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Light absorption by phytoplankton and the filter amplification correction: cell size and species effects

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Abstract

Filter amplification corrections are presented for eight marine centric diatoms. These functions are required to correct the amplified optical path-length associated with the glass-fiber filter used in the measurement of phytoplankton absorption. Correction factors constructed from phytoplankton cultures in the laboratory are often applied to phytoplankton assemblages in the field. This study demonstrates significant differences in the filter amplification correction correlated to species and cell volume. This variation in the filter amplification correction can result in significant error in estimated absorption coefficients, compromising subsequent estimates of quantum yield and primary production.

Keywords: Beta-factor; Cell size; Filter amplification; Light absorption; Phytoplankton

1. Introduction

Light absorption by phytoplankton can be used to analyse community structure and as an input into bio-optical estimates of primary production (Keifer and Mitchell, 1983; Sathyendranath et al., 1996). Unfortunately, the concentration of phytoplankton in the

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water column is often too low to quantify absorption in solution using standard methods. This makes the quantitative determination of light absorption by phytoplankton difficult. The quantitative filter technique (QFT) concentrates phytoplankton on glass-fiber filters and measures absorption in a spectrophotometer. Subsequently, a correction—the β-correction—must be applied for the increased path-length the light travels due to the scattering of the filter. There is controversy regarding the value of this correction.

Initially, workers used the ratio of the optical (optical density in suspension OD_s) to geometric (optical density on the filter OD_f) path-length as the β-factor. Kishino et al. (1985) found this ratio to be variable, and suggested that this was due to spectrophotometer, filter and particle type. Mitchell and Keifer (1984, 1988) found the β-factor varied with sample optical density. They estimated optical density in suspension from the optical density on the filter as:

$$OD_s(\lambda) = aOD_f(\lambda) + bOD_f^2(\lambda). \quad (1)$$

This filter amplification correction has been used extensively over the last decade (Cleveland and Weidemann, 1993; Hoepffner and Sathyendranath 1992, 1993; Arbones et al., 1996). Despite its wide application, the β-correction remains the largest source of error in estimated absorption coefficients (Roessler, 1998).

In laboratory studies, β-correction polynomials can be determined for all samples, but it is often impractical or impossible to determine these corrections in the field. Therefore β-correction functions determined from laboratory studies are often applied to new field data (Hoepffner and Sathyendranath, 1992, 1993; Babin et al., 1996; Sathyendranath et al., 1996). This practice may be problematic since different species can have radically different β-factors (Arbones et al., 1996). Moore et al. (1995) found that the prochlorophyte *Prochlorococcus marinus* has a very different β-correction from previously documented species. They suggest that cell size may be a factor in this difference. This variability in the correction can result in significant differences in reported absorption coefficients and affect subsequent estimates of quantum yield (Moore et al., 1995).

The β-correction polynomials (Eq. (1)) were determined for eight marine centric diatoms that range in cell volume from ~ 10 to 250 000 μm^3; in aggregate, the eight separate β-correction factors are subsequently referred to as the species model. A β-correction size class model is constructed from the grouping of species into size-classes, representing small, medium and large cells. A pooled, or general diatom β-correction polynomial is calculated from all the data. We show that there is a statistical difference between the species, size and pooled models. Then, we show that a significant error is incurred in the calculation of the absorption coefficient if a single, general β-correction is applied to the optical density spectra of the specific diatom species. Finally, the correlation between the β-correction and cell volume is discussed.

2. Methods

Eight centric diatom species were chosen to represent a wide spectrum of cell volumes: *Chaetoceros calcitrans* (CCMP1315), *Thalassiosira pseudonana* (CCMP1335),

two *Chaetoceros* spp. (Labrador Sea and St. Andrews isolates), *T. weissflogii* (CCMP1336), *Hyalodiscus* sp. (CCMP1679), and two *Coscinodiscus* spp. (CCMP312 and CCMP1583). All cultures were obtained from the Provasoli-Guillard National Center for the Culture of Marine Phytoplankton (CCMP), with the exception of the *Chaetoceros* spp.—one isolate came from the Huntsman Marine Science Center in St. Andrews, New Brunswick (J. Jellett, personal communication; B.I.O., Dartmouth, NS) and the second was isolated from a Bedford Institute of Oceanography cruise on the Labrador Sea (V. Lutz, personal communication; B.I.O., Dartmouth, NS). Cultures were grown in f/2-enriched, 0.45-μm-filtered Bedford Basin seawater as described in Finkel (2001). Before the spectrophotometric analysis, cultures were diluted with nutrient media to provide a range of optical densities.

The optical density of phytoplankton both on filters and in suspension was obtained from a Shimadzu UV-2101 PC spectrophotometer equipped with an integrating sphere. Optical densities were determined from 350 to 750 nm with a 1-nm spectral bandwidth; OD at 750 nm was adjusted to zero. Only OD from 400 to 700 nm were used in the construction of the β-correction. The optical density of the phytoplankton in suspension was determined by placing phytoplankton culture in a 1-cm quartz cuvette with the same volume of filtered media in a second cuvette serving as a blank. To determine the optical density of the phytoplankton on the filter (OD_f), phytoplankton culture was filtered under low pressure onto a Whatman GF/F filter. Culture volumes were chosen so that the geometric path-length of the filtered samples matched the path-length in the cuvette. The blank was obtained from the same volume of filtrate, filtered under low pressure onto a second filter. The two filters were placed in the spectrophotometer on special filter holders immediately after the filtration step.

The β-correction was determined by an ordinary least-squares fit of Eq. (1) to experimentally determined OD data for each species. Following Cleveland and Weidemann (1993), optical densities above 0.4 were not included in the analysis. Eq. (1) was also fitted to the pooled data to construct a general diatom correction factor, and sub-sets of the data representing different size-classes of phytoplankton. The size model is constructed of three size-classes: the small cells (*C. calcitrans*, *T. pseudonana* and the two *Chaetoceros* spp.), the medium cells (*T. weissflogii* and *Hyalodiscus* sp.), and the

Table 1
β-correction and cell volume (μm^3) for the diatom taxa

Taxa	Volume (μm^3)	*a*	SE	*b*	SE	r^2	*n*
C. calcitrans	$1.02*10^1$	0.378	0.004	0.726	0.025	0.995	1505
T. pseudonana	$5.73*10^1$	0.386	0.003	0.910	0.017	0.993	1500
Chaetoceros (St. Andrews)	$1.44*10^2$	0.396	0.006	0.577	0.060	0.996	1204
Chaetoceros (Labrador Sea)	$1.47*10^2$	0.373	0.004	0.733	0.021	0.994	900
T. weissflogii	$1.99*10^3$	0.409	0.007	0.510	0.072	0.990	1204
Hyalodiscus sp.	$6.21*10^3$	0.355	0.003	0.595	0.015	0.990	1505
Coscinodiscus CCMP312	$1.35*10^5$	0.461	0.002	0.065	0.008	0.982	2568
Coscinodiscus CCMP1583	$2.56*10^5$	0.384	0.002	0.201	0.008	0.993	1722

Table 2
β-corrections for the pooled data and four size-classes

Experimental organisms	*a*	SE	*b*	SE	r^2	*n*
Pooled model: all eight species	0.446	0.001	0.122	0.006	0.985	12 108
Small cells: four species, ~10–150 μm^3	0.381	0.002	0.805	0.012	0.992	5109
Small and medium cells: six species, ~10–7000 μm^3	0.388	0.001	0.616	0.008	0.984	7818
Medium cells: two species, ~2000–7000 μm^3	0.381	0.003	0.473	0.014	0.880	2709
Large cells: two species, ~135 000–250 000 μm^3	0.435	0.002	0.072	0.006	0.985	4290

large cells (the *Coscinodiscus* spp.). A fourth pooled small–medium model that eliminated the two *Coscinodiscus* spp. was also generated. To determine if there were significant differences between the β-corrections of different species, the standard *F*-test was used to compare a model which allowed the β-correction coefficients to vary among species to the pooled and size-class models.

To assess the effect of error in the β-correction factor on the phytoplankton absorption coefficient, the β-correction factors of Mitchell (1990), Cleveland and Weidemann (1993), Hoepffner and Sathyendranath (1992) and Arbones et al. (1996)

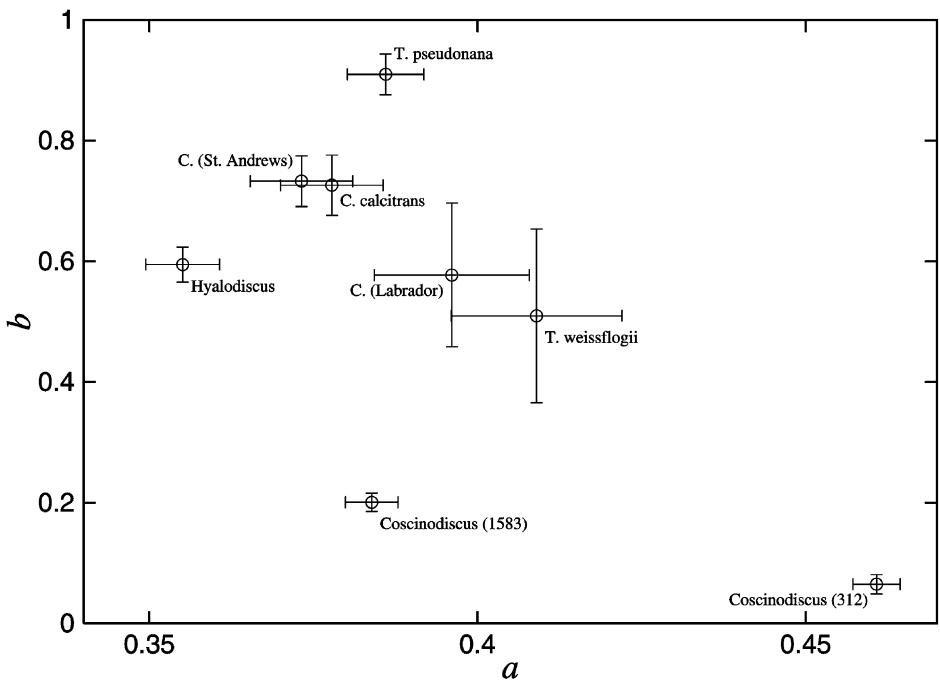

Fig. 1. The β-correction coefficients (Eq. (1)) for each diatom taxon. Error bars indicate 95% confidence intervals.

were applied to the $OD_f(\lambda)$ data of each diatom species to predict $OD_s(\lambda)$. Optical density in suspension was converted to absorption as in Cleveland and Weidemann (1993),

$$a_p = \frac{2.3OD_s(\lambda)}{(V/A)}, \quad (2)$$

where 2.3 converts from $\log_{10}$ to $\log_e$, V is the volume filtered (m^3), and A is the clearance area of the filter (m^2). The percent relative error in the spectrally averaged absorption coefficients due to the different β-correction factors were tabulated.

3. Results

There are significant differences between the pooled, size-class, and individual species β-corrections, $p < 10^{-4}$ (Tables 1 and 2). The species model is significantly

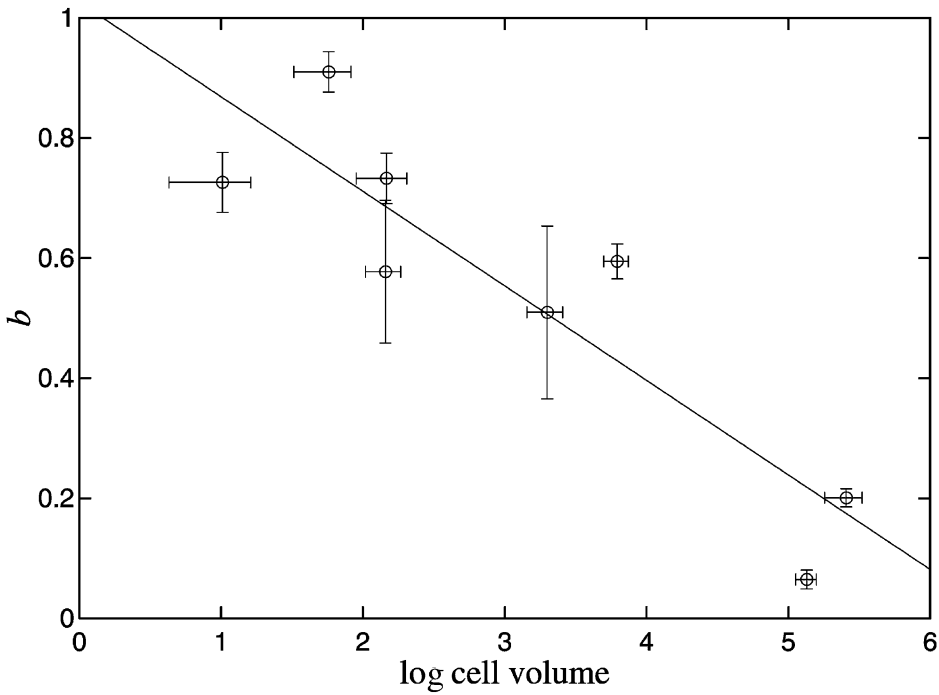

Fig. 2. The size-dependence of the β-correction quadratic term. Open circles represent the quadratic coefficient, error bars represent the 95% confidence interval, and the line is the best ordinary least-squares fit with slope -0.16 ± 0.03, $r^2 = 0.77$. Cell volume is in μm^3.

Table 3
Common β-corrections from the literature

Experimental organisms	*a*	SE	*b*	SE	r^2	*n*	References
Dunaliella sp., *Chlorella* sp., *Nanachloroxis* sp., *T. fluviatilis*, *Synechococcus* sp.	0.392	0.006	0.655	0.023	n.a.	6981	Mitchell (1990)
C. gracilis, *P. tricornutum*, *I. galbana*	0.31	n.a.	0.57	n.a.	n.a.	n.a.	Hoepffner and Sathyendranath (1992, 1993)
C. gracilis, *T. weissflogii*, *A. carterae*, *Dunaliella* sp.	0.378	0.004	0.523	0.014	0.988	14 400[a]	Cleveland and Weidemann (1993)
Synechococcus WH8103	0.304	n.a.	0.450	n.a.	0.947	16 123	Moore et al. (1995)
Prochlorococcus marinus	0.291	n.a.	0.051	n.a.	0.951	9750	Moore et al. (1995)
Diatoms, chlorophytes, prymnesiophytes, cyanophytes and rhodophytes (nine cultures)	0.38	n.a.	0.42	n.a.	0.97	15 600	Arbones et al. (1996)

[a] Forty-eight spectrophotometric scans reported.

Table 4
Percent relative error in the spectrally averaged absorption coefficient using different β-correction factors from the literature

β-correction factor	*C. calcitrans*	*T. pseudonana*	*Chaetoceros* sp. [a]	*Chaetoceros* sp.[b]	*T. weissflogii*	*Hyalodiscus* sp.	*Coscinodiscus* 312	*Coscinodiscus* 1583
Mitchell (1990)	6.62	0.82	−1.10	13.02	2.14	8.14	35.26	37.17
Hoepffner and Sathyendranath (1992)	−14.80	−19.04	−20.68	−9.79	−18.15	−12.24	11.50	12.56
Cleveland and Weidemann (1993)	0.96	−5.34	−6.95	7.26	−3.74	−0.40	20.96	23.73
Moore et al. (1995)— *P. marinus*	−28.28	−35.42	−35.86	−23.03	−33.14	−38.47	−37.53	−32.39
Arbones et al. (1996)	−0.32	−7.34	−8.71	6.14	−5.41	−4.43	12.38	16.07

[a] St. Andrew's isolate.
[b] Labrador Sea isolate.

different from the pooled β-correction, indicating that a minimum of one of the species corrections differ from the general diatom model. The β-correction of many of the diatom taxa differ—there is little overlap of the 95% confidence intervals between the coefficients of the different species (Fig. 1). Although coefficient *a* is similar for all species, with a mean value of 0.39 ± 0.03, coefficient *b* is quite variable between species, ranging from 0.06 to 0.91, with a mean of 0.54 ± 0.28. In addition, the value of *b* tends to decrease with increasing cell volume. Ordinary least squares linear regression of *b* against $\log_{10}$ cell volume yields a significant slope of -0.16 ± 0.03, $r^2 = 0.77$, $p < 0.01$ (Fig. 2).

The β-correction size model is significantly different from the pooled model. Coefficient *a* shows little variation between size-classes, but coefficient *b* decreases with average cell size. The two *Coscinodiscus* spp., the two largest species, have extremely low values of *b*, and unlike all the other species examined, exhibited an hysteresis effect (Arbones et al., 1996). To eliminate possible bias of the *Coscinodiscus* spp. on the size model, a pooled model was constructed from the six remaining small and medium species. The small and medium model is significantly different from the small-medium pooled β-correction, indicating cell volume has a significant impact on the β-correction.

The β-correction determined for the combination of the small and medium diatom cells is the most similar of all our β-corrections to the corrections suggested by Mitchell (1990), Hoepffner and Sathyendranath (1992) and Cleveland and Weidemann (1993), who used, predominately, cells in this size range in the generation of their coefficients (Table 3). There are differences between the β-corrections of Mitchell (1990), Hoepffner and Sathyendranath (1992), Cleveland and Weidemann (1993), Moore et al. (1995) and Arbones et al. (1996), which can result in large errors in the estimate of light absorption by phytoplankton assemblages (Table 4).

Application of standard β-corrections from the literature to the optical density on the filter of the individual diatom species results in notable error in the estimate of the light absorption coefficient. Percent relative error in the estimated spectrally averaged absorption in suspension, using correction factors from the literature, ranges from as low as 0.3% to over 38%, depending on the species and correction function. In general, for our species, the correction taken from Mitchell (1990) tended to over-estimate absorption in suspension, while the correction factor suggested by Hoepffner and Sathyendranath (1992) tended to underestimate absorption in suspension. On average, Cleveland and Weidemann (1993) and Arbones et al. (1996) provided the best estimate of spectrally averaged absorption for the diatom cultures, with an average 1.5% and 4% overestimate, respectively.

The error due to different correction factors depends on the species to which it was applied. There is a correlation between the error in estimated absorption and cell volume likely due to coefficient *b* (Fig. 2). Predictions using Mitchell (1990) and Cleveland and Weidemann (1993) were relatively good for small and medium cells, but absorption tended to be overestimated for the largest cells. In contrast, the correction determined by Hoepffner and Sathyendranath (1992) tended to underestimate the absorption of the small and medium cells, but gave better estimates for the larger cells.

4. Discussion

Cleveland and Weidemann (1993) suggested that a single β-correction generated from a wide range of algal cultures might be adequate for the calculation of light absorption by phytoplankton from field samples, if a consistent method was used between investigations. The large variation between the β-corrections in this study precludes the use of a single correction factor. Although Mitchell (1990) suggested that any differences in particle type due to refractive index or cell size would be overshadowed by the scattering of the filter, this study adds to the accumulating evidence of significant species and size variation in the β-correction (Moore et al., 1995; Arbones et al., 1996).

Differences in the β-correction could be due to the physical properties of the cells such as the silica frustule of the diatoms, the calcite liths of the coccolithophorids, or the organic armour of the dinoflagellates. Arbones et al. (1996) found differences between the β-corrections of nine different algal cultures representing several algal classes. Moore et al. (1995) found the small prochlorophytes have dramatically different β-factors than the other species previously examined by Mitchell (1990) and Cleveland and Weidemann (1993). The importance of species composition and cell size on the estimate of absorption by the QFT and β-correction is confirmed by the significant differences between the β-corrections of the centric marine diatoms species and size-classes in this study.

We demonstrate that the variation between β-corrections is correlated with cell volume, lending support to the argument that cells of different sizes may lie differently within or on the filter, resulting in different ratios of optical density on the filter to that in suspension (Moore et al., 1995). For species that form large colonies such as *Trichodesmium* spp. the amplification of optical path-length due to the filter is assumed negligible (Subramaniam et al., 1999; personal communication). This implies that large cells or colonies are optically equivalent to a single leaf that covers the glass fibers of the filter. Thus, we expect, and our data shows, that coefficient a will tend to 1 and b will tend to 0 as cell volume or colony size increases. Seemingly in contrast, very small cells have very small values of b (Moore et al. 1995). The similarity in b between the small cells in Moore et al. (1995) and large cells in this study may be because the very small cells embed themselves between the glass fibers, resulting in a decrease in b similar to that due to the increased coverage of the filter by the large cells. Although these explanations are plausible, the β-correction is poorly understood and other possibilities for the correlation between cell volume and the coefficients in the filter amplification correction should be considered.

Variation in the β-correction can alter the estimate of absorption coefficients and quantum yield and, thus, bio-optical estimates of primary production. The application of a β-correction, determined from a set of cultures in the laboratory applied to a different set of species, can result in large errors in predicted absorption. Moore et al. (1995) found that the absorption of *P. marinus* could be overestimated as much as two-fold in the blue part of the spectrum. Our examples show that the application of corrections from Mitchell (1990), Hoepffner and Sathyendranath (1992, 1993), Cleveland and

Weidemann (1993), or Arbones et al. (1996) to diatom cells can over- or underestimate spectrally averaged absorption between +35% and −38%.

Several approaches have been suggested to reduce this error. One can improve the correction algorithm by ensuring it is representative of the sample to which it is being applied. For example Kyewalyanga et al. (1998) used a weighted combination of correction factors, depending on the species composition of the field sample as determined by HPLC and flow cytometry. The assumption that a linear combination of species-specific correction factors will be appropriate for an assemblage of those species still needs to be tested. Other improvements attempt to remove the problem of filter amplification altogether. Field samples can be concentrated on a filter, then, re-suspended in solution or placed frozen on a slide, although incomplete recovery and cellular damage are still problematic (Allali et al., 1995).

5. Conclusion

The β-correction is still widely used to determine light absorption by phytoplankton assemblages from optical densities measured on a glass-fiber filter. If the β-correction is to be used, it is imperative that investigators confirm it does not introduce large systematic errors into their final calculation of absorption. This study confirms that the use of an inappropriate β-correction can lead to gross errors in the estimation of the absorption coefficient. At present, the choice of β-correction is an important determinant to the error in the estimates of light absorption, quantum yield and bio-optical estimates of primary production.

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