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Determination of chlorophyll in seawater

Report of intercalibration tests

sponsored by SCOR and carried out in September-October 1978

by C.J. Lorenzen

Department of Oceanography,
University of Washington, Seattle,
Washington, USA 98195

and S.W. Jeffrey

CSIRO Division of Fisheries & Oceanography
P.O.B. 21, Cronulla, N.S.W. 2230, Australia

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PREFACE

This series, the Unesco Technical Papers in Marine Sciences, is produced by the Unesco Division of Marine Sciences as a means of informing the scientific community of recent developments in oceanographic research and marine science affairs.

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I. INTRODUCTION AND TERMS OF REFERENCE

The intercalibration tests of chlorophyll oceanographic methods reported here were carried out at the laboratories of the CSIRO Division of Fisheries and Oceanography, Cronulla, N.S.W., Australia, from 29 September to 12 October 1978.

The terms of reference were : To evaluate spectrophotometric and fluorometric methods commonly used for chlorophyll *a* determinations in oceanography".

The past 25 years have seen a gradual development of routine methods for estimating phytoplankton chlorophylls (*a, b* and *c)* in the oceans. Richards and Thompson (1952) offered the first trichromatic spectrophotometric equations based on Mac Kinney's (1941) early extinction coefficients of chlorophylls *a* and *b,* and these were superseded by improved versions by Parsons and Strickland (1963) and SCOR-Unesco (1966) based on extinction coefficients of Smith and Benitez (1955) and Vernon (1960) for chlorophylls *a* and *b,* and that of Jeffrey (1963) for chlorophyll *c*. A set of equations was later published by Jeffrey and Humphrey (1975) which used the most recently determined extinction coefficients for chlorophylls *a* and *b* (Jeffrey, unpublished, reported in Jeffrey and Humphrey, 1975) and c_1 and c_2 (Jeffrey, 1972).

A fifty to one hundred-fold increase in sensitivity (though not necessarily accuracy) of the chlorophyll measurement resulted from the introduction of fluorescence techniques by Holm-Hansen *et al.* (1965), and when Lorenzen (1966) adapted the fluorescence method to *in vivo* studies, using a "flow through" system, a very useful technique for scanning phytoplankton populations at sea was obtained.

An awareness that chlorophyll degradation products from senescent phytoplankton and detritus could be giving over-estimates of the "true" chlorophyll values resulted in the introduction of an acidification step to both the spectroscopic (Lorenzen, 1967 ; Marker, 1972) and fluorometric (Holm-Hansen *et al.*, 1965) techniques to help correct this source of error. (Vernon (1960) first introduced this acidification step for pheophytin analysis in higher plant extracts.)

About the same time, development of thin-layer chromatographic techniques for algal pigments (Jeffrey, 1968) eventually allowed the full spectrum of chlorophylls, carotenoids and chlorophyll degradation products in seawater samples to be recognised (Jeffrey 1974, 1976). The true nature of the interfering chlorophyll degradation products (pheophytin *a,* chlorophyllide *a* and pheophorbide *a),* which absorb and fluoresce in the same region as the parent chlorophylls, emphasized the kind of interference that these compounds must be having on the accuracy of the simple routine spectrophotometric and fluorometric methods. Attempts to deal with these by phase separation techniques (Jeffrey, 1977 ; Whitney and Darley, 1979) may help, but are new, untried developments.

Uncertainties regarding the reliability of the various extinction coefficients for chlorophylls *a, b* and *c* used in the different methodologies (only some of which are cited here) represent a further problem "plaguing" the oceanographer searching for a reliable method for estimating phytoplankton chlorophylls in his samples.

Thus the need for careful intercalibration tests has become obvious. The report presented here, initiated and funded by SCOR, represents an initial evaluation based on our accumulated experimental and field experience, as well as the present brief series of intercalibration tests. Because time was limited to 12 days, we confined our tests to methodology and procedures subsequent to extraction of the pigments. Thus technical problems concerned with collection of samples, harvesting the phytoplankton, filter storage conditions, choice of solvents for extraction, length of extraction time and homogenization techniques, although important, could not be experimentally examined during the 12 days. We are currently examining these and other developments. Further details of the present tests as well as the results of our on-going research will be reported elsewhere.

Both authors wish to thank SCOR for providing the opportunity for them to work together on these problems.

II. METHODS TESTED

The methods tested included various trichromatic spectrophotometric equations ; spectrophotometric equations using acidification techniques for "pheopigments" ; fluorometric *in vitro* methods using acidification techniques for "pheopigments", and fluorometric *in vivo* methods. These are listed below :

1. Spectrophotometric - Trichromatic chlorophylls *a*, *b* & *c* (no corrections for breakdown products)
   1. Jeffrey & Humphrey (1975)
   2. SCOR-Unesco (1966)
   3. Parsons & Strickland (1963)
   4. Richards with Thompson (1952)
2. Spectrophotometric - Chlorophyll *a* and "pheopigments"
   5. Lorenzen (1967)
   6. Marker (1972)
3. Fluorometric *(in vitro)* - Chlorophyll *a* and "pheopigments"
   7. Holm-Hansen *et al.* (1965)
4. Fluorometric *(in vitro)* - Chlorophylls *a*, *b* & *c* and "pheopigments"
   8. Boto & Bunt (1978)
5. Fluorometric *(in vivo)* - Chlorophyll *a*
   9. Lorenzen (1966)
   10. Slovacek & Hannan (1977) (uses DCMU for maximum fluorescence).

The Jeffrey & Humphrey (1975) trichromatic method was used as the standard in these tests, since it supersedes all previous equations by using the most accurate extinction coefficients available for the four chlorophylls *a* and *b* (Jeffrey & Humphrey, 1975) and c_1 and c_2 (Jeffrey, 1972). Both SCOR-Unesco (1966) and Parsons & Strickland (1963) use old Jeffrey (1963) extinction coefficients for chlorophyll *c* which over-estimates this chlorophyll by about 100 %.

III. EXPERIMENTAL STRATEGY

Highly purified pigments, chlorophylls *a*, *b*, *c*, pheophorbide *a* and chlorophyllide *a*, were prepared by standard methods, their purity was checked by chromatographic and spectroscopic techniques, and mixtures of these in known concentrations were then analysed by the various *in vitro* techniques. All comparisons were based on pigments in 90 % acetone, the most commonly used solvent, since there was no time for evaluation of other solvents (e.g.,methanol, DMSO, etc.).

IV. METHODS

a) Pigment purifications. Chlorophylls *a* and *b* were obtained from Swiss chard using cellulose column chromatography and purification techniques of Strain *et al.* (1963). Chlorophyll *c* was isolated from the brown seaweed, *Sargassum fallax*, by the methods of Jeffrey (1969, 1972) using cellulose column chromatography and polyethylene thin layer chromatography. The chlorophyll *c* preparation used was a mixture of c_2 and c_2 in the proportion 1:1. Chlorophyllide *a* was isolated from cultures of diatoms using the methods of Barrett and Jeffrey (1964, 1971). Care was taken to eliminate harmful oxidative activity during the initial extraction, since this can give rise to atypical chlorophyllides (Barrett and Jeffrey, 1964). Pheophorbide *a* was prepared from Swiss chard by a modification of the method of Holt and Jacobs (1954). Preparation of all pigments was done in the dark, and storage was under nitrogen at - 15° C to eliminate any possibility of breakdown occurring in these highly labile pigments. Immediately before use, the purity of each pigment was rechecked by sensitive thin layer chromatography (Jeffrey, 1968) and absorption spectroscopy. Full details of the preparative methods used will be given elsewhere (Jeffrey and Lorenzen, in preparation).

b) Extinction coefficients used for quantitative determinations. The specific absorption coefficients chosen for chlorophylls *a*, *b* and *c* were those of Jeffrey as cited in Jeffrey and Humphrey (1975). These were obtained from highly purified crystalline preparations. The specific absorption coefficient of chlorophyllide *a* was calculated assuming identical molar extinctions of chlorophyll *a* and chlorophyllide *a*. Specific absorption coefficients of pheophytin *a* and pheophorbide *a* were calculated from observed acid factors for conversion of chlorophyll *a* and chlorophyllide *a* respectively to its derivative. The fluorometric *in vitro* method of Holm-Hansen *et al.* (1965) was calibrated using these extinction coefficients (Table 1).

c) Instruments. A Cary Model 17 spectrophotometer was used for all spectrophotometric measurements. A Turner (Model III) filter fluorometer and a Farrand (Mark I) spectrofluorometer were used for the fluorometric measurements.

Table 1

Extinction coefficients of chlorophylls and derivatives used for quantifying all pigment solutions

(Solvent 90 % acetone)

	Mol. Wt	M	α	
chlorophyll $a_{664\ nm}$	893.48	7.83×10^4	87.67	Jeffrey & Humphrey (1975)
pheophytin $a_{667\ nm}$	869.16		51.2	calculated (acid factor 1.76)
chlorophyllide $a_{664\ nm}$	614.97	7.83×10^4	127	calculated
pheophorbide $a_{667\ nm}$	590.65		74.2	calculated (acid factor 1.79)
chlorophyll $b_{647\ nm}$	907.46		51.36	Jeffrey & Humphrey (1975)
chlorophyll c_1 & c_2 631 nm	611 & 609		42.6	Jeffrey (1972)

$\frac{E}{M} \cdot 1000 = \mu M$; $\frac{E}{\alpha} \cdot 1000 = \mu g$

M = molar extinction coefficient ; α = specific absorption coefficient

V. JUSTIFICATION FOR CHOICE OF PIGMENT MIXTURES USED FOR TESTS

Selection of the particular chlorophyll pigments for the tests was based on our experience of thin layer chromatography of field samples (Jeffrey, 1974, 1976 ; Shuman and Lorenzen, 1975 ; Lorenzen (unpublished)). Our findings, at the time of our tests, October 1978, were that chlorophylls *a*, *b* and *c* and the degradation products chlorophyllide *a* and pheophorbide *a* are the most common chlorophylls encountered. At that time we had not encountered degradation products of chlorophylls *b* or *c* in oceanic samples, and pheophytin *a* was found only in traces. These pigments were therefore considered an unimportant source of error, and were neglected. /However, field work carried out in the East Australian Current since October 1978 has shown that pheophytin *a* can occur in amounts up to 20 % of the total chlorophyll *a* under certain conditions, and "new" unidentified derivatives of chlorophylls *a* and *b* have also been noted (Jeffrey and Hallegraeff, in preparation). Further tests will therefore be needed to assess the interference to the chlorophyll *a* measurement from these sources./

Artificial mixtures of pure pigments in different concentrations and proportions were made to correspond to known combinations of pigments found in seawater samples by thin layer chromatography. Six standards and fourteen mixtures were prepared, some with extreme ratios to test the equations (Tables 2, 3). The series 1-14 represents mixtures prepared with one batch of highly purified chlorophyll *a* ; the series 15-20 represents mixtures prepared from a second batch of pure chlorophyll *a*.

Table 2

Simulation of marine phytoplankton samples by various combinations of pure pigments

Mixture No.	Pigments	Simulation
1-4 ; 15,16	chlorophylls *a*, *b*, *c*, pheophorbide *a* chlorophyllide *a* } single pigments	Pure pigment standards
5-6	chlorophylls *a*+*b*	Green algae
	" " " + pheophorbide	
7-8	chlorophylls *a*+*c*	Diatoms, dinoflagellates, etc.
	" " " + pheophorbide	
9-10	chlorophyll *c* + pheophorbide	"Deep" euphotic zone pigments
11	chlorophyll *a* + pheophorbide	Faecal pellet
12	chlorophylls *a* + *b* + *c*	Green algae and diatoms
13-14	" " " " + pheophorbide	Mixed algal population with pheophorbide
17	chlorophylls *a* + *b* + chlorophyllide	Green algae with chlorophyllide
18	chlorophylls *a* + *c* + chlorophyllide	Diatoms with chlorophyllide
19	chlorophyllide + pheophorbide	"Deep" euphotic zone with chlorophyllide & pheophorbide
20	Chlorophylls *a* + *b* + *c* + chlorophyllide + pheophorbide	All pigments

TABLE 3

COMPARISON OF SPECTROPHOTOMETRIC AND FLUOROMETRIC (*IN VITRO*) METHODS OF CHLOROPHYLL ANALYSIS, USING MIXTURES OF PURE PIGMENTS

Mixture No.	Known concentrations µg ml.$^{-1}$					Jeffrey & Humphrey (1975) Spectrophotometric			Holm-Hansen et al. (1965) Fluorometric			Lorenzen (1967) Spectrophotometric		Marker (1972) Spectrophotometric	
	C_A	C_B	C_C	P	$C_{chlideA}$	C_A	C_B	C_C	* C_A	C	P	C_A	P	C_A	P
1	1.836	-	-	-	-	1.840	-0.034	-0.007	1.76	1.72	0.07	1.84	-0.25	1.84	-0.09
2	-	1.947	-	-	-	0.024	2.014	-0.711	0.36	-0.55	1.74	-0.13	0.50	-0.13	0.52
3	-	-	1.972	-	-	0.002	-0.024	1.980	0.59	0.75	0.31	0.00	0.35	0.00	0.04
4	-	-	-	1.765	-	1.485	-0.010	-0.115	1.20	0.14	2.03	0.16	2.05	0.15	2.21
5	1.836	0.779	-	-	-	1.869	0.771	-0.21	1.87	1.45	0.74	1.79	-0.03	1.78	0.15
6	1.836	0.389	-	0.833	-	2.583	0.241	-0.044	2.34	1.63	1.35	1.79	0.98	1.78	1.22
7	1.836	-	0.493	-	-	1.823	-0.042	0.494	1.87	1.90	-0.07	1.82	-0.23	1.81	-0.06
8	1.836	-	0.296	0.833	-	2.550	-0.100	0.192	2.32	1.77	1.05	1.84	0.84	1.86	1.04
9	-	-	0.957	1.7114	-	1.481	-0.087	0.784	1.48	0.54	1.78	0.23	1:90	0.23	2.06
10	-	-	1.972	1.765	-	1.528	-0.097	1.736	1.87	0.95	1.74	0.23	1.99	0.23	2.15
11	0.184	-	-	1.765	-	1.697	-0.086	-0.153	1.40	0.27	2.14	0.34	2.05	0.34	2.22
12	1.836	0.389	0.296	-	-	1.850	0.392	0.227	1.87	1.63	0.45	1.74	-0.06	1.73	0.11
13	1.836	0.389	0.296	0.883	-	2.558	0.081	0.284	2.52	1.82	1.33	1.82	0.92	1.81	1.16
14	1.836	0.097	0.394	0.883	-	2.584	-0.031	0.308	2.47	1.77	1.33	1.87	0.85	1.84	1.12
15	1.700	-	-	-	-	1.702	-0.029	-0.011	1.69	1.72	-0.05	1.69	0.20	1.68	-0.05
16	-	-	-	-	1.264	1.840	-0.199	0.088	1.69	1.76	-0.11	1.84	-0.25	1.84	-0.09
17	1.700	1.947	-	-	0.632	2.703	1.940	-0.078	2.56	1.79	1.46	2.47	0.27	2.46	0.53
18	1.700	-	1.972	-	0.632	2.608	-0.091	1.977	2.56	2.77	-0.40	2.57	0.26	2.56	-0.02
19	-	-	-	0.883	0.632	1.698	-0.121	-0.023	1.31	0.88	0.82	1.07	0.83	1.06	0.99
20	0.170	1.947	1.972	0.883	0.632	3.435	1.768	1.869	3.49	2.30	2.26	2.52	1.35	2.51	1.69

C_A = concentration chlorophyll *a*

C_B = " " *b*

C_C = " " *c*

P = concentration pheophorbide *a*

$C_{chlideA}$ = " chlorophyllide *a*

*C_A = " chlorophyll *a* calculated from the initial fluoroescence reading

VI. RESULTS OF TESTS OF *IN VITRO* METHODS

Table 3 gives detailed quantitative results of comparisons between the various spectrophotometric and fluorescence methods with the 6 standards, and the 14 mixtures outlined in Table 2.

- All techniques adequately estimate chlorophyll *a* in mixtures of pure chlorophylls with errors of less than 6 %. An exception is the fluorometric method with mixtures containing chlorophyll *b*. In this case, No. 5 in the Table, which would be characteristic of a green algal population, the chlorophyll *a* estimate is in error by - 21 %.

- Chlorophyllide is measured as chlorophyll *a* in all the techniques tested and the error is directly proportional to its abundance relative to chlorophyll *a* (and the difference in their extinction coefficients). The worst case, No. 19, which represents a sample from some depth below the euphotic zone, measures significant quantities of chlorophyll *a* when none is present.

- Chlorophyll degradation products, of which pheophorbide *a* appears to be the most abundant in the water column, will be measured as chlorophyll *a* unless a technique is used that specifically takes this into account, e.g., methods in groups 2, 3 and 4, on page 7. The precision of the chlorophyll *a* determination of the three methods listed on the right-hand side of the Table is not impaired by the presence of pheophorbide unless chlorophyll *a* is a minor constituent of the mixture. In Mixture No. 11, where the ratio of the pigments is similar to fecal pellets, chlorophyll *a* is overestimated by 85 %. Although not demonstrated in this Table, the pigment least abundant is usually the most poorly estimated.

Accuracy of chlorophyll *a* determination by the methods listed in Table 3

a) All methods measure chlorophyll *a* satisfactorily when chlorophyll *a* is the only pigment present.

b) All spectrophotometric methods perform satisfactorily for chlorophyll *a* when only chlorophylls are present in any combination.

c) All methods err when chlorophyllide *a* is present since none differentiate between chlorophyll *a* and chlorophyllide *a*.

d) The presence of chlorophyll *b* interferes with the fluorometric technique since it is partially calculated as "pheopigment". Chlorophyll *b* also adversely affects the chlorophyll *a* calculation.

e) Pheophorbide *a* can only be handled by methods designed to account for it, and then only with varying degrees of precision. The chlorophyll *a* determination is not necessarily affected.

f) Extinction coefficients for chlorophyll *a* used in the spectrophotometric methods (Table 3) are very similar. Marker (1972) used Vernon's (1960) absorption coefficient with corrections applied for average interference of accessory chlorophylls. Marker's (1972) optical density factor for chlorophyll *a* is 10.48 ; Lorenzen's (1967) is 11.0 Jeffrey and Humphrey (1975) would be 11.4 if only one wavelength was measured. Marker uses a different mathematical procedure from Lorenzen. However, comparing chlorophyll mixtures only in Table 3, Lorenezn and Marker are 1.6 % and 4.6 % less respectively for chlorophyll *a* than Jeffrey and Humphrey.

Note 1 : re *in vitro* fluorescence technique of Boto and Bunt (1978)

This selective excitation technique (using a spectrofluorometer) gives the same kind of accuracy as the spectrophotometric equations. Although it uses an acidification ratio,, it cannot solve for degradation products, since it uses five measurements for six unknowns. This is not a good selective method as presently published.

Effect of carotenoids on chlorophyll fluorescence *in vitro*

The effect of carotenoids on quenching or enhancing chlorophyll fluorescence was tested separately. Chlorophyll *a* : fucoxanthin in the ratio 2:1 (w/w) was tested with the Turner fluorometer. No effect of the carotenoid on the fluorescence emission of chlorophyll *a* was observed.

VII. EVALUATION OF TRICHROMATIC SPECTROPHOTOMETRIC EQUATIONS IN CURRENT USE

Four sets of trichromatic spectrophotometric equations have been used extensively in oceanography. These are Richards with Thompson (1952), Parsons and Strickland (1963), SCOR-Unesco (1966) and Jeffrey and Humphrey (1975). Humphrey (1966) modified the Richards-Thompson equations by correcting the chlorophyll *c* term (Jeffrey, 1963).

The accuracy of these equations was tested with the most favourable chlorophyll mixture (12, see Table 3) which contained only chlorophylls *a*, *b* and *c* in the proportions 8 : 1.7 : 1. No degradation products were present. The results and % errors of these analyses are given in Table 4.

TABLE 4

Comparison of accuracy of trichromatic spectrophotometric equations using Mixture 12 (Table 3)

Equations	Concentrations of chlorophylls (C_A C_B C_C) ($\mu g\ ml.^{-1}$)			% Errors C_A	% Errors C_B	% Errors C_C
Known chlorophylls mixture	1.836	0.389	0.296			
Jeffrey-Humphrey (1975)	1.85	0.394	0.225	<1 %	<1 %	24 % low
Parsons-Strickland (1963)	1.78	0.26	0.672	3 % low	33 % low	208 % high
SCOR-Unesco (1966)	1.80	0.35	0.55	2 % low	11 % low	169 % high
Richards-Thompson (1952)	2.38	0.21	1.00	30 % high	54 % low	300 % high
Richards-Thompson modified Humphrey (1966)	2.38	0.21	0.43	30 % high	54 % low	45 % high

Conclusions

a) Chlorophyll *a* is satisfactory when calculated by Jeffrey-Humphrey, SCOR-Unesco or Parsons-Strickland (1-3 % error). Richards-Thompson methods are 30 % high.

b) Chlorophyll *b* is satisfactory only with Jeffrey-Humphrey or SCOR-Unesco.

c) Chlorophyll *c* is satisfactory only with Jeffrey-Humphrey, but even this set of equations can give errors, if chlorophyll *c* is low compared to chlorophyll *a*. However, good accuracy was obtained with other chlorophyll *c*-containing mixtures (Table 3, Mixture 3, 7, 10, 13, 14, 18, 20).

d) Errors involved in using different wavelengths for chlorophyll *a* are small (e.g.,663 nm (SCOR-Unesco) ; 664 nm (Jeffrey-Humphrey) ; 665 nm (Parsons-Strickland

and Richards-Thompson)). In 90 % acetone, the chlorophyll *a* in Mixture 12 red band shows a plateau at 663-664 nm and drops 3 % in optical density at 665 nm. The small errors in wavelength reading are insignificant for chlorophyll *a*. For chlorophyll *b* the wavelengths used are 647 nm (Jeffrey-Humphrey ; SCOR-Unesco) and 645 nm (Parsons-Strickland and Richards-Thompson). In 90 % acetone there is an 11 % drop in otpical density at 645 nm compared to 647 nm (in Mixture 12) which results in the greater errors observed.

VIII. EVALUATION OF *IN VIVO* FLUOROMETRY

In vivo fluorometry of cultures of different algal taxonomic groups in different stages of their life cycle yield a wide range of calibration factors in respect of their chlorophyll *a* content. This is further compounded by past light history, diurnal cycles, nutrient status and temperature at which the analysis is carried out. Limited testing of fluorescence enhancement of four cultures of unicellular marine algae (*Skeletonema costatum*, diatom ; *Amphidinium carterae*, dinoflagellate ; *Dunaliella tertiolecta*, green flagellate ; and *Olisthodiscus luteus*, chrysomonad) by the addition of the herbicide dichlorophenyl dimethyl urea (DCMU) did not seem to decrease the variation of the calibration factor. These results were obtained with both the Turner filter fluorometer and the Farrand (Mark I) spectrofluorometer. This is at variance to the results reported by Slovacek and Hannan (1977), but is in agreement with their most recent investigations (Slovacek, 1978) and those of Esais (1978).

Past and present experience of shipboard *in vivo* phytoplankton fluorescence (without DCMU) yields a $\pm$ 50 % envelope around the calibration line (Lorenzen, 1966), with night values lying above the line, day values below the line. This *in vivo* technique can give differences in chlorophyll that are significant above a factor of 2. Other investigators need to work out their own limits of confidence with the *in vivo* technique, using their own particular instruments and technical set-up. For example, Loftus and Seliger (1975) reported a 10-fold variation in the ratio of *in vivo* fluorescence to extractable chlorophyll *a*, whereas Herman and Denman (1977) achieved a much closer correlation between *in vivo* fluorometry (Turner, and the *in situ* Variosens fluorometer) and chlorophyll *a*.

Our recommendation is that *in vivo* fluorometry is invaluable as a "search" method for phytoplankton populations at sea. It should be used as a guide to the appropriate location for the field experiment. It should not be substituted for an accurate measurement of chlorophyll *a* as the method stands at present.

IX. PRINCIPLES FOR THE SELECTION OF AN APPROPRIATE CHLOROPHYLL TECHNIQUE

The selection of a particular chlorophyll method must be based on judgment of the particular marine biological region being sampled :

1. Estuarine
2. Coastal
3. Open ocean
4. Sediments

Each of the above may also be subdivided further, e.g.,upper and lower euphotic zone, surface layer, "deep" ocean, etc. Different types of pigments will be encountered, depending on which of the above oceanographic regions is being investigated. Thus the takers and users of chlorophyll measurements must carefully select a method appropriate to the water type, and the problem being studied, taking into account Tables 2 and 3 and Section X below.

X. RECOMMENDATIONS FOR THE USE OF SIMPLE ROUTINE METHODS FOR CHLOROPHYLL *a* IN OCEANOGRAPHY

1. Samples collected in the near-surface portion of the euphotic zone can be analysed by either spectrophotometric or fluorometric methods because degradation products are normally absent, or present only in insignificant amounts.

2. The trichromatic spectrophotometric equations of Jeffrey and Humphrey (1975) are the most accurate for chlorophylls *a*, *b* and *c* in near surface euphotic zone waters. However, errors may occur even with these equations if *b* and *c* are low compared to *a*. If only chlorophyll *a* is required, the three sets of equations, Jeffrey-Humphrey, SCOR-Unesco and Parsons-Strickland, are satisfactory (1-3 % error).

3. Using the trichromatic equations for chlorophyll *a* only, and neglecting corrections for *b* and *c* will cause errors only if *b* is present in significant quantities. Neglect of these corrections is not recommended, however, since chlorophyll *b* is frequently present.

4. Samples collected further down in the water column should be analysed by an acidification technique since degradation products from senescent cells, detritus and faecal pellets can be expected.

5. Sediments, or samples containing sediments, could be analysed by the acidification techniques, but caution should be exercised since these normally contain a variety of unidentified pigments, and chlorophyll derivatives.

6. *In vivo* fluorometry (Lorenzen, 1966) should be used only as a "search" method at sea for locating fronts, defining patch areas, locating chlorophyll maxima, etc. Neither this method nor its DCMU modification (Slovacek and Hannan, 1977) used to maximize *in vivo* fluorescence should be used as a substitute for an accurate chlorophyll *a* measurement at present. Tests of these techniques show that correlation with chlorophyll *a* concentrations can vary by at least an order of magnitude.

7. We would like to emphasize the need for the investigator to "know" their particular instruments. Our experience shows that large variations occur between instruments, not only in calibration but also in other aspects which could make pigment measurements a sham. For example, the maximum acid factor (particularly in fluorometric acidification techniques) is a function of the response curve of the particular photomultiplier used. Also, the proportionality between sensitivity scales may not be what the manufacturer says. Some instruments need to be "zeroed" when shifting scales. These and other problems emphasize the importance of "knowing your instrument" through extensive calibration.

8. If precise knowledge of pigment composition is required, chromatography is the only method. Either cellulose thin layer chromatography, or high pressure liquid chromatography (HPLC) is appropriate. These methods far surpass the simple routine spectrophotometric and fluorometric methods for the complete knowledge of all photosynthetic pigments which they display in a single operation. From our own experience we would like to recommend these techniques for wider use in oceanography, since they are already bringing new insights to our understanding of phytoplankton processes in the sea.

XI. EVALUATION OF WORLD OCEAN CHLOROPHYLL DATA ANALYSED BY TRICHROMATIC SPECTROPHOTOMETRY

1. Oceanographic data for chlorophyll *a* in surface waters is valid if calculated by Jeffrey-Humphrey, SCOR-Unesco or Parsons-Strickland. Old data calculated by Richards-Thompson should be corrected (Wartenberg, 1978 ; Humphrey, 1978).

2. All oceanographic data for chlorophylls *b* and *c* are suspect in surface waters unless calculated by Jeffrey-Humphrey equations, or converted to them (Wartenberg, 1978 ; Humphrey, 1978).

3. Trichromatic spectrophotometric equations are less useful for deeper water layers, because of detrital chlorophyll interference (chlorophyllide, pheophorbide). No conversions (Wartenberg, 1978 ; Humphrey, 1978) can correct these errors.

4. Since the Jeffrey-Humphrey equations are the most accurate (they are based on the most accurately determined specific absorption coefficients for crystalline preparations of chlorophylls *a*, *b* and *c)*, their use is recommended for surface waters, and for calibration of the *in vivo* and *in vitro* fluorescence methods.

RECOMMENDATION TO SCOR

In view of ongoing technological developments (HPLC, phase separation, deconvolution techniques, etc.), a further review and "up-date" of chlorophyll methodology will be required in about five years time.

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