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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.[1,2]

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ABSTRACT

Collection of plankton samples on "AA" (aerosol assay) type "Millipore" membrane filters has been substituted for centrifugation in the method of Richards with Thompson for estimating plankton pigments. Although the filter dissolves in the 90% acetone that is subsequently used for extracting the pigments, its solution does not interfere with the spectrophotometric determination of the chlorophyll and carotenoid components. Comparison between retention by Millipore filters and the Foerst plankton centrifuge indicates that the filters are significantly more efficient. Plankton samples can be stored dry, in the dark, under vacuum, for at least three weeks without affecting the pigments for subsequent analysis.

During the work reported in the second paper of this series (5), various methods for removing plankton from water were investigated, and the Foerst plankton centrifuge was selected as the most satisfactory. Since that time, Millipore membrane filters[3] have become

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[3] Manufactured under the name "Millipore" by the Lovell Chemical Company, Watertown, Massachusetts (4); available in two grades, designated "HA" (hydrosol assay) and "AA" (aerosol assay).

readily available and the procedures previously described have been varied to incorporate their use.

The method, as revised, is as follows: filter an adequate quantity of water containing about 0.1 g of powdered magnesium carbonate through an AA Millipore filter, trim the filter to the size of its effective filtering area, fold and roll it into a cylinder and drop it into a 16 × 125 mm screw-cap test tube. Place the tube in a vacuum desiccator to be dried and stored if immediate extraction is impractical. Preliminary investigations indicate that dried plankton samples may be stored at least three weeks without apparent change in the absorption spectra of their extracts by refrigerating the samples in a vacuum desiccator. This procedure protects them from oxygen, moisture, light and high temperatures. Extraction and estimation are carried out according to the procedures of Richards with Thompson (5).

Membrane filters have been described by Goetz and Tsuneishi (2) and by Bush (1), and their use in plankton collection has been described by Goldberg, Baker and Fox (3).

Despite their designation as AA filters, they have proved applicable for use with aqueous suspensions. According to a personal communication from W. B. Krabek of the Lovell Chemical Company, a suspension of *Serratia marcescens* (coccoid, 0.5 μ, or short rods, 0.5 × 0.5 − 1.0 μ) containing 8×10^6 organisms was almost completely retained by AA filters, 25 or fewer colonies appearing in the filtrate. In this laboratory, aerosol filters have retained completely the cells of a *Nannochloris* species culture (1.0 − 2.5 μ). These observations as well as those on the retention of a large number of natural plankton populations lead to the conclusion that AA filters will retain all natural plankton organisms down to 1 μ in diameter. The HA filter, though it has a somewhat smaller pore size, filters less freely, is more brittle and bulky, and has a somewhat greater absorbancy when placed in 90% acetone solution (Table I); therefore, the AA filter is now used exclusively in this laboratory.

Table I. Absorbancies (log I_0/I) of solutions of Millipore filters in five ml of 90% acetone, in absorption cells of 5 cm light path.

Wavelength $m\mu$	*AA Type (Entire, 4.7 cm. diam.)*	*AA Type (Trimmed to ca. 3.9 cm diam.)*	*HA Type (Entire, 4.7 cm diam.)*
480	.005	.002	.012
510	.004	.001	.010
630	.002	.000	.006
645	.001	.000	.006
665	.001	.000	.005
750	.001	.000	.004

The volume to be filtered varies with the type of water under investigation. The filters act as two-dimensional screens, permitting water to pass through them rapidly until all pores are covered; after that time, additional water can be filtered only very slowly. The amount of water which can be filtered in a reasonable time depends on the amount and kind of particulate matter present. Small volumes can be filtered when the plankton crop is large, large volumes when the crop is small. Nonpigmented detritus can also clog the filters, and in extreme cases sufficient amounts of plankton for a satisfactory analysis might not be collected. The volumes of natural waters which have been used in this laboratory have varied from a few hundred milliliters to more than six liters. Much smaller volumes of algal cultures have been used. To insure representative sampling, precautions must be taken against the settling onto the membrane of particulate matter from water which will not be filtered.

Absorbancy of Millipore Filters. Millipore filters are almost completely soluble in 90% acetone. Clear solutions are obtained on centrifuging for about 5 minutes in a clinical centrifuge in the presence of magnesium carbonate. Solutions of AA Millipore filters have negligible absorbancies at all wavelengths that are used to compute pigment concentrations (Table I). The absorbancy is decreased when the filters are trimmed to remove the unexposed edge; this removes about 30% of the total bulk of the filter.

Millipore filters were added to a large number of solutions containing plankton pigments, and the resultant spectra were compared with those of the original pigment solutions. Examples of two such experiments are shown in Table II. There is no evidence of chemical interaction between the filter and the pigments to create discrepancies in the absorption spectra. The small effects which the filters have on the pigment spectra are similar to Rayleigh scattering and probably result from incomplete solution of the filter and (or) gel-stabilized colloidal suspensions of particulate matter. In these studies, representing a variety of concentrations, average deviation of chlorophyll *a* estimates, either with or without added filters, was less than 5%.

Scattering can be detected from the absorbancy at 750 mμ, where pigment absorbancies are nearly negligible. If the absorbancy at this wavelength is appreciable, further centrifugation in the presence of magnesium carbonate is probably necessary. Even slight turbidities cause erroneously high estimates of components other than chlorophyll *a*. The chlorophyll *a* estimates are not as greatly affected but tend to be slightly low.

Table II. Absorbancies (log I_0/I) in 5 cm absorption cells, computed pigment concentrations of plankton extracts with and without added Millipore (AA) filter.

	ABSORBANCY			
	Station 11		*Station 12*	
Wavelength, mμ.	*Without M.F. added*	*With M.F. added*	*Without M.F. added*	*With M.F. added*
480	1.020	1.015	.455	.468
510	.390	.395	.185	.202
630	.197	.197	.076	.075
645	.220	.220	.089	.088
665	.790	.795	.239	.238
750	.009	.015	.009	.012
	COMPUTED CONCENTRATIONS			
Chlorophyll *a*,mg/l	11.72	11.80	3.49	3.48
Chlorophyll *b*,mg/l	0.05	0.02	0.43	0.42
Chlorophyll *c*,MSPU/L	5.28	5.23	2.75	2.70
Non-astacin carotenoids, MSPU/L	3.41	3.56	1.33	1.24
Astacin-type carotenoids, MSPU/L	1.16	1.21	0.75	0.78

Comparisons of Retention by the Foerst Plankton Centrifuge and Millipore Filters. It is well known that the recovery of plankton organisms by the continuous-feeding plankton centrifuge depends on several factors, including the speed of the instrument, the size and relative density of the plankton organisms, and the rate of flow through the centrifuge. The membrane filters provide a good means for investigating the retention by the centrifuge.

Aliquot samples of sea water were passed through AA Millipore filters and through the Foerst plankton centrifuge at a rate of about seven minutes per liter. The material passed by the centrifuge was subsequently filtered. The differences in retention by the two methods were large, the chlorophyll *a* retained by the centrifuge varying between 69 and 86% of that retained by the filters. The sum of the pigment contents of the material retained and passed by the centrifuge agreed well with the pigments retained by filtration.

In the examples in Table III, the material retained by the centrifuge was comparatively poor in chlorophyll *c* whereas the material recovered on filtration of the centrifugate was rich in this compound.

Table III. Comparison of retention by Foerst plankton centrifuge and AA type Millipore filters.

Sample			*Chlorophyll a* mg/M^3	*Chlorophyll b* mg/M^3	*Chlorophyll c* $MSPU/M^3$		$\frac{Chph\ b}{Chph\ a}$	$\frac{Chph\ c}{Chph\ a}$
21	Retained by Centrifuge		2.52	0.76	3.33		0.30	1.32
	Passed by Centrifuge		1.04	0.48	2.17		0.46	2.05
		Sum	3.56	1.24	5.50	$\Sigma/\Sigma Ca$	0.35	1.54
	Retained by M.F.		3.58	1.05	5.80		0.29	1.62
15	Retained by Centrifuge		4.68	0.76	4.83		0.16	1.03
	Passed by Centrifuge		0.97	0.48	2.65		0.50	2.74
		Sum	5.65	1.24	7.48	$\Sigma/\Sigma Ca$	0.22	1.32
	Retained by M.F.		5.94	1.40	8.00		0.24	1.35
8	Retained by Centrifuge		8.04	2.31	2.51		0.29	0.31
	Passed by Centrifuge		1.06	0.57	1.67		0.54	1.58
		Sum	9.10	2.88	4.18	$\Sigma/\Sigma Ca$	0.32	0.46
	Retained by M.F.		11.60	2.26	5.56		0.19	0.48

This suggests that the centrifuge is effecting a separation of plankton species.

Other work, now in progress, shows that dinoflagellates are particularly rich in this compound and that they are thrown down by centrifuging less readily than diatoms and other plankton organisms. The samples in Table III contained an abundance of dinoflagellates, and the enrichment of chlorophyll *c* in the material passed by the centrifuge could reflect the failure of the instrument to retain dinoflagellates or fragments of them. Similar though less marked changes in the ratios of chlorophyll *b* to *a* could be interpreted as a selective failure of the centrifuge to retain small green algae.

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