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Daniel Estrella and Eric O. Hartwig

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Prepared for the U.S. Department of Energy
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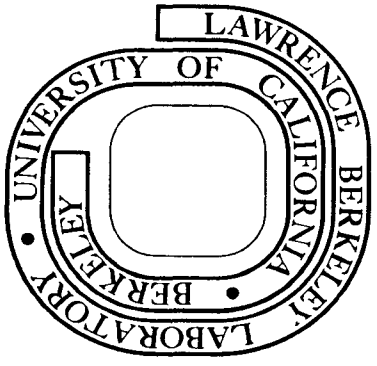

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A SELECTED BIBLIOGRAPHY OF CHLOROPHYLL a DETERMINATION METHODOLOGY

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Introduction

The Marine Science Group at LBL is measuring chlorophyll a as part of the environmental assessment and measurement program of the Ocean Thermal Energy Conversion (OTEC) Program. Chlorophyll a is a green pigment which serves as a photocatalyst in photosynthesis. Marine environmental field studies typically utilize chlorophyll a concentrations as a measure of phytoplankton biomass. The ratio of total phytoplankton carbon (biomass) varies between 25 and 110 to 1 depending upon the class of phytoplankter and its state of nutrition (Fogg, 1975; Strickland, 1965; Subba Rao and Platt, 1969). As the knowledge of the chlorophyll a content is important to the understanding of the distribution of plant material in the ocean the methods used to gain this knowledge is also of importance.

Several accepted methods for the determination of chlorophyll a in seawater have been described: Holm-Hansen, 1965 & 1978; Strickland and Parsons, 1972; and Yentsch and Menzel, 1963. Tolstoy (1977) presents an excellent review of current methods of chlorophyll a determination as well as phytoplankton collection, sample storage, pigment extraction and their various sources of error. The spectrophotometric method described by Richards and Thompson (1952) has been widely accepted for analysis of waters rich in phytoplankton. An improved version of this method is outlined in Strickland and Parsons (1972). The fluorometric method, described by Yentsch and Menzel (1963), has the advantage of speed and greater sensitivity thereby requiring much smaller sample volumes although it is not as accurate or precise as the spectrophotometric method (Strickland and Parsons, 1972). The precision of the spectrophotometric method is ±5% at the 5 microgram level and, for the fluorometric method, better than ±8% of any value of chlorophyll a exceeding 0.5 microgram/liter (Strickland and Parsons, 1972).

Further discussion on fluorometric methodology, including light filter selection, is found in the Turner Designs paper. The physical/chemical characteristics, including the absorption spectra and excitation/emission spectra of chlorophyll a are discussed in Harris and Zscheile (1943), Parsons (1963), Richards (1952), Tolstoy (1977), Vernon and Seely (1966), White, Jones, Gibbs and Butler (1972) and Yentsch and Menzel (1963). Holm-Hansen and Riemann (1978) present some of the most recent improvements to chlorophyll a determination methodology including extraction solvent selection, sample storage and filter type selection.

A method for the continuous measurement of the *in vivo* chlorophyll *a* concentration is described by Lorenzen (1966). Studies on some of its problems and limitations are reported on by Cullen and Renser (1979), Kiefer (1973), Loftus and Seliger (1975), Maerker and Szekielda (1976) and Strickland (1968).

Phaeopigment, a degradation product of the different chlorophylls can interfere with the spectrophotometric or fluorometric determination of chlorophyll *a* because it absorbs light and fluoresces in the same region of the spectrum as chlorophyll *a* and if present, may cause errors in chlorophyll *a* values (Patterson and Parsons, 1963; Vernon, 1960). Therefore, when performing chlorophyll *a* determinations, measurements of phaeopigment must be included.

The following is a list of references compiled for researchers of chlorophyll *a* determination methodology. The references have been gathered from marine and freshwater research journals, texts and handbooks. The list is organized by aspects of analysis:

Some articles overlap in coverage and appear in more than one category.

This work was supported by the U. S. Department of Energy under Contract W-7405-ENG-48.

References

Spectrophotometric Methods

BANSE, K. and G.C. ANDERSON (1967) Computations of chlorophyll concentrations from spectrophotometric readings. Limnology and Oceanography, 12(4), 696-697.

FITZGERALD, G.P. and S.L. FAUST (1967) A spectrophotometric method for the estimation of percentage degradation of chlorophylls to phaeo-pigments in extracts of algae. Limnology and Oceanography, 12, 335.

KIEFER, D.A., R.J. OLSON and W.H. WILSON (1979) Reflectance spectroscopy of marine phytoplankton. Part 1. Optical properties as related to age and growth rate. Limnology and Oceanography, 24(4), 664-672.

LIVINGSTONE, R., R. PARISER, L. THOMPSON and A. WEBBER (1953) Absorption spectra of solutions of phaeophytin a in methanol containing acid or base. Journal of the American Chemical Society, 75, 3025-3026.

LORENZEN, C.J. (1967) Determination of chlorophyll and phaeopigments: Spectrophotometric equations. Limnology and Oceanography, 12, 343-346.

MAERKER, M. and K.H. SZEKIELDA (1976) Chlorophyll determination of phytoplankton: A comparison of in vivo fluorescence with spectrophotometric absorption. Journal du Conseil. Conseil permanent International Pour l'Exploration de la Mer, 36(3), 217-219.

MOSS, B. (1967a) A note on the estimation of chlorophyll a in freshwater algal communities. Limnology and Oceanography, 12(2), 340-342.

MOSS, B. (1967b) A spectrophotometric method for the estimation of percentage degradation of chlorophylls to phaeopigments in extracts of algae. Limnology and Oceanography, 12, 335-340.

PARSONS, T.R. (1963) A new method for the microdetermination of chlorophyll c in seawater. Journal of Marine Research, 21, 164.

PARSONS, T.R. and J.D. STRICKLAND (1963) Discussion of spectrophotometric determination of marine plant pigments with revised equations for ascertaining chlorophylls. Journal of Marine Research, 21(3), 155-163.

RICHARDS, F.A. (1952) The estimation and characterization of plankton populations by pigment analyses. I. The absorption spectra of some pigments occuring in diatoms, dinoflagellates and brown algae. Journal of Marine Research, 11(2), 147-155.

RICHARDS, F.A. and T.G. THOMPSON (1952) The estimation and characterization of plankton populations by pigment analyses. II. A spectrophotometric method for the estimation of plankton pigments. Journal of Marine Research, 11(2), 156-171.

STRICKLAND, J.D.H. and T.R. PARSONS (1972) Spectrophotometric determination of chlorophylls and total carotenoids. In: A Practical Handbook of Seawater Analysis, Fisheries Research Board of Canada, Bulletin 167. (second edition) pp. 185-206.

TALLING, J.F. and D. DRIVER (1963) Some problems in the estimation of chlorophyll a in phytoplankton. In: Proceedings, Conference On Primary Productivity Measurement, Marine and Freshwater, Hawaii, 1961. M.S. Doty, editor, U.S. Atomic Energy Commission TID-7633, pp. 142-146.

TETT, P.M., M.G. KELLY and G.M. HORNBERGER (1975) A method for the spectrophotometric measurement of chlorophyll a and phaeophytin a in benthic microalgae. Limnology and Oceanography, 20, 887-896.

VERNON, L.P. (1960) Spectrophotometric determination of chlorophylls and phaeophytins in plant extracts. Analytical Chemistry, 32, 1144-1150.

WHITNEY, D.E. and W.M. DARLEY (1979) A method for the determination of chlorophyll a in samples containing degradation products. Limnology and Oceanography, 24(1), 183-186.

ZSCHEILE, F.P., Jr. (1934) An improved method for the purification of chlorophylls a and b; quantitative measurement of their absorption spectra; evidence for the existence of a third component of chlorophyll. Botanical Gazette, 95, 529-562.

ZSCHEILE, F.P., Jr. (1934) A quantitative spectro-photoelectric analytical method applied to solutions of chlorophylls a and b. Journal of Physical Chemistry, 38, 95-102.

Fluorometric Methods

BOARDMAN, N.K. and S.W. THORNE (1971) Sensitive fluorescence method for the determination of chlorophyll a /chlorophyll b ratios. Biochemica Biophysica Acta, 253, 222-231.

CULLEN, J.J. and E.H. RENGER (1979) Continuous measurement of the DCMU-induced fluorescence response of natural phytoplankton populations. Marine Biology, 53(1), 13-20.

GOEDHEER, J.C. (1966) Visible absorption and fluorescence of chlorophyll and its aggregates in solution. In: The Chlorophylls, L.P Vernon and G.R. Seely editors, Academic Press, pp. 147-184.

HERBLAND, A. (1978) The soluble fluorescence in the open sea: Distribution and ecological significance in the equatorial Atlantic Ocean. Journal of Experimental Marine Biology and Ecology, 32(3), 275-284.

HOLM-HANSEN, O, C.J. LORENZEN, R.W.HOLMES and J.D.H. STRICKLAND (1965) Fluorometric determination of chlorophyll. Journal du conseil. Conseil Permanent International Pour l'Exploration de la Mer, 30(1), 3-15.

HOLM-HANSEN, O. and B. RIEMANN (1978) Chlorophyll a determination: improvements in methodology. Oikos, 30(3), 438-447.

JARRETT, O., Jr., C.A. BROWN, J.W. CAMPBELL, W.M. HOUGHTON and L.R. POOLE (1979) Measurement of chlorophyll a fluorescence with an airborne fluorosensor. NASA, Langley Research Center, Hampton, VA 23365

KIEFER, D.A. (1973) Fluorescence properties of natural phytoplankton populations. Marine Biology, 22(3), 263-269.

LINCOLN, A. (1975) Problems involved in calibrating a fluorometer for measuring phytoplankton variability of chlorophyll fluorescence with species. British Phycological Journal, 10(3), 312.

LOFTUS, M.E. and J.H. CARPENTER (1971) A fluorometric method for determining chlorophylls a, b and c. Journal of Marine Research, 29(3), 319-338.

LOFTUS, M.E. and H.H. SELIGER (1975) Some limitations of the in vivo fluorescence technique. Chesapeake Science, 16(2), 79-92.

LORENZEN, C.J. (1966) A method of continuous measurement of the in vivo chlorophyll concentration. Deep Sea Research, 13, 223-227.

MAERKER, M. and K.H. SZEKIELDA (1976) Chlorophyll determination of phytoplankton: A comparison of in vivo fluorescence with spectrophotometric absorption. Journal du Conseil. Conseil permanent International Pour l'Exploration de la Mer, 36(3), 217-219.

STRICKLAND, J.D.H. (1968) Continuous measurement of in vivo chlorophyll; a precautionary note. Deep Sea Research, 15, 225-227.

STRICKLAND, J.D.H and T.R. PARSONS (1972) Fluorometric determination of chlorophylls. In: A Practical Handbook of Seawater Analysis, Fisheries Research Board of Canada, Bulletin 167. (second edition) pp. 185-206.

TURNER ASSOCIATES (1974) TURNER Filter Fluorometer Model 111, Operating Instructions and Service Manual. Palo Alto, California 94303.

TURNER DESIGNS In: Fluorometric facts, chlorophyll and phaeophytin. Turner Designs, Palo Alto, California 94303.

TURNER, G.K. and ASSOCIATES (1973) Determination of algae in natural waters by fluorometry. Turner Associates, Palo Alto, California 94303.

WHITE, R.C., I.D. JONES, E. GIBBS and L.S. BUTLER (1972) Fluorometric estimation of chlorophylls, chlorophyllides, pheophytins and pheophorbides in mixtures. Journal of Agriculture and Food Chemistry, 20, 773-778.

YENTSCH, C.S. and D.W. MENZEL (1963) A method for the determination of phytoplankton, chlorophyll and phaeophytin by fluorescence. Deep Sea Research, 10, 221-231.

Filtration Methodology

CORCORAN, E.F., J.F. KIMBALL, Jr. and J.E. ALEXANDER (1964) An improved method of filtration for chlorophyll analyses. Bulletin of Marine Science of the Gulf and Caribbean, 14(4), 545-548.

CREITZ, C.I. and F.A. RICHARDS (1955) The estimation and characterization of plankton populations by pigment analysis. III. A note on the use of "Millipore" membrane filters in the estimation of plankton pigments. Journal of Marine Research, 14, 211-216.

HOLM-HANSEN, O. and B. RIEMANN (1978) Chlorophyll a determination: improvements in methodology. Oikos, 30(3), 438-447.

LASKER, R. and R.W. HOLMES (1957) Variability in retention of marine phytoplankton by membrane filters. Nature, 180, 1295.

SAND-JENSEN, K. (1976) A comparison of chlorophyll a determinations of unstored and stored plankton filters extracted by methanol and acetone. Vatten, 32(4), 337-341.

Extraction Methodology

NELSON, D.J. (1960) Improved chlorophyll extraction method. Science, 132, 351.

SHOAF, W.T. and B.W. LIUM (1976) Improved extraction of chlorophyll a and b using dimethyl sulfoxide. Limnology and Oceanography, 21(6), 926-928.

STRAIN, H.H. and W.A. SVEC (1966) Extraction, separation, estimation and isolation of the chlorophylls. In: The Chlorophylls, L.P. Vernon and G.R. Seely, editors, Academic Press, pp. 21-66.

SUBBA RAO, D.V. and T. PLATT (1969) Optimal extraction conditions of chlorophylls from cultures of five species of marine phytoplankton. Journal of the Fisheries Research Board of Canada, 26(6), 1625-1630.

TUCKER, A. (1949) Pigment extraction as a method of quantitative analysis of phytoplankton. Transactions of the American Microscopic Society, 68, 21-33.

Pigments and Solvents Effects

FINENKO, Z.Z. and G.P. BERSENEVA (1976) Quantitative determination of chlorophylls a and e in marine planktonic algae by paper chromatography. Oceanology, 15(1), 124-127.

FOGG, G.E. (1975) Primary Productivity. In: Chemical Oceanography, J,P. Riley and G. Skirrow, editors. Academic Press, pp. 412-413.

GRAHAM, H.W. (1943) Chlorophyll-content of marine plankton. Journal of Marine Research, 5, 153-160.

HARRIS, D.G. and F.P. ZSCHEILE (1943) Effects of solvent upon absorption spectra of chlorophylls a and b, their ultraviolet absorption spectra in ether solutions. Botanical Gazette, 104, 515-527.

JONES, I.D., R.C. WHITE, E. GIBBS and C.D. DENARD (1968) Absorption spectra of copper and zinc complexes of pheophytins and pheophorbides. Journal of Agriculture and Food Chemistry, 16, 80-83.

LEHNINGER, A.L. (1975) The photosynthetic pigments. In: Biochemistry, The molecular basis of cell structure and function. (second edition) Worth Publishers, pp. 595-597.

LIVINGSTONE, R., R. PARISER, L. THOMPSON and A. WEBBER (1953) Absorption spectra of solutions of phaeophytin a in methanol containing acid or base. Journal of the American Chemical Society, 75, 3025-3026.

MADIGAN, M.T. and T.D. BROCK (1976) Quantitative bacterio-chlorophyll c in the presence of chlorophyll a in aquatic environments. Limnology and Oceanography, 21(3), 462-467.

MARKER, A.F.H. (1972) The use of acetone and methanol in the estimation of chlorophyll in the presence of phaeophytin. Freshwater Biology, 2, 361-385.

MARKER, A.F.H. (1977) Some problems arising from the estimation of chlorophyll a and phaeophytin a in methanol. Limnology and Oceanography, 22(3), 578-579.

MOSS, B. (1968) Studies on the degradation of chlorophyll a and carotenoids in freshwaters. New Phytology, 67, 49-59.

PARSONS, T.R. (1961) On the pigment compositions of eleven species of marine phytoplankters. Journal of the Fisheries Research Board of Canada, 18, 1017-1025.

PARSONS, T.R. (1963) A new method for the microdetermination of chlorophyll c in seawater. Journal of Marine Research, 21, 164.

PARSONS, T.R. (1963) The determination of photosynthetic pigments in seawater. A survey of methods. UNESCO NS/89J.

PATTERSON, J. and T.R. PARSONS (1963) Distribution of chlorophyll a and degradation products in various marine materials. Limnology and Oceanography, 8, 355-356.

RABINOWITCH, E.I. and GOVINDJEE (1968) The role of chlorophyll in photosynthesis. In: The molecular basis of life, readings from Scientific American. R,H. Haynes and P.C. Hanawalt, editors. W.H. Freeman and Company, pp.307-315.

RICHARDS, F.A. (1952) The estimation and characterization of plankton populations by pigment analyses. I. The absorption spectra of some pigments occuring in diatoms, dinoflagellates, and brown algae. Journal of Marine Research, 11(2), 147-155.

RICHARDS, F.A. and T.G. THOMPSON (1952) The estimation and characterization of plankton populations by pigment analyses. II. A spectrophotometric method for the estimation of plankton pigments. Journal of Marine Research, 11, 156-171.

SAND-JENSEN, K. (1976) A comparison of chlorophyll a determinations of unstored and stored plankton filters extracted by methanol and acetone. Vatten, 32(4), 337-341.

SEELY, G. R., M.J. DUNCAN and W.E. VIDAVER (1972) Preparative and analytical extraction of pigments from brown algae with dimethyl sulphoxide. Marine Biology, 12, 184-188.

SHOAF, W.T. and B.W. LIUM (1976) Improved extraction of chlorophyll a and b using dimethyl sulfoxide. Limnology and Oceanography, 21(6), 926-928.

SPENCER, C.P. (1964) The estimation of phytoplankton pigments. Extrait du Journal du Conseil International Pour l'Exploration de la Mer, 28(3), 327-334.

STRICKLAND, J.D.H. (1965) Production of organic matter in the primary stages of the marine food chain. In: Chemical Oceanography, J.P Riley and G. Skirrow, editors, Academic Press, 1, pp. 477-610.

WHITNEY, D.E. and W.M. DARLEY (1979) A method for the determination of chlorophyll a in samples containing degradation products. Limnology and Oceanography, 24(1), 183-186.

YENTSCH, C.S. (1965) Distribution of chlorophyll and phaeophytin in the open ocean. Deep Sea Research, 12, 653-666.

ZSCHEILE, F.P., Jr. (1934) An improved method for the purification of chlorophylls a and b; quantitative measurement of their absorption spectra; evidence for the existence of a third component of chlorophyll. Botanical Gazette, 95, 529-562.

ZSCHEILE, F.P., Jr. (1934) A quantitative spectro-photoelectric analytical method appled to solutions of chlorophylls a and b. Journal of Physical Chemistry, 38, 95-102.

Method Manuals and Surveys

AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, WATER POLLUTION CONTROL FEDERATION (1975) In: Standard Methods for the Examination of Water and Wastewater, 14th edition, American Public Health Association, 1015 Eighteenth St. NW, Washington, D.C. 20036, pp. 1029-1034.

PARSONS, T.R. (1963) The determination of photosynthetic pigments in seawater. A survey of methods. UNESCO NS/89J.

RYDING, S.O. (1976) Intercalibration of methods for determining chlorophyll a in water. Vatten, 31(4), 327-332.

TOLSTOY, A. (1977) Methods of Determining Chlorophyll a in Phytoplankton. National Swedish Environmental Protection Board, Limnological Survey, Uppsala SNV PM 831 NLU Rapport 91. 62 p.

VERNON, L.P. and G.R. SEELY, editors, (1966) The Chlorophylls, Academic Press.

VOLLENWEIDER, R.A. (1969) A manual on methods for measuring primary production in aquatic environments. IBP Handbook 12, Blackwell Science Publications, England. XVI, 213p.

WEBER, C.I. (1973) Recent developments in the measurement of the response of plankton and periphyton to changes in their environment. In: Bioassay Techniques and Environmental Chemistry, G. Glass, editor, Ann Arbor Science Publications Incorporated.

ZINGMARK, R.G. (1979) Are chlorophyll measurements meaningful indicators of phytoplankton biomass? (abstract) Journal of Phycology, 15(19), supplement.

This report was done with support from the Department of Energy. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the Department of Energy.

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