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The presence of chlorophyll *b* and the estimation of phaeopigments in marine phytoplankton

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Abstract. A reverse-phase h.p.l.c. technique was used to estimate the concentration of chlorophyll *b* in phytoplankton cultures, fecal pellets of *Calanus pacificus*, and suspended particulate matter from the Central North Pacific, Oregon coastal waters, and Dabob Bay (a temperate fjord in Puget Sound, WA, USA). The purpose was to assess the distribution of this pigment in the euphotic zone and its effect on the fluorometric estimation of phaeopigments. Analyses of natural waters confirm high chlorophyll *b* concentrations (median mass ratio of chl *b*:*a* > 0.3) at the depth of the chlorophyll *a* maximum in tropical waters while values for temperate plankton are relatively low (median mass ratio of chl *b*:*a* = 0.05) and patchy. Zooplankton fecal pellets showed a significant enrichment in chlorophyll *b*, suggesting grazing as a mechanism to explain high concentrations of this pigment at the bottom of the euphotic zone. It is estimated that the presence of chlorophyll *b* could cause an average overestimation of phaeopigment concentration by the fluorometric technique of 38% between 0 and 200 m in the Central North Pacific. This effect is more pronounced at the layer of chlorophyll *b* maximum (120–140 m).

Introduction

Chromatographic analyses by t.l.c. of open-ocean (Jeffrey, 1976; Gieskes *et al.*, 1978) and coastal waters (Jeffrey, 1974; Hallegraeff, 1981) have indicated the presence of chlorophyll *b* as a cosmopolitan feature. Furthermore, the mass ratio of chlorophyll *b* to chlorophyll *a* (chl *b*:*a*) in equatorial and tropical plankton reaches a maximum of 0.55, a surprisingly high value when compared to laboratory cultures of chlorophytes and terrestrial plants which have ratios <0.3 (but see Ricketts, 1970; Wood, 1979). If chlorophyll *a* is not being degraded preferentially over chlorophyll *b* during the chromatographic analysis, these results imply the dominance of green algae which have a high cellular chlorophyll *b* content or an accumulation of chlorophyll *b* in other particles, such a detritus (Jeffrey, 1976).

The presence of chlorophyll *b* in marine waters is also of interest because of its influence on the fluorometric estimation of chlorophyll *a* and phaeopigments. The method introduced by Yentsch and Menzel (1963) and modified by Holm-Hansen *et al.* (1965) assumes that the only pigments present in the acetone extract are chlorophyll *a* and phaeophytin *a*. Chlorophyll *b*, however, may decrease the estimated concentration of chlorophyll *a* by a factor equal to 30% of its concentration (Gibbs, 1979; Lorenzen, 1981) due to its lower acid ratio (relative fluorescence before and after acidification). Furthermore, Gibbs (1979) demonstrated that the concentration of phaeopigments is overestimated by a factor of 2.5 times the chlorophyll *b* concentration in the sample while Loftus and Carpenter (1971) estimated a factor of 1.9 times the chlorophyll *b* concentration, and Lorenzen and Jeffrey (1980) showed the overestimation to be equal to the chlorophyll *b* concentration (factor of 1.0).

Lorenzen (1981), using h.p.l.c., tested the hypothesis of chlorophyll *b* interference for field samples. The concentrations of chlorophyll *b* found would not cause, on the average, an underestimation of chlorophyll *a* > 10%, but his sampling was mostly confined to surface samples in North East Pacific. The present study originated when we found consistently high concentrations of chlorophyll *b* at the bottom of the euphotic zone in the tropical North Pacific. We report here abundances of chlorophyll *b* measured by h.p.l.c. in Central and North East Pacific, in unialgal cultures, and in fecal pellets of *Calanus pacificus*. Analyses of chlorophyll *a*, *b* and *c* by h.p.l.c. were compared to simultaneous measurements of chlorophyll *a* and phaeopigments by filter fluorometry. Errors incurred in the fluorometric determination by the presence of the accessory chlorophylls is estimated and the ecological implications discussed.

Methods

Several areas of the North Pacific were sampled for water column pigments during 1981 and 1982. Tropical waters are represented by four stations visited during March 1982 south of the Hawaiian Islands (lat. 13° and 18°N; long. 157°W; cruise R/V *Thomas G. Thompson* # 165). Forty-five samples were taken between surface and 200 m (0, 30, 60, 100, 110, 120, 150 and 200 m). Shelf waters were sampled off the coast of Oregon during August 1981 (cruise R/V *T. G. Thompson* # 160) and June 1982 (cruise R/V *Wecoma* 8206a). Transects ran betwen 124°13′ and 125°08′W on parallel 47°07′N). Depths sampled corresponded approximately to surface, 1% incident radiation, and depth of minimum transmission. The latter was interpreted as the depth of the chlorophyll *a* maximum (Kitchen *et al.*, 1978). The third area studied was Dabob Bay, a fjord-like embayment in northern Puget Sound, WA (lat. 47°50′N, long. 122°55′W). Depths corresponding to 100, 10 and 1% incident radiation were sampled from April 1981 to October 1982.

Water column samples were collected with Niskin bottles. Large sinking particles (i.e. zooplankton fecal pellets) were collected from Dabob Bay using sediment traps (Lorenzen *et al.*, 1981) placed at midwater (55 m) below the euphotic zone. Deployments lasted for either 1 day or 1 month. Pigments associated with macrozooplankton fecal pellets were also produced under laboratory conditions. *C. pacificus* were fed either *Thalassiosira weissflogii* (diatom) as a source of chlorophyll *a* and *c* or *Dunaliella tertiolecta* (chlorophyte) for chlorophylls *a* and *b*. Zooplankton and their pellets were kept separated by a 300-μm mesh screen placed near the bottom of the vessel. The experiments ran for 48 h in the dark at 8°C after which the pellets were concentrated, rinsed and filtered.

All samples were filtered through glass-fiber filters (Whatman GF/F or GF/C) under 7.5 mm Hg differential vacuum. Filtered samples for fluorometric analysis were processed in the field immediately after sampling; filters for h.p.l.c. analysis were stored in liquid nitrogen (−196°C) for an average of 2 weeks. For both analyses, pigments were extracted with 90% acetone; cells and filters were either ground with a tissue grinder or lysed by ultrasound and the extract centrifuged until clear.

Chlorophylls *a*, *b* and *c*, phaeophytin *a* and phaeophorbide *a* were analyzed by h.p.l.c. with a reverse-phase C-18 Bondapak column using a stepwise solvent elution program. Ninety-percent methanol in water (v/v) eluted chlorophyll *c* and phaeophorbide *a*; 100%

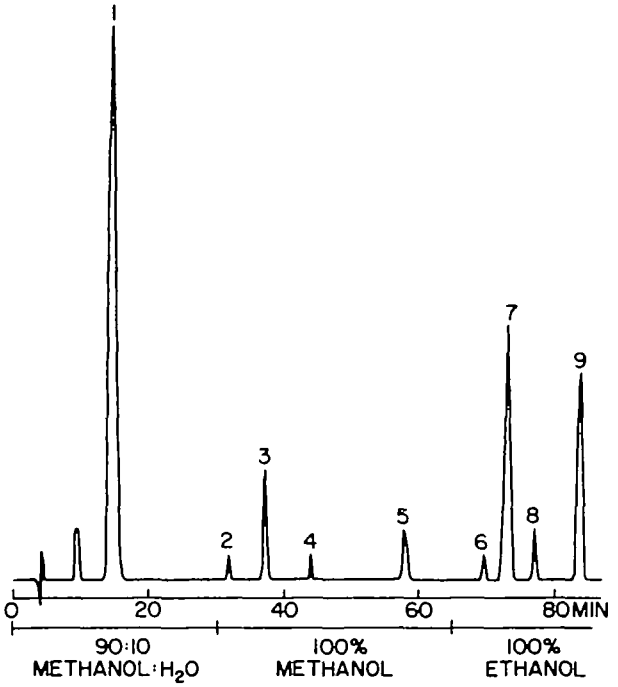

Fig. 1. Separation of pigments and phaeopigments by reverse-phase h.p.l.c.: pigments present in faecal pellets of *C. pacificus* feeding on the flagellate *D. tertiolecta*. Pigments monitored at 410 nm. Peak identities: **1.** phaeophorbide *a*, **2.** neoxanthin, **3.** xanthophyll, **4.** chlorophyll *b*, **5.** chlorophyll *a*, **6.** β,β-carotene, **7.** phaeophytin *a*, **8** and **9.** phaeophytin-like pigments.

methanol eluted chlorophylls *b* and *a*; and 100% ethanol eluted phaeophytin *a* (Figure 1). Characteristics of the system are summarized in Table I. Each sample was analyzed in two consecutive runs: chlorophylls were monitored at 440 nm and phaeopigments at 410 nm.

The column was calibrated by injecting known amounts of pigments to the column. Pigments from axenic cultures were purified in cellulose (Mackerel Nagel MN-300) t.l.c. following the procedures of Jeffrey (1968) and Guillard and Lorenzen (1972). Concentrations of purified pigments were estimated spectrophotometrically in a Unicam SP-1800 using the specific absorption coefficients (α) shown in Table I.

Fluorometric measurements of chlorophyll *a* and phaeopigments were taken with a Sequoia-Turner Model 112 fluorometer with a red photomultiplier and filters as specified in Lorenzen (1966). The instrument was calibrated with an axenic culture of *Isochrysis galbana* growing exponentially in f/2 medium (Guillard and Ryther, 1962).

A quantitative estimation of the interference of chlorophylls *b* and *c* in the filter fluorometric technique was performed using extracts with known ratios of chlorophylls *b*:*a* and *c*:*a*. Unialgal cultures of phytoplankton species containing either chlorophyll *b* or *c* as accessory pigment were analyzed for chlorophylls and phaeopigments by h.p.l.c. and filter fluorometer. Cells were harvested during exponential growth to avoid chlorophyll *a* degradation. Cultures of *D. tertiolecta* and three unidentified flagellates (Prasinophytes strains BB-1, Pras-G and 29-a) were used as a source of chlorophyll

Table I. Spectrophotometric and chromatographic characteristics of pigments analyzed in the h.p.l.c. system.

	Pigments				
	Chlorophylls			Phaeophytin	Phaeophorbide
	a	*b*	*c*	*a*	*a*
Absorption maxima (nm)	432 663	457 646	448 634	410 668	409 665
Solvent	100% acetone	100% acetone	100% methanol	100% acetone	100% methanol
Specific absorption coefficient (α) (1 g^{-1} cm^{-1})	92.0[a]	53.5[a]	15.2[a]	51.0[b]	74.0[b]
Wavelength (nm) monitored in h.p.l.c.	440	440	440	410	410
H.p.l.c. peak analysis	height	area	area	area	area
Acid ratio (fluorescence before acidification)/(fluorescence after acidification)	2.32 (100% MeOH) 2.14 (100% acetone)	0.60 (100% MeOH) 0.61 (100% MeOH)	2.30 (90% acetone)	1.20 (100 EtOH)	1.30 (90% MeOH)

[a]Jeffrey (1968).
[b]Lorenzen and Jeffrey (1980).

b. Phytoplankton species for chlorophyll *c* included two dinoflagellates (*Scrippsiella* 276, D-166), one diatom (*T. weissflogii*), and four flagellates (strains CHR-245, CriLi, CP, Cr-65). All cultures were grown in f/2 medium, under constant illumination (250 μE m^{-2} s^{-1}), and at a temperature of either 13 or 16°C. Innocula were provided by Beatrice Booth at the University of Washington. In addition, chlorophylls *a* and *b*, previously purified by t.l.c. and estimated by spectrophotometry, were mixed in different proportions. The range of chl *b:a* included all values found in natural samples ($0.0 <$ chl *b:a* < 0.8). The total concentration of (chl *a* + chl *b*) was kept constant at a value of 2.28 μg l^{-1} of extract for the series of dilutions.

Results

Abundance of chlorophyll 'b' in phytoplankton

The concentration of chlorophyll *b* is expressed in μg l^{-1} and as the mass ratio of chlorophyll *b* to chlorophyll *a* (chl *b:a*). The ratio is convenient as an index of the relative concentration of both pigments and reduces the variance between consecutive chromatographic runs when comparing different samples.

Fifty-five out of 93 samples from Dabob Bay had measurable concentrations of chlorophyll *b* (Figure 2). Chlorophyll *a* ranged from 0.2 to 1.3 μg l^{-1} and chlorophyll *b* from undetectable to 0.2 μg l^{-1}. The ratio of chl *b:a* had a median of 0.05 and was never observed >0.2 (Table II). Chlorophyll *b* was least abundant during winter and spring months and presented a subsurface maximum between 3 and 13 m in summer.

Sediment traps from Dabob Bay had very low chlorophyll *b* concentrations. Only four samples out of 21 showed any measurable amounts (Table II).

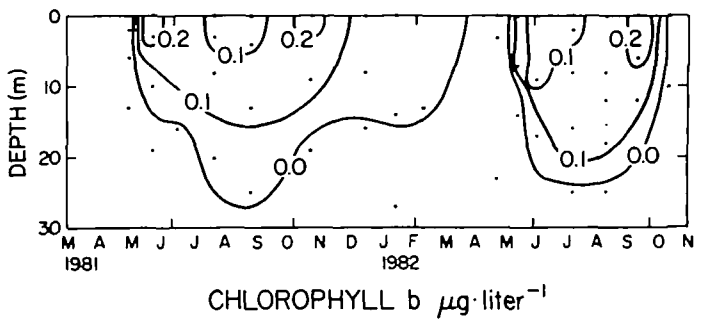

Fig. 2. Temporal and spatial distribution of chlorophyll *b* concentration in μg l^{-1} in Dabob Bay as measured by h.p.l.c. during monthly cruises.

Table II. The mass ratio of chlorophyll *b:a* in phytoplankton: tropical waters of the North Pacific Ocean; temperate waters off the coast of Washington and Dabob Bay, Washington; and in sediment traps (Dabob Bay).

Area	Chlorophyll *b:a*		Total	Chlorophyll $b \neq 0$
	Median	Range	*n*	*n*
Tropical waters south of Hawaii	0.15	0.00–0.43	45	32
Washington coast	0.00	0.00–0.28	47	11
Dabob Bay plankton	0.00	0.00–0.19	93	55
Dabob Bay sediment traps	0.00	0.00–0.57	21	4

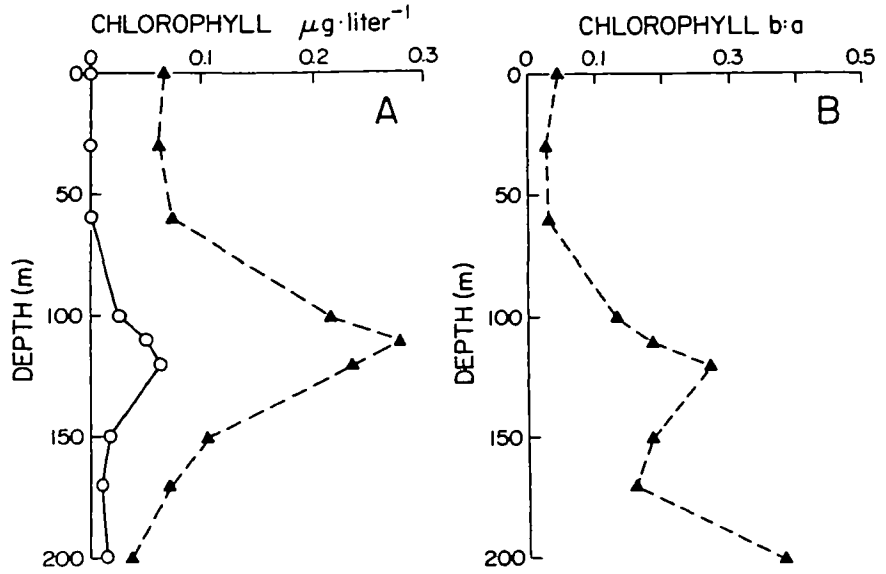

Fig. 3. Chlorophylls in tropical waters. (a) Profiles of chlorophyll *a* (triangles) and chlorophyll *b* (open circles) with depth; (b) profile of the ratio of chlorophyll *b*:*a* with depth.

The presence of chlorophyll *b* off the Washington coast was usually limited to offshore stations over the continental slope. During an event of strong upwelling (22–24 June 1982), no chlorophyll *b* was found over the continental shelf and only two stations had measurable amounts during a period of relaxation (18–22 August 1981). In summary, from a total of 47 samples in the euphotic zone, chlorophyll *b* was measured in 11 samples, 9 of which were taken over the slope (Table II).

Tropical waters showed a different picture. Chlorophyll *b* was always found from 100 to 200 m but was not detected in several samples of the mixed layer (0–60 m). Chlorophyll *b* concentrations peaked in coincidence with chlorophyll *a* maxima (Figure 3).

Feeding experiments showed that in fecal pellets of *C. pacificus* the average chl *b*:*a* increased by a factor of two with respect to the pigment ratio in the food source, *D. tertiolecta*. Chlorophyll *b*:*a* in algae was 0.16 (range 0.13–0.17) while in the fecal pellets the ratio was 0.34 (0.20–0.48, $n = 4$).

Chlorophylls 'b' and 'c' and the estimation of phaeopigments

Simultaneous determinations of chlorophylls *a* and *b* from cultures by spectrophotometry and chlorophyll *a* and phaeopigments by fluorometry revealed that chlorophyll *b* would cause an overestimation of phaeopigment concentration in the filter fluorometer proportional to chlorophyll *b* concentration. The overestimation of $[\text{phaeopigment}]_{\text{FLUOR}}$ in $\mu g\ ml^{-1} = 0.03 + 1.64$ [chlorophyll *b*], $n = 13$, $r^2 = 0.81$. This effect is due to the decrease of the ratio of fluorescence before and after acidification (F_o/F_a) that is proportional to chlorophyll *b* concentration for a constant [chl*a* + chl*b*] (Figure 4a). When the same measurements were repeated in several species of flagellates with chlorophyll *b* (chlorophylls *a* and *b* estimated by h.p.l.c.), it was found that, on the average,

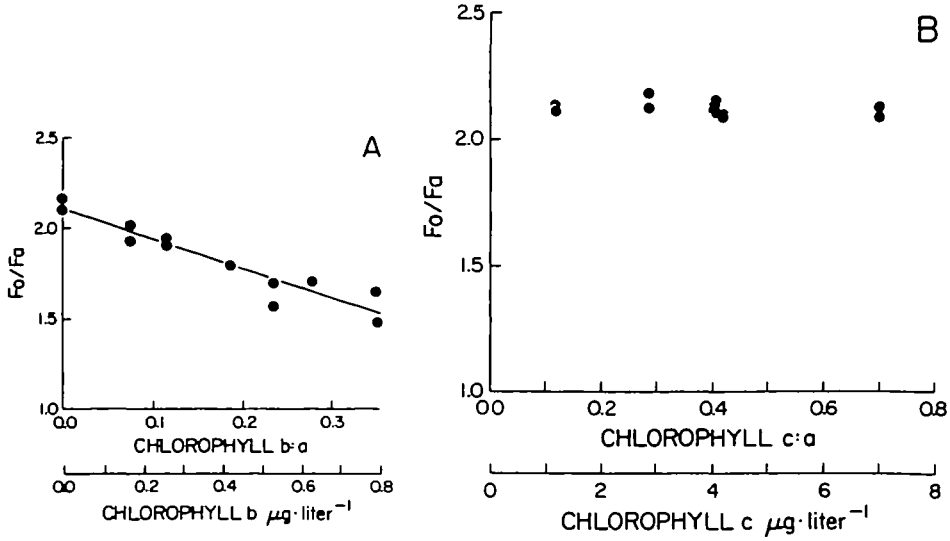

Fig. 4. Effect of chlorophyll *b* and *c* on measurements with the filter fluorometer. (a) The decrease of the ratio of fluorescence before and after acidification (F_o/F_a) as a function of the ratio of chl *b*:*a*. Chlorophylls *a* and *b* were purified by t.l.c. and mixed in different proportions with [chl *a* + chl *b*] = 2.28 μg l^{-1} of extract. (b) The ratio of fluorescence before and after acidification $(F_o/F)_a$ as a function of the ratio of chl *c*:*a*. Ninety-percent acetone:water (v/v) extracts of species with different chl *c*:*a* ratios were injected to the column with simultaneous estimations of the *F* ratio in the filter fluorometer.

Table III. The effect of chlorophyll *b* concentration on the estimation of phaeopigments in the filter fluorometer for four chlorophyll *b*-containing marine algae harvested during exponential growth and with undetectable phaeopigment concentration by h.p.l.c.

Culture	[*chl b*] h.p.l.c.	[phaeo] FLUOR	[phaeo FLUOR] / [chl *b*]
Dunaliella tertiolecta	4.57	8.16	1.78
Dunaliella tertiolecta	1.48	3.18	2.14
Dunaliella tertiolecta	1.75	3.48	1.98
Prasinophyte 1 (BB-1)	0.39	0.81	2.08
Prasinophyte 2 (Pras-G)	1.79	2.36	1.32
Prasinophyte 3 (29-a)	4.66	5.35	1.15

phaeopigment concentration was overestimated by a factor 1.75 ± 0.38 the concentration of chlorophyll *b* in the sample (Table III). These samples were free of either phaeophytin or phaeophorbide.

No measurable change in the acid ratio of extracts of chlorophyll *c*-containing algae was observed for a range of values of chl *c*:*a* from 0.1 to 0.71 (Figure 4b). In consequence, the effect of chlorophyll *c* on the calculated phaeopigment concentration was not significant: overestimation $[\text{phaeopigment}]_{\text{FLUOR}}$ in μg l^{-1} = −0.14 + 0.11 [chlorophyll *c*], $n = 8$, $r^2 = 0.32$, $\alpha = 0.05$.

The effect of chlorophyll *b* on the concentration of phaeopigments in the water column, as measured by filter fluorometer, was calculated for the four stations in tropical

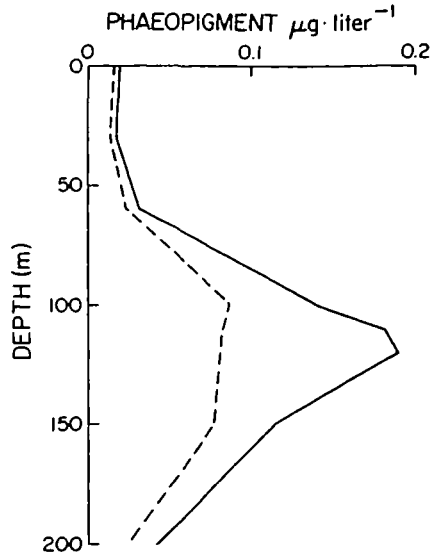

Fig. 5. Vertical profile of phaeopigment concentration in tropical waters South of Hawaii. Composite profile for four stations (n = 45). Solid line: phaeopigments as estimated in the fluorometer; dashed lines: phaeopigment concentrations after correcting for chlorophyll *b* concentration in the sample.

waters South of Hawaii, using the 1.75 factor described above. For each sample, percent correction was calculated as 1 − [corrected phaeopigment concentration × (initial phaeopigment concentration)$^{-1}$] × 100. The average decrease for all samples was 38%. Figure 5 shows the estimated decrease for each depth sampled. When the same correction was applied to Dabob Bay, it was found that chlorophyll *b* did not affect more than half the samples (median = 0.0) and that only a few cases (12 samples out of 93) showed an overestimation >20%.

Discussion

Abundance of chlorophyll 'b' in phytoplankton

The high mass ratio of chl *b:a* systematically found at the depth of the chlorophyll *a* maximum in open ocean (Table II) confirms preliminary results for tropical waters found by Jeffrey (1976) and Gieskes *et al.* (1978). Three possible mechanisms may contribute to the existence and maintenance of this chlorophyll *b* subsurface maximum: a higher abundance of phytoplankton with chlorophyll *b* as accessory pigment with respect to surface waters coupled with taxa rich in chlorophyll *b* content (Wood, 1979), cells adapted to low-light conditions, and the presence of tripton enriched with chlorophyll *b*.

There is no independent evidence that phytoplankton with chlorophyll *b* (Chlorophytes and Prasinophytes) form a larger proportion of the plankton community at the depth of the chlorophyll *a* maximum. Quantitative cell counts by light microscopy have not identified these taxa (Beers *et al.*, 1975; Fryxell *et al.*, 1979; Venrick, 1982) in the Central North Pacific although transmission electron microscopy has shown their association with the picoplankton fraction (Takahashi and Hori, 1984).

Phytoplankton species containing chlorophyll *b* and adapted to low-light conditions

have shown higher pigmentation per cell and a higher chl *b*:*a* ratio (Halldal, 1970; Prézelin, 1981) when compared to cells grown at high light. In the area studied, chlorophyll *a* and *b* maxima were ≤1% incident radiation level so that cells are expected to be light limited (Dugdale, 1967; Eppley *et al.*, 1973). *Chlorella*-type cells in the Western North Pacific have been observed to be more pigmented at the depth of the subsurface chlorophyll maximum (Takahashi and Hori, 1984), suggesting also that a higher chl *b*:*a* ratio may be expected.

The association of undegraded pigments with detrital material has been well documented for macrozooplankton fecal pellets (Patterson and Parsons, 1963; Jeffrey, 1974; Hallegraeff, 1981). Chlorophyll *b* can also be present in these pellets; and due to its higher stability with respect to chlorophyll *a*, it is possible to see an increase in the ratio of chl *b*:*a* of the pellets of macrozooplankton compared to the food source (Daley, 1973; this study). If similar mechanisms are acting in suspended detrital material that results from microzooplankton grazing activity (SooHoo and Kiefer, 1982; Welschmeyer and Lorenzen, 1985), then this source of chlorophyll *b* in tropical plankton samples could be considerable.

The ratios of chl *b*:*a* found off the coast of Washington and in Dabob Bay are consistent with values published by Lorenzen (1981) for the same area and by Hallegraeff (1981) for the east coast of Australia. Two features are of particular interest. First, although chlorophyll *b* was present all year in Dabob Bay, its distribution was more patchy than the other chlorophylls. The patchiness was both spatial and temporal. Secondly, chlorophyll *b* was not found during periods of phytoplankton bloom, either during spring or fall in Dabob Bay or in areas of upwelling off the coast of Washington.

In conclusion, for the three areas studied, the plankton (and perhaps detritus) associated with the chlorophyll *a* maximum in tropical waters of the open ocean are richest in chlorophyll *b*. In temperate waters both water-column samples and macrozooplankton fecal pellets typically show lower chlorophyll *b* concentrations.

Chlorophylls 'b' and 'c' and the estimation of phaeopigments

The overestimation of phaeopigments by the presence of chlorophyll *b* measured in this study (1.75 [chl *b*]) is lower than the 2.5 factor found by Gibbs (1979) using the spectrofluorometer, higher than the results of Lorenzen and Jeffrey (1980) who measured the increase in phaeophorbide concentration to be equal to that of chlorophyll *b*, and similar to the 1.95 factor of Loftus and Carpenter (1971). The difference in the instruments seems to be the more plausible explanation for these discrepancies (Yentsch, 1965) and shows that no generalization with respect to the magnitude of the error can be made.

The discrepancy between the phaeopigment concentration measured in the water column by fluorometry and its chl *b*-corrected value (Figure 5) is most pronounced between 100 and 150 m, the depth of the phaeopigment maximum. The presence of these maxima, associated with or below the chlorophyll *a* maxima, is a feature that has been described for several areas of the ocean (Hobson and Lorenzen, 1972), including the area of our study (Eppley *et al.*, 1973; Bienfang and Szyper, 1981). The chlorophyll *b* correction does not remove this feature but does decrease the overall concentration of phaeopigment suspended in the euphotic zone and flattens the phaeopigment subsurface maximum.

Chlorophyll *b* does not cause a substantial error in the calculation of phaeopigments in the temperate waters of the North East Pacific sampled in this study. It is only during months with a thermally stratified water column (May – August) that a correction might be necessary.

A lower phaeopigment concentration in the water column implies that estimates of microzooplankton grazing from pigment budgets (SooHoo and Kiefer, 1982; Welschmeyer and Lorenzen, 1985) would be lower than previously believed for certain parts of the ocean. It appears that at least for tropical communities it would be more accurate to measure phaeopigments independently (i.e. with h.p.l.c.) or, if not possible, to estimate chlorophyll *b* concentrations and apply a correction to the original results.

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References

Beers,J.R., Reid,R.M.H. and Stewart,G.L. (1975) Microplankton of the North Pacific Central Gyre. Population structure and abundance, June 1973. *Int. Rev. Gesamten Hydrobiol.*, **69**, 607–638.

Bienfang,P.K. and Szyper,J.P. (1981) Phytoplankton dynamics in the subtropical Pacific Ocean off Hawaii. *Deep-Sea Res.*, **28A**, 981–1000.

Daley,R.J. (1973) Experimental characterization of lacustrine chlorophyll diagenesis. II. Bacterial, viral and herbivore grazing effect. *Arch. Hydrobiol.*, **72**, 409–439.

Dugdale,R.C. (1967) Nutrient limitation in the sea: dynamics, identification and significance. *Limnol. Oceanogr.*, **12**, 196–206.

Eppley,R.W., Renger,E.H., Venrick,E.L. and Mullin,M.M. (1973) A study of plankton dynamics and nutrient cycling in the Central gyre of the North Pacific Ocean. *Limnol. Oceanogr.*, **18**, 534–551.

Fryxell,G.A., Taguchi,S. and El-Sayed,S.Z. (1979) Vertical distribution of diverse phytoplankton communities in the Central Pacific. In Bischoff,J.L. and Piper,D.Z. (eds), *Marine Geology and Oceanography of the Pacific Manganese Nodule Province*. Plenum Press, New York, pp. 203–239.

Gibbs,C.F. (1979) Chlorophyll *b* interference in the fluorometric determination of chlorophyll *a* and 'phaeopigments'. *Austr. J. Mar. Freshwater Res.*, **30**, 597–606.

Gieskes,W.W.C., Kraay,G.W. and Tijssen,S.B. (1978) Chlorophylls and their degradation producs in the deep pigment maximum layer of the tropical North Atlantic. *Neth. J. Sci. Res.*, **12**, 195–204.

Guillard,R.R.L. and Lorenzen,C.J. (1972) Yellow-green algae with chlorophyllide *c*. *J. Phycol.*, **8**, 10–14.

Guillard,R.R.L. and Ryther,J.H. (1962) Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervaceae* (Cleve) Gran. *Can. J. Microbiol.*, **8**, 229–239.

Halldal,P. (1970) The photosynthetic apparatus of microalgae and its adaptation to environmental factors. In Halldal,P. (ed.), *Photobiology of Microorganisms*. Wiley-Interscience, London, pp. 17–54.

Hallegraeff,G.M. (1981) Seasonal study of phytoplankton pigments and species at a coastal station off Sydney: importance of diatoms and the nanoplankton. *Mar. Biol.*, **61**, 107–118.

Hobson,L.A. and Lorenzen,C.J. (1972) Relationship of chlorophyll maxima to density structure in the Atlantic Ocean and Gulf of Mexico. *Deep-Sea Res.*, **19**, 297–306.

Holm-Hansen,O., Lorenzen,C.J., Holmes,R.W. and Strickland,J.D.H. (1965) Fluorometric determination of chlorophyll. *J. Cons. Perm. Int. Expl. Mer*, **30**, 3–15.

Jeffrey,S.W. (1968) Quantitative thin-layer chromatography of chlorophylls and carotenoids from marine algae. *Biochim. Biophys. Acta*, **162**, 271–285.

Jeffrey,S.W. (1974) Profiles of photosynthetic pigments in the ocean using thin-layer chromatography. *Mar. Biol.*, **26**, 101–110.

Jeffrey,S.W. (1976) A report of green algal pigments in the Central North Pacific Ocean. *Mar. Biol.*, **37**, 33–37.

Kiefer,D.A., Olson,R.J. and Holm-Hansen,O. (1976) Another look at the nitrite and chlorophyll maxima in the Central North Pacific. *Deep-Sea Res.*, **23**, 1199–1208.

Kitchen,J.C., Zaneveld,J.R.V. and Pak,H (1978) The vertical structure and size distributions of suspended particles off Oregon during the upwelling season. *Deep-Sea Res.*, **25**, 453–468.

Loftus,M.E. and Carpenter,J.H. (1971) A fluorometric method for determining chlorophylls *a*, *b*, and *c*. *J. Mar. Res.*, **29**, 319–338.

Lorenzen,C.J. (1966) A method for the continuous measurement of *in vivo* chlorphyll concentration. *Deep-Sea Res.*, **13**, 223–227.

Lorenzen,C.J. (1981) *Chlorophyll b* in the ocean. *Deep-Sea Res.*, **28**, 1049–1056.

Lorenzen,C.J. and Jeffrey,S.W. (1980) Determination of chlorophyll in seawater. *UNESCO Tech. Pap. Mar. Sci.*, 35.

Lorenzen,C.J., Shuman,F.R. and Bennett,J.T. (1981) In situ calibration of a sediment trap. *Limnol. Oceanogr.*, **26**, 580–585.

Patterson,J. and Parsons,T.R. (1963) Distribution of chlorophyll *a* and degradation products in various marine materials. *Limnol. Oceanogr.*, **8**, 355–356.

Prézelin,B.B. (1981) Light reactions in photosynthesis. In Platt,T. (ed.), *Physiological Bases of Phytoplankton Ecology. Can. Bull. Fish. Aquat. Sci.*, **210**, 346 pp.

Ricketts,T.R. (1970) The pigments of the Prasinophyceae and related organisms. *Phytochemistry*, **9**, 1835–1842.

SooHoo,J.B. and Kiefer,D.A. (1982) Vertical distribution of phaeopigments. I. A simple grazing and photooxidative scheme for small particles. *Deep-Sea Res.*, **29**, 1539–1551.

Takahashi,M. and Hori,T. (1984) Abundance of picoplankton in the subsurface chlorophyll maximum layer in subtropical and tropical waters. *Mar. Biol.*, **79**, 177–186.

Venrick,E.L. (1982) Phytoplankton in an oligotrophic ocean: observations and questions. *Ecol. Monogr.*, **52**, 129–154.

Welschmeyer,N.A. and Lorenzen,C.J. (1985) Chlorophyll budgets: zooplankton grazing and phytoplankton growth in a temperate fjord and the Central Pacific Gyres. *Limnol. Oceanogr.*, **30**, 1–21.

Wood,A.M. (1979) Chlorophyll *a:b* ratios in marine planktonic algae. *J. Phycol.*, **15**, 330–332.

Yentsch,C.S. (1965) The relationship between chlorophyll and photosynthetic carbon production with reference to the measurement of decomposition products of chloroplastic pigments. *Mem. Ist. Ital. Idrobiol.*, **18** (suppl.), 323–346.

Yentsch,C.S. and Menzel,D.W. (1963) A method for the determination of phytoplankton chlorophyll and phaeophytin fluorescence. *Deep-Sea Res.*, **10**, 221–231.

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