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A comment on "The measurement of bacterial chlorophyll and algal chlorophyll *a* in natural waters" (Caraco and Puccoon)

Caraco and Puccoon (1986) describe a method for determining algal and bacterial chlorophylls and pheophytins in acidified 90% acetone extracts. The method was proposed to distinguish algal pigments (Chl *a*) from those of green sulfur bacteria (GSB) with absorbance measurements of their pheopigments. This approach avoids the labor and time required for chromatographic separations and analysis (e.g. Steenbergen and Korthals 1982). In attempting to apply this method, however, we obtained questionable results that cause us to suspect that the ability of the method to distinguish algal and bacterial pigments is less than Caraco and Puccoon believed.

The most serious problem is that the similarity between algal Chl *a* and bacteriochlorophyll (BChl) *c* is not considered. Bacteriochlorophyll *c* (originally called chlorobium chlorophyll-660) is a major pigment in some green-colored varieties of GSB (Stanier and Smith 1960; Takahashi and Ichimura 1968; Pfennig 1978). Like algal Chl *a*, its pheophytin has a red absorbance peak at about 665 nm in 90% acetone (Gloe et al. 1975; Lorenzen 1967; Baker et al. 1983) so that absorbance measurements alone will not reliably distinguish this pigment from those of algal origin.

Caraco and Puccoon seem unaware of the problems created by BChl *c*. They assumed that their bacterial samples contained some BChl *c*, but their absorbance spectra (p. 891, figure 1) and absorbance ratios calculated for samples of bacterial pigment (determined from equation 2, p. 891) suggest BChl *c* may have been absent or present in only small quantities.

As formulated, the proposed method cannot accurately estimate total algal and bacterial chlorophylls unless BChl *c* is absent. This determination will still require chromatographic separation, so a major impetus for using the method is lost. The method might, however, be useful in estimating BChl *d* and *e*, which have red absorbance maxima 6–10 nm lower than Chl *a* and BChl *c* (Gloe et al. 1975).

To assess the problem we have tabulated absorbance data for algal and bacterial pheophytins from the literature and our own measurements and simulated an analysis of different mixtures of algal and bacterial chlorophylls (Table 1). As anticipated, the method of Caraco and Puccoon will not distinguish BChl *c* from algal pigment but provides some differentiation of BChl *d* and *e*. The simulations suggest, however, that determinations of these pigments will not be highly accurate.

The errors predicted by these simulations may result partly from assumptions we made about absorbance spectra. We have not obtained purified bacteriochlorophylls and base these simulations on published spectra (Stanier and Smith 1960; Jensen et al. 1964; Gloe et al. 1975) and our work with purified Chl *a* and unpurified bacterial pigments. The greatest source of error, however, seems to be the approach used to calibrate the method. Caraco and Puccoon did not determine the specific pigment content of their calibration samples, which probably contained several pigments with different spectral properties. Equations for estimating concentrations of individual pigments in mixtures should be formulated with data from purified pigments of known concentration. This approach, for example, has been used to develop the familiar trichromatic methods used for determining Chl *a*, *b*, and *c* in mixtures (Parsons and Strickland 1963; SCOR-UNESCO 1966; Jeffrey and Humphrey 1975).

Chromatographic methods for separation and measurement of pigments have recently been greatly improved with major reductions in the time and sample volume required for analysis of natural waters. Be-

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Table 1. Estimated concentrations of total Chl *a* and total BChl by the method of Caraco and Puccoon (1986) for five hypothetical samples containing one or more pigments.

	Sample concn (mg liter^{-1})				
	1	2	3	4	5
True concn*					
Chl *a*	1	0	0	0	1
BChl *c*	0	1	0	0	1
BChl *d*	0	0	1	0	1
BChl *e*	0	0	0	1	1
Estimated concn					
Total Chl *a*	1.4	1.3	0.3	−0.1	2.1
Total BChl	−0.1	−0.1	0.6	1.3	1.1

* Values were determined assuming absorbance ratios (A_{658}/A_{663}) of 0.73, 0.73, 1.12, and 1.49 for the pheophytins of Chl *a* and BChl *c*, *d*, and *e*. Red absorbance maxima were assumed to occur at 665, 665, 659, and 655 nm, respectively, with specific absorption coefficients of 56 liter g^{-1} cm^{-1} for algal pheophytin *a* (the value used by Caraco and Puccoon) and 54 liter g^{-1} cm^{-1} for bacteriopheophytins. These values are based on published spectra (Stanier and Smith 1960; Golterman and Clymo 1969; Gloe et al. 1975) or unpublished data. Absorbance peaks of bacteriopheophytins were corrected 1 nm from values reported for 100% acetone (Gloe et al. 1975) to account for red shift in 90% acetone (e.g. Jeffrey and Humphrey 1975).

cause of the availability of chromatographic methods and the great uncertainty involved in spectral analysis of mixtures containing bacterial and algal chlorophyll, we cannot recommend spectrophotometric methods as a means of distinguishing pigments of the two types. Caraco and Puccoon's equations could be reformulated, however, for the purpose of distinguishing the approximate sum of algal Chl *a* and BChl *c* from the sum or individual concentrations of BChl *d* and *e*. In samples with high pigment concentrations, such equations could provide quick and simple approximations of pigment content. Nonetheless, the close similarity of absorbance spectra of these pigments means that even small errors in measuring peak heights or wavelength settings can produce large errors in concentration estimates, particularly for samples with low pigment concentrations. Interference from algal Chl *b*, for example, could give false positive results for bacteriochlorophylls. For precise identification and determination of pigments, chromatography remains the method of choice.

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