

This Week's Citation Classic

Richards F A & Thompson T G. The estimation and characterization of plankton populations by pigment analyses. II. A spectrophotometric method for the estimation of plankton pigments. *J. Marine Res.* **11**:156-72, 1952. [Oceanographic Labs., Univ. Washington, Seattle, WA]

A semimicro spectrophotometric method is described for the estimation of chlorophyll a, b, and c and of astacin and nonastacin type carotenoids in acetone extracts of plant and animal material. Developed specifically for use in estimating plankton samples, spectrophotometric measurements, computation of results, and errors are discussed. [The SCI® indicates that this paper has been cited over 160 times since 1961.]

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"At the University of Washington's Friday Harbor Laboratories, where this research was done, the general exuberance of the newly returned GI's and the camaraderie among students and faculty provided a cross-fertilization of ideas about the sea from chemical and physical oceanographers, zoologists, botanists, meteorologists—even anthropologists.

"Primarily an analytical chemist, I was introduced to marine problems through the infectious enthusiasm of Thomas C. Thompson, director, and the first American chemist to work in marine problems.¹ I was his graduate student, and, as a class project in algology, I separated algal extracts using column chromatography, then a little known technique.

"Talks with Martin W. Johson, a biological oceanographer from Scripps Institution of Oceanography, and John P. Tully, a Canadian physical oceanographer, suggested that estimating phytoplankton populations and their photosynthetic potential was a most important oceanographic problem and that pigment determinations might be a reasonable basis for the estimates.

"The idea of measuring chlorophyll in phytoplankton was not new, but I thought

that a knowledge of the other pigments would tell more about the productive potential and physiological state of plankton than an estimate of chlorophyll alone. The idea of 'pigment units' had been introduced,² they were estimated by visual comparison of plankton extracts with different concentrations of NiSO₄ and K₂Cr₂O₇ solutions, mixed in a constant ratio to each other. I tried varying the ratios of green to yellow, but the extracts always came close to Harvey's original values.

"My aim was to determine major pigments without separating them, as had been necessary before. Spectrophotometry offered better sensitivity than the colorimetry used earlier, and, in his enthusiastic support, Thompson purchased a newly introduced Beckman electronic spectrophotometer for my work.

"The method required knowledge of the absorption spectra of the individual pigments involved. A few were commercially available; important ones were not, and thus separation, purification, and spectral characterization of the pigments was a major effort. The greatest frustration was isolation of chlorophyll c, reported to be important in diatoms but never, at that time, determined in natural populations.

"The compound was finally separated from the colonial diatom *Navicula araneosum* Cl., its absorption spectrum determined, and the simultaneous equations for five different pigments (or pigment groups) could then be written.

"The method was published when primary productivity was recognized as a major oceanographic concern, it used instrumentation that had just become readily available, and it was relatively simple and reliable. It was a method whose time had come. Also, it was easily applicable to the fisheries' problem of measuring ocean richness to find feeding grounds for the catches. Others saw in it a technique for identifying water masses and tracing their movement.

"There have been many modifications and improvements, most importantly the better definition of spectral characteristics of the chlorophylls. A critical review of the method and its modifications was published in a Unesco monograph."³

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1. **Redfield A C, Barnes C A & Richards F A.** Thomas Gordon Thompson 1888-1961. *National Academy of Sciences, biographical memoirs*. New York: Columbia University Press, 1973. Vol. 43. p. 237-60.
 2. **Harvey H W.** Measurement of phytoplankton population. *J. Mar. Biol. Ass. UK* **19**:761-73, 1934.
 3. **United Nations Educational, Scientific and Cultural Organization.** *Determination of photosynthetic pigments in sea-water*. Paris: Unesco. 1966. 69 p.