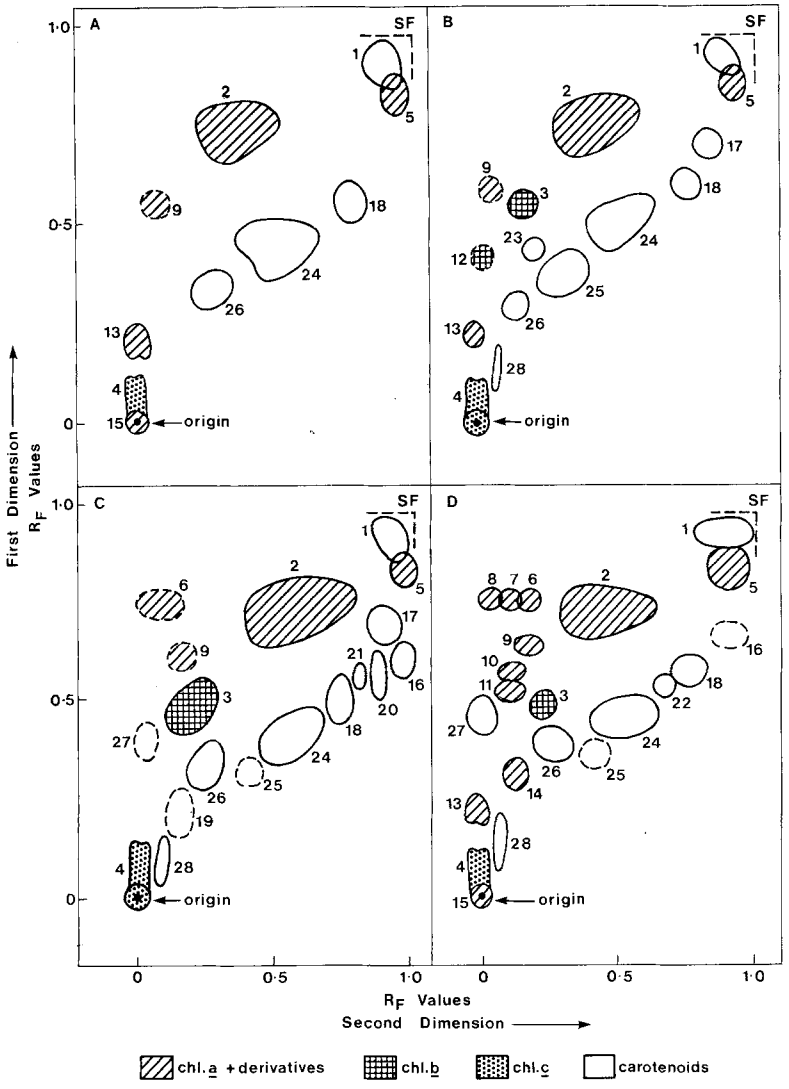

Fig. 1. Photosynthetic pigments of phytoplankton from Port Hacking station separated on thin-layers of cellulose. (A) Bloom of diatom *Nitzschia seriata*, 8 January 1979, 30 m; (B) dinoflagellates (*Gymnodinium* spp., *Prorocentrum micans*), with diatoms and green flagellates, 28 February 1979, surface sample; (C) green flagellates, diatoms and dinoflagellates, 16 March 1979, surface sample; (D) phytoplankton plus small copepods, 7 February 1979, 50 m. Solvent system: first dimension, *n*-propanol in light petroleum (60° to 80 °C) = 2.5:97.5 (v/v); second dimension, chloroform:light petroleum:acetone = 30:70:0.5 (v/v/v). Pigment fractions: 1, carotene (orange); 2, chlorophyll *a* (blue-green); 3, chlorophyll *b* (olive green); 4, chlorophyll *c* (light green); 5, pheophytin *a* (grey); 6,7,8,9, 10,11, unidentified chlorophyll *a* derivatives (blue-green); 12, unidentified chlorophyll *b* derivative (olive green); 13, chlorophyllide *a* (blue-green); 14, pheophorbide *a* (grey); 15, unidentified origin material (brown); 16, astaxanthin (pink); 17, lutein (yellow); 18, diadinoxanthin (yellow); 19, neoxanthin (yellow); 20,21,22,23, unidentified yellow xanthophylls; 24, fucoxanthin (orange); 25, peridinin (red); 26, neofucoxanthin (orange); 27,28, unidentified fucoxanthin derivatives (orange). SF: solvent front

and rapid (10 min) development. The solvent systems for the washed cellulose were: first dimension, n-propanol in light petroleum (60° to 80 °C) = 2.5:97.5 (v/v); second dimension, chloroform:light petroleum:acetone = 30:70:0.5 (v/v/v). Plates were dried in the dark (5 min) between the first and second dimension. After development, pigment chromatograms were examined in both visible and ultraviolet light, and the position of pigment zones recorded. Identification of pigments was by R_f values and absorption spectra (Jeffrey, 1968). For quantitative analysis, pigments were eluted with acetone (chlorophylls *a*, *b* and derivatives) or ethanol (carotenoids). Extinctions were read in 1 cm microcells (1 ml capacity) in a Unicam SP 500 spectrophotometer at the wavelength of maximum absorption, and pigment concentrations were calculated using the extinction coefficients given by Jeffrey (1968) and Jeffrey and Humphrey (1975). Control tests, applying known amounts of pigments to chromatograms, showed 90 to 95% recovery after two-dimensional development. In the routine studies, pigment fractions were recorded as abundant, present or absent (see Fig. 5). All operations involving pigment extraction and chromatography were done in darkened fume cupboards to prevent damage by photo-oxidative processes.

Pigment chromatograms of selected phytoplankton samples are shown in Fig. 1. Fig. 1A, taken during a bloom of *Nitzschia seriata*, shows the full complement of diatom pigments [carotene (Fraction 1), chlorophylls *a* and *c* (Fractions 2 and 4, respectively), diadinoxanthin (18), fucoxanthin (24) and neofucoxanthin (26)]. Small amounts of the chlorophyll degradation products pheophytin *a* (Fraction 5), chlorophyllide *a* (13), an unidentified blue-green derivative (9) and unidentified brown origin material (15) were also present. In Fig. 1B and C, green algal pigments [chlorophyll *b* (Fraction 3), lutein (17)] and the dinoflagellate pigment peridinin (25) were present in addition to diatom pigments. In Fig. 1D, a variety of known [pheophytin *a* (Fraction 5), chlorophyllide *a* (13), pheophorbide *a* (14)] and unknown

(Fractions 6,7,8,9,10,11,15) chlorophyll *a* degradation products were present in addition to the normal pigments of diatoms, dinoflagellates and green flagellates.

Phytoplankton Species Composition

The phytoplankton from 5 l of seawater were concentrated in a plankton centrifuge (Davis, 1957; 15 000x *g*, flow rate 1 l per 6 min) to about 10 ml. This was done within 2 to 5 h after collection. The harvested algae were immediately examined in the living state with an inverted microscope. Quantitative species counts on unpreserved samples were made by allowing 2.5 ml of concentrated sample to settle overnight at 5 °C in counting chambers (Utermöhl, 1958). Most of the delicate species thus retained their morphological form, flagellar structures and chloroplast color. Overnight phytoplankton growth or incomplete settling of living algae did not pose serious problems. The whole bottom area of the counting chamber (5 mm deep) was counted at low magnification (x 200) for rare and large species (such as large dinoflagellates). A portion (10 to 30 random fields) was counted for the abundant diatoms (x 200) and small flagellates (x 200). A counting error of 10 to 15% was accepted for the small flagellates, and 15 to 25% for the large diatoms and dinoflagellates.

Zooplankton Feeding Experiments

Salps and copepods were collected from the Port Hacking station by making vertical hauls with a 330 µm-mesh plankton net. The animals were used for feeding experiments in the laboratory within 2 h of collection. With the salp *Thalia democratica*, attempts to obtain fecal strings of sufficient purity were unsuccessful. Therefore, the stomachs of about 100 freshly collected individuals were dissected in seawater buffered with 0.01 M phosphate, pH = 7.8, and the pigments they contained extracted with acetone neutralized with $MgCO_3$. With the copepods (mainly *Temora, Acartia* or *Calanoides* species), about 150 individuals were incubated in a jar (4 l) with seawater enriched with natural phytoplankton or with cultures of *Chroomonas* sp. and *Olisthodiscus luteus* (1 to 3 µg chlorophyll *a* l^{-1}). The vessels were kept on a laboratory bench under natural light-dark cycles, gently stirred and aerated, and after 1 to 3 d fecal pellets were collected from the bottom of the jar, and the pigments they contained extracted with acetone.

Results

Chlorophyll *a* Determined by Spectrophotometry

The seasonal variation of phytoplankton chlorophyll *a* at the Port Hacking 100 m station is shown in Fig. 2. The chlorophyll *a* concentration, summed for the water column, ranged from 20 to 280 mg m^{-2}. Both > 15 and < 15 µm size-fractions are shown. These varied between 5 and 215 mg m^{-2} (> 15 µm fraction) and 20 to 50 mg m^{-2} (< 15 µm fraction), representing a 43-fold and 2.5-fold variation, respectively. The short-lived (2 to 6 wk) increases in the large fraction occurred in May, July, September, January and February. The nanoplankton fraction formed a more constant background over the period studied (Table 2), normally contributing 50 to 80% of the total chlorophyll except when the large phytoplankton chlorophyll peaks occurred (10 to 20%).

Typical chlorophyll depth profiles are shown in Fig. 3. In nonstratified waters chlorophyll varied up to 4-fold with depth. This was primarily due to variation in the large (> 15 µm) fraction, with the nanoplankton fraction (< 15 µm) being more uniformly distributed (Table 2). In stratified waters chlorophyll varied up to 30-fold with depth, and distinct maxima (up to 15 µg l^{-1}) at 20 to 40 m often occurred. This was primarily due to

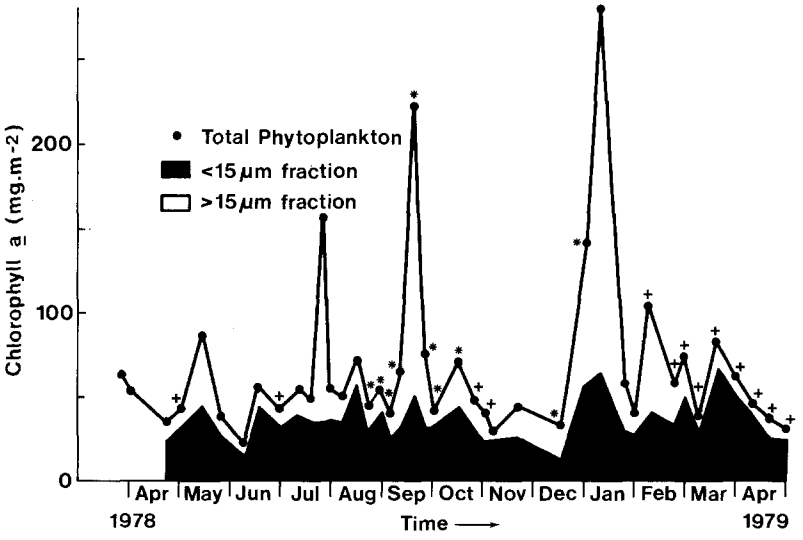

Fig. 2. Seasonal variation of phytoplankton chlorophyll *a* at Port Hacking 100 m station. Asterisks indicate a salp swarm, crosses indicate many small copepods

Table 2. Statistics of chlorophyll concentration of total phytoplankton and of > 15 and < 15 μm fractions at Port Hacking, 100 m station. Both range and coefficient of variation (CV) are shown

Source of variation	Total chlorophyll *a*	> 15 μm fraction	< 15 μm fraction	No. of observations
Season (cf. Fig. 2) (water-column-integrated data)	20–280 mg m^{-2}	5–215 mg m^{-2} CV = 140%	20–50 mg m^{-2} CV = 36%	41
Depth (cf. Fig. 3A, B) (non-stratified period, June-Sept. 1978)	0.05–3.2 μg l^{-1}	0.02–2.3 μg l^{-1} CV = 83%	0.08–0.90 μg l^{-1} CV = 47%	108
Depth (cf. Fig. 3C, D) (stratified period, Apr. - May 1978, Oct. 1978 - Apr. 1979)	0.05–15.0 μg l^{-1}	0.02–11.5 μg l^{-1} CV = 282%	0.03–3.5 μg l^{-1} CV = 92%	172

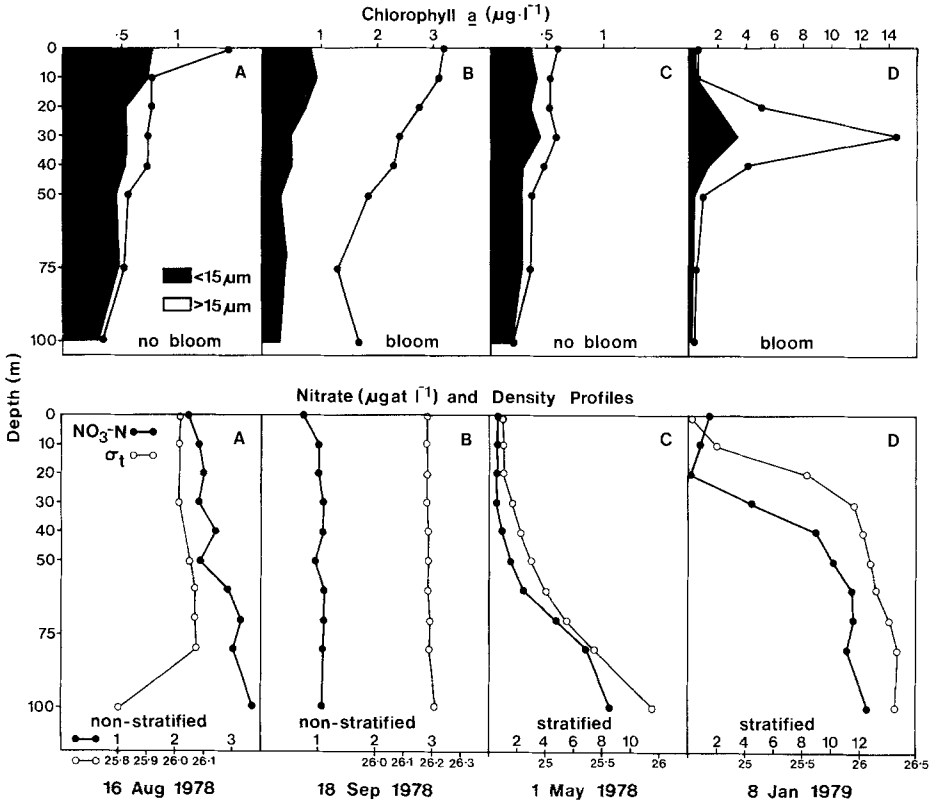

Fig. 3. Typical chlorophyll depth profiles in non-stratified (A,B) and stratified (C,D) waters, and under bloom (B,D) and non-bloom (A,C) conditions, compared with nutrient and density structure of the water column

peaks of the large size-fraction, which appeared just above the pycnocline (Fig. 3D). Nanoplankton chlorophyll was less variable (6-fold variation; Table 2).

Phytoplankton Species

Maximal cell numbers of the dominant species in the > 15 and < 15 μm fractions are listed in Table 3. The large size-fraction was made up primarily of chain-forming diatoms, and the nanoplankton fraction consisted mostly of small flagellates (non-thecate dinoflagellates, prymnesiophytes, prasinophytes, cryptomonads). Small diatom cells (e.g. *Skeletonema costatum*) were also found, but these never accounted for more than 10 to 20% of the < 15 μm fraction. Seasonal occurrence of the 16 most abundant species-groups is shown in Fig. 4. Diatom blooms occurred in May, July, September, January and February. *Nitzschia seriata* and to a lesser extent also *Chaetoceros* spp. were present in all blooms observed. Different diatom species occurred as co-dominants: *Leptocylindrus danicus, Skeletonema costatum, Asterionella glacialis, Detonula pumila* (= *Schroederella delicatula*), and *Eucampia zoodiacus*. The dinoflagellates *Peridinium trochoideum* and *Prorocentrum micans* were of some importance in September-October and February-March. Variations in the number of small flagellates also occurred. Non-thecate dinoflagellates (*Gymnodinium* spp.) showed peaks in February-March, coccolithophorids (*Gephyrocapsa oceanica* and *Emiliania huxleyi*) were particularly abundant in October-April, and minute green flagellates (similar to *Micromonas* sp.)

Table 3. Maximum cell numbers of dominant phytoplankton species at Port Hacking station, 1978–1979

Species	Average cell dimensions (μm)	Maximal abundance (cells l^{-1})
Chain-forming diatoms (> 15 μm fraction)		
Nitzschia seriata[a]	3 × 54 - 5 × 80; chains 200–500 length	2.10^6
Leptocylindrus danicus	5 × 40; chains 80–250 length	7.10^4
Skeletonema costatum	3 × 14 - 8 × 12; chains 30–150 length	10^5
Asterionella glacialis	30–50 × 3–5; chains 90–250 length	4.10^4
Detonula pumila	20–40; chains 120–500 length	2.10^4
Chaetoceros sp.	15 × 30; chains 100–300 length	3.10^4
Eucampia zoodiacus	30 × 40; chains 100–400 length	2.10^4
Phytoflagellates (< 15 μm fraction)		
Dinoflagellates		
Gymnodinium (10–15 spp.)	5–15	7.10^5
Prymnesiophytes		
Gephyrocapsa oceanica + *Emiliania huxleyi*	5–15	2.10^5
cf. *Dicrateria* sp.	6–10	3.10^4
Prasinophytes		
cf. *Micromonas* sp.	1–3	10^6
Pyramimonas (2 spp.)	5–7.5	3.10^4
Platymonas sp.	7.5×10.5	8.10^4
Cryptomonads		
Cryptomonas (2 spp.)	7 × 20	10^4
cf. *Plagioselmis* sp.	4 × 7	2.10^6

[a] The taxonomy of the *Nitzschia seriata* -complex is at present under revision (see Hasle, 1972)

bloomed in March-April. The green flagellates *Pyramimonas* spp. and *Platymonas* sp., and the cryptomonads *Cryptomonas* spp. and *Plagioselmis* sp. were of secondary importance. As a group, the diatoms ranged in cell number from 10^7 to 10^{10} m^{-2} for the total water column, and the nanoflagellates from 10^9 to 10^{11} cells m^{-2}.

Pigments Determined by Thin-Layer Chromatography

Fig. 5 summarizes the major pigments at the 41 stations worked. The seasonal and depth distribution of chlorophylls *a*, *b*, and *c*, the chlorophyll *a* degradation products pheophytin *a*, pheophorbide *a*, chlorophyllide *a*, and unidentified blue-green derivatives, and the carotenoids carotene, fucoxanthin, peridinin and astaxanthin are shown. Chlorophylls *a* and *c*, carotene, and fucoxanthin were dominant fractions throughout the year. Chlorophyll *a* and fucoxanthin occurred in all but a few deep samples, whereas chlorophyll *c* and carotene were present in all samples analyzed. Chlorophyll *b* was particularly abundant in March-August, when it was found throughout the water column, and less abundant in September-February. Peridinin was of some importance in May-July, September-October and March-April, and was insignificant at other times of the year. The ratio of chlorophyll *b*, fucoxanthin and peridinin to chlorophyll *a* was measured from selected chromatograms taken throughout the year, e.g.: chlorophyll *b*: chlorophyll *a* ratio = 0.29 ± 0.20 SD (n = 35); fucoxanthin:chlorophyll *a* ratio = 0.43 ± 0.26 SD (n = 56); peridinin:chlorophyll *a* ratio = 0.06 ± 0.05 SD (n = 11). The results show the relative abundance of the accessory pigments.

Chlorophyll *a* degradation products were frequently observed. Pheophytin *a* often occurred in small amounts (5 to 10% of the total chlorophyll *a*), and was observed in significant quantities (20 to 45%) throughout the water column in September-October and January-March. Pheophorbide *a* was less frequently observed than pheophytin *a*. It was of some importance (10 to 30%) at greater depths at the end of October and in January-February. Chlorophyllide *a* was significant (20 to 45%) throughout the water column in July and September and in the upper 50 m in January, and was associated with blooms of *Skeletonema costatum* and *Chaetoceros* spp., respectively (Fig. 4). Other unidentified blue-green chlorophyll derivatives, intermediate in polarity between chlorophyll *a* and chlorophyllide *a*, were often noticed. Their absorption spectra, chromatographic properties and possible identity will be discussed elsewhere (Hallegraeff and Jeffrey, in preparation). Normally they were detected only in small amounts (5 to 10%), but they were more abundant (15 to 20%) throughout July,

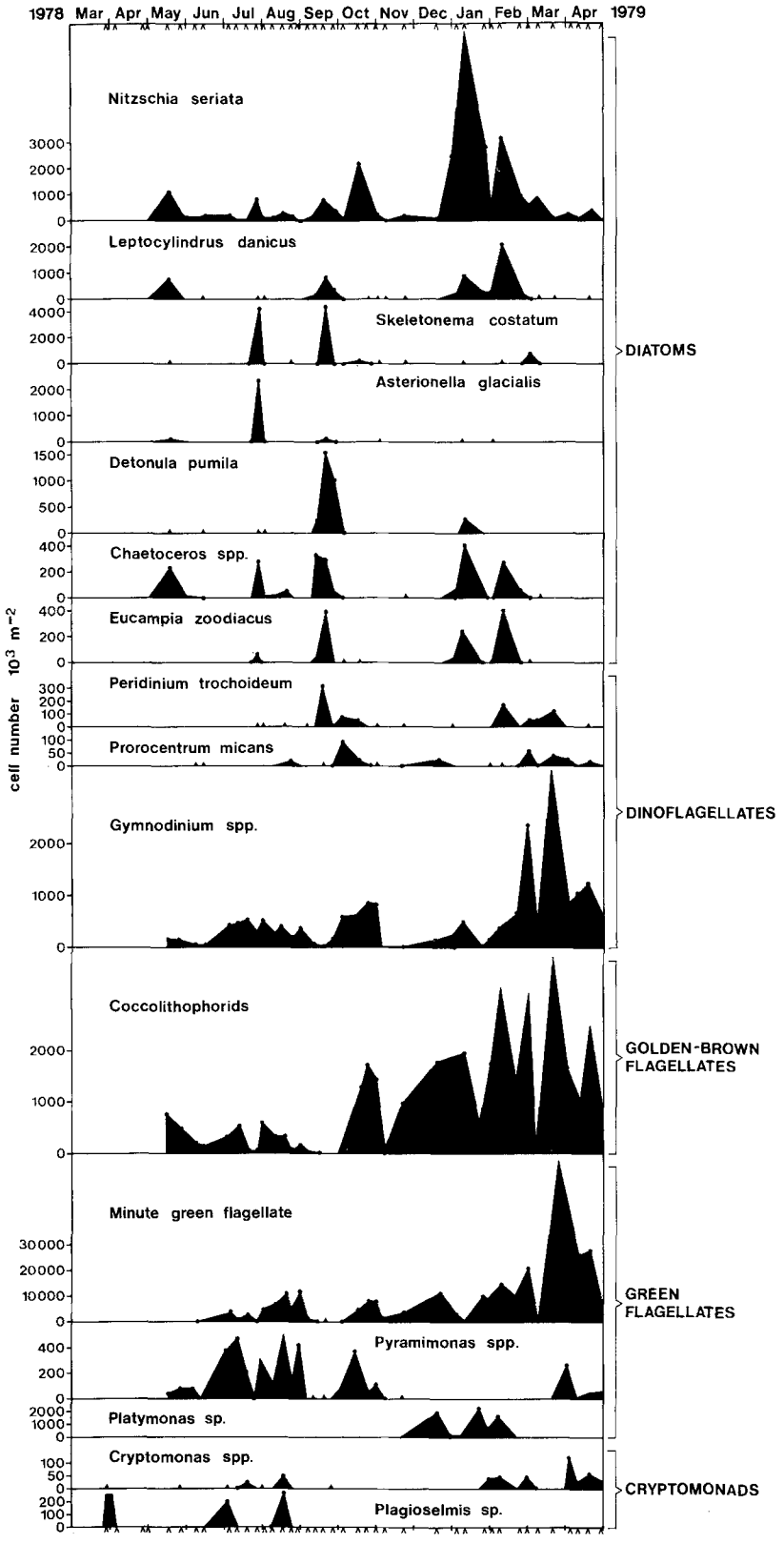

Fig. 4. Seasonal occurrence of 16 most abundant phytoplankton species-groups at Port Hacking 100 m station

September and January. Pink-coloured astaxanthin-type carotenoids (originating primarily from small copepods) often occurred at the end of October and in January-April.

Chromatographic data for two stations are replotted in Fig. 6 to show associations of pigments with living cells (hatched bars) and pigments from detrital material (black bars). Relative proportions of pigments are also shown. This presentation shows clearly the location of algal types, copepods and detrital material in the water column.

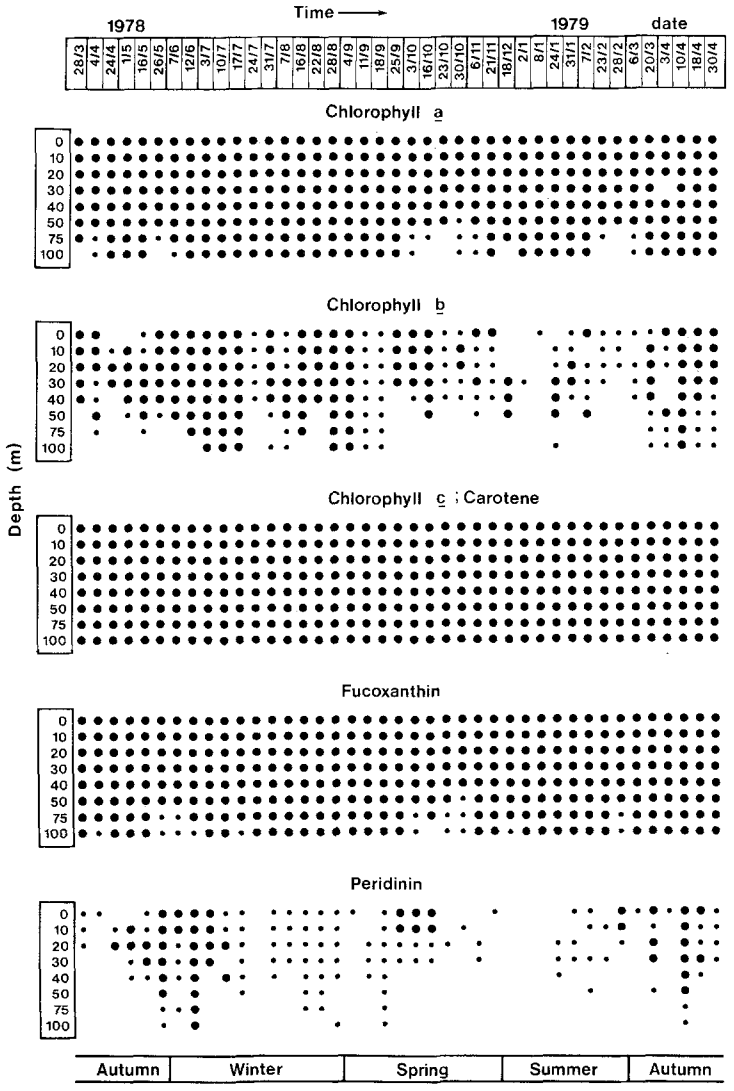

Fig. 5. Seasonal occurrence of important pigments at 41 stations off Port Hacking. Large data points indicate abundant = approximately 0.4 to 0.8 μg chlorophyll *a* per chromatogram, 0.1 to 0.4 μg chlorophyll *b*, 0.1 to 0.3 μg chlorophyll *c*, 0.1 to 0.2 μg carotene, 0.2 to 0.7 μg fucoxanthin, 0.03 to 0.08 μg peridinin, and chlorophyll *a* degradation products > 10% of the total chlorophyll *a*. Small data points indicate present = one-fifth to one-half of the above amounts

Zooplankton Feeding Experiments

Since pheophytin *a* was abundant when salp swarms or many small copepods were present in the water samples (Fig. 2), feeding experiments with salps and copepods were carried out in the laboratory. Pheophytin *a* (Fraction 5) was identified as the dominant chlorophyll breakdown product by both animals (chromatograms in Fig. 7). In salp stomachs, chlorophyll *a* was never completely degraded. Copepod fecal pellets typically contained 50 to 80% pheophytin *a*, 20 to 50% pheophorbide *a* and 10 to 20% unidentified chlorophyll derivatives.

Degradation products of carotenoids were also observed. For example, an unidentified blue pigment (Fig. 7A, Fraction 29) was found in salp stomachs. The pigment is chromatographically identical to the acid rearrangement product of fucoxanthin (Jensen, 1964), and may indicate an acidification history for fucoxanthin after its passage through the gut of zooplankton.

Discussion

The irregular short-term fluctuations in phytoplankton chlorophyll found in previous studies at the Port Hacking 100 m station (Humphrey, 1960, 1963) were fully confirmed in the present work. In addition, fractionation of the phytoplankton showed that the large chlorophyll peaks were primarily due to the > 15 μm fraction, superimposed upon a nanoplankton fraction (< 15 μm) which formed a more constant background level throughout the year (Fig. 2; Table 2).

The species analyses indicated that these chlorophyll peaks were due to short-lived diatom blooms (Fig. 4). Taxonomic composition confirmed the observations of Dakin and Colefax (1940), Grant and Kerr (1970) and Jeffrey and Carpenter (1974), but it also appeared that the dominants and subdominants may vary from bloom to bloom and from year to year. A statistical analysis of the species succession patterns and their relationship to

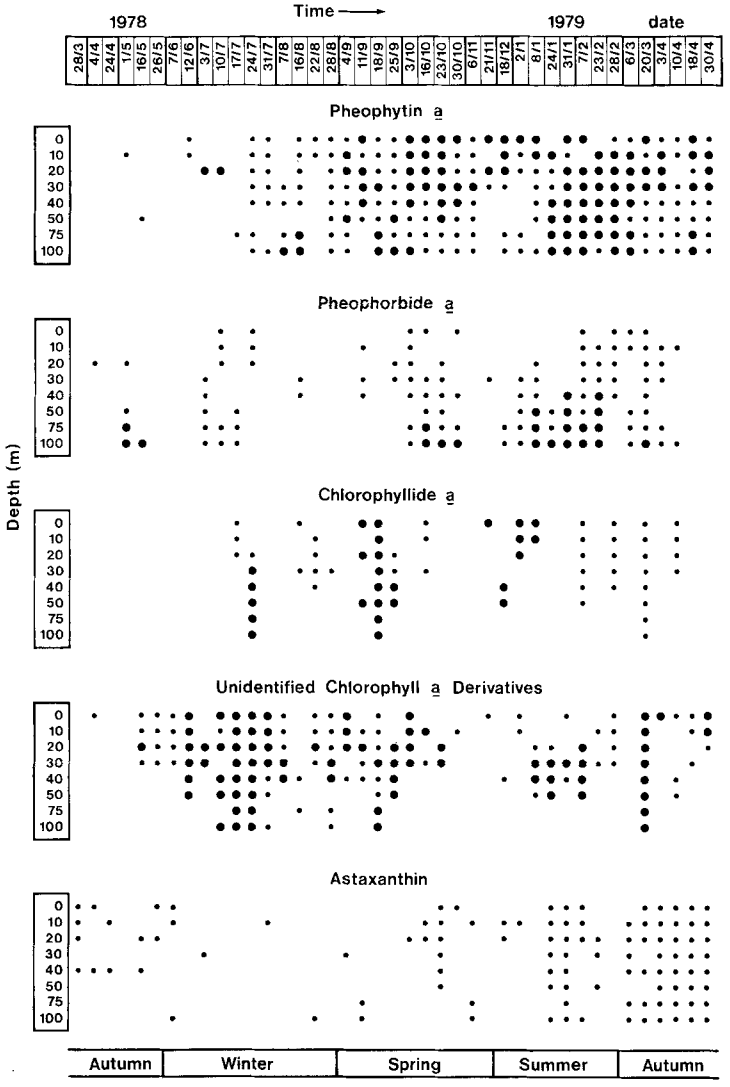

Fig. 5. (continued)

the hydrological environment will be examined separately (Hallegraeff and Reid, in preparation).

In periods not dominated by diatom blooms, small coccolithophorids, green flagellates and non-thecate dinoflagellates were found to be the main components of the phytoplankton, demonstrating for the first time their quantitative importance in Australian coastal waters. Jeffrey and Hallegraeff (1980) have also recorded their importance at oceanic stations in the East Australian Current. The presence of nanoplankton is now recognised in many other parts of the world ocean (Throndsen, 1976; Eppley and Weiler, 1979), and they may account for some 40 to 90% of the total phytoplankton chlorophyll (Table 4). Although at the Port Hacking 100 m station nanoplankton chlorophyll levels were remarkably constant (Fig. 2), their cell numbers fluctuated widely, reaching highest concentrations in the period December-March (summer, early autumn; Fig. 4). Estimates of chlorophyll content per cell gave average values of 0.7 to 4 pg for nanoflagellates and 10 to 20 pg for diatom blooms, which would tend to make nanoplankton chlorophyll concentrations fluctuate less sharply than diatom chlorophyll (Table 2).

Whether diatoms or flagellates dominate an area may be due to their differences in nutrient-uptake kinetics and specific growth rates (Eppley *et al.*, 1969), suspension properties (Lännergren, 1979) and suitability as food for filter feeders (Skjoldal and Lännergren, 1978; Malone and Chervin, 1979). At the Port Hacking coastal station, it is possible that an intrusion of nutrient-rich slope water (NO_3-N > 5 μg-at l^{-1}; Fig. 3D) initiates a bloom of diatoms, which then divide more rapidly than flagellates (Parsons *et al.*, 1978). Due to a higher chlorophyll content per cell, a large chlorophyll peak is produced (Fig. 2), and the diatom bloom then becomes subject to rapid dissipation by sinking, coastal currents and high grazing pressure. The role of sinking is also suggested by the frequent association of diatom chlorophyll with density structure in the water column (Newell and Bulleid, 1975; and present Fig. 3D). The importance of

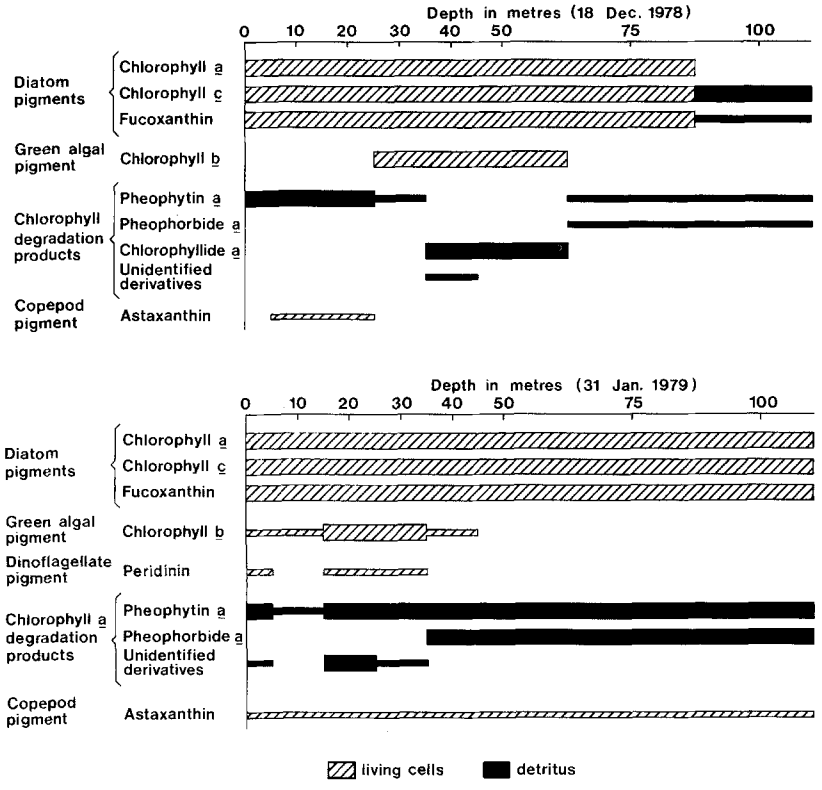

Fig. 6. Summary of pigment profiles with depth on two occasions in 1978. Hatched bars: pigments associated with living cells; black bars: pigments associated with detrital material. Broad and narrow bars indicate pigments which are abundant or present, respectively (see Fig. 5)

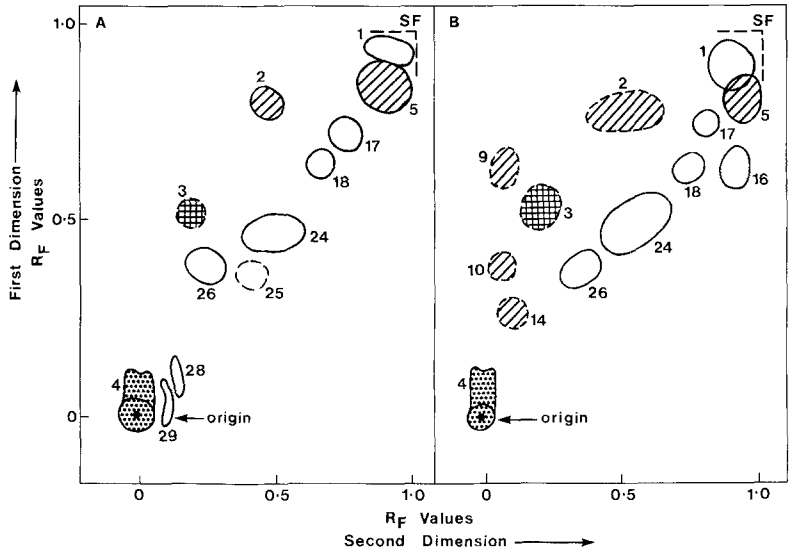

Fig. 7. Thin-layer chromatograms of pigments contained in (A) stomach contents of freshly collected salps (*Thalia democratica*), and (B) fecal pellets of small copepods (mainly *Temora* spp.) fed in the laboratory with natural phytoplankton. Fraction 29:unidentified blue pigment; other pigment fractions as in Fig. 1

grazing by salps and copepods (Fig. 2) is indicated by the abundance of pheophytin *a* and pheophorbide *a* in the samples, particularly from September-October and January-March (Fig. 5). Depletion of nitrate (and silicate) then provides a competitive advantage to the nanoplankton (Eppley *et al.*, 1969), which can thrive under low-nutrient conditions (NO_3-N $\leqslant$ 1 μg-at l^{-1}; Fig. 3B). Being flagellated, they are also better able to maintain their position in the water column than diatoms (Lännergren, 1979; and present Fig. 3). Critical studies on productivity of the nanoplankton and on grazing response by the local zooplankton species are needed to understand their significance in the food-chain dynamics of these waters.

The dominance of the chlorophylls *a*, *b*, *c* and fucoxanthin on the pigment chromatograms (Fig. 5) indicates that diatoms, coccolithophorids, and green flagellates are the main primary producers at the Port Hacking 100 m station (cf. Jeffrey, 1974). Photosynthetic dinoflagellates, containing peridinin, are of minor importance. Microscopic observations showed that the majority of the gymnodinioid dinoflagellates

Table 4. Contribution of nanoplankton to total chlorophyll *a* in different parts of the world oceans

Area	Season	Nanoplankton as % of total chlorophyll	Mesh-size used for fractionation	Source
Polar region				
Barents Sea	late summer	at times > 90%	25 μm	Reynolds (1973)
Temperate waters				
Narragansett Bay	spring + fall summer-winter	~ 20% 44–75%	20 μm	Durbin *et al.* (1975)
New York Bight	winter summer	~ 20% ~ 80%	22 μm	Malone and Chervin (1979)
Subtropics				
North Pacific Central Gyre	–	typically > 90%	10 μm	Eppley and Weiler (1979)
California Current	upwelling events other occasions	~5–~15% 60–99%	22 μm	Malone (1971a)
Port Hacking, Sydney	intrusion events other occasions	10–20% 50–80%	10 μm	Present study
East Australian Current	early summer	45–76%	10 μm	Jeffrey and Hallegraeff (1980)
Tropics				
Caribbean, Tropical Pacific	–	45–98%	22 μm	Malone (1971b)

were colourless, and presumably had a heterotrophic mode of nutrition (Kofoid and Swezy, 1921).

Other chromatographic pigment studies have shown that in some waters pheophytin *a* (Neveux, 1975), pheophorbide *a* (Yentsch, 1970, Jeffrey, 1974), chlorophyllide *a*, or unidentified blue-green derivatives (Jensen and Sakshaug, 1973; Gieskes *et al.*, 1978) may be the major decomposition products of chlorophyll *a*. All these pigments were observed at the Port Hacking station over one annual cycle (Fig. 5). Pheophytin *a* and chlorophyllide *a* were quantitatively the most important, and pheophorbide *a* was important only at greater depths. Unidentified chlorophyll derivatives often occurred, but were quantitatively less important. The results again showed (Jeffrey, 1974; Jeffrey and Hallegraeff, 1980) that degradation products can at times account for more than 50% of the total chlorophyll *a* of seawater samples.

Chlorophyllide *a* is produced by certain marine algae with a highly active enzyme chlorophyllase, which hydrolyses the phytol from the chlorin system of the chlorophyll *a* molecule (Barrett and Jeffrey, 1964, 1971). It was previously thought that chlorophyllide *a* occurred only when senescent diatoms were present (Jeffrey, 1974), but recent studies have shown that it can also be formed during the filtration procedure, when harvesting fragile phytoplankton species which contain an active chlorophyllase (Jeffrey and Hallegraeff, in preparation).

Pheophorbide *a* (loss of magnesium and phytol) is known to be a major pigment in copepod fecal pellets (Jeffrey, 1974; Shuman and Lorenzen, 1975). In the present work, pheophytin *a* (loss of magnesium) was the major form of degraded chlorophyll, both in salp stomachs and in fecal pellets from Port Hacking copepods (Fig. 7). The results demonstrate that species-specific differences in feeding behaviour, and in gut acidity and in activity of digestive enzymes may affect the outcome of chlorophyll degradation by herbivore grazing processes.

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