# PLANKTON PIGMENT NOMOGRAPHS[1]

By

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## ABSTRACT

Nomographs for computing the amounts of plankton pigments as determined by the spectrophotometric method have been constructed. The methods used for alignment and scaling are discussed. The use of the nomographs shortens computation time considerably.

*Introduction.* The semi-micro method described by Richards with Thompson (1952) for analysis of plankton samples may be used to determine pigment types in plankton populations. In this method the total absorbencies of plankton extracts are measured at specified wave lengths. In brief, the plankton samples are concentrated, the pigments are extracted in 90% acetone, and the optical density of the extract is measured at wave lengths 665, 645 and 630 mμ for chlorophylls *a*, *b*, and *c* and at 480 and 510 mμ for animal and plant carotenoids. Where large numbers of samples are involved, considerable time is spent in computing the pigment types because of the numerous steps involved in solving the simultaneous equations of three or four variables. This time may be shortened considerably by converting the Richards with Thompson equations into nomographic form.

*Development of the Nomographs.* The nomographs designed for solving these equations comprise a series of alignment diagrams which are readily constructed and which can be used rapidly for determining the amounts of pigment types. The nomographs consist of a series of parallel lines which are spaced and scaled by means of ratios between scale moduli. The scale modulus is a factor which will reduce the coefficient of a component of an equation, such as that of Richards with Thompson, to a unit value. The use of scale moduli allows the different components to be treated with equal scale spacing and

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facilitates the construction of uniform scales for these components. The method used in preparing these nomographs is taken, in part, from Levens (1948) and Kulmann (1954).

The Richards with Thompson equations for determining chlorophyll are:

$$C_a \text{ (mg/L)} = 15.6\ D_{665} - 2.0D_{645} - 0.8D_{630}$$
$$C_b \text{ (mg/L)} = 25.4\ D_{645} - 4.4D_{665} - 10.3D_{630}$$
$$C_c \text{ (MSPU/L)} = 109\ D_{630} - 12.5D_{665} - 28.7D_{645},$$

where the $D$ terms to the right are optical densities. The equations for determining animal and plant carotenoids, knowing the optical densities and chlorophyll values, are:

$$D_{res510} = D_{510} - 0.0026C_a - 0.0035C_b - 0.0021C_c$$
$$D_{res480} = D_{480} - 0.0019C_a - 0.0136C_b - 0.0054C_c$$

or

$$\text{Nonastacian (MSPU/L)} = 7.6\ (D_{res480} - 1.49\ D_{res510})$$
$$\text{Astacian (MSPU/L)} = 2(4.45\ D_{res510} - D_{res480})$$

The nomograph of the equation for determining chlorophyll *a* is set up in the following manner. Since the equation has three components or variables that are multiplied by constant coefficients, scale moduli that reduce or change these coefficients to equal values must be determined. This can be done by multiplying each coefficient by its reciprocal, which reduces all coefficients in the equations to unity. These reciprocals are the scale moduli. The equation now has all components weighted equally.

A vertical line is drawn for $D_{645}$ near the left side of the paper, and then another line at some distance is drawn parallel to it for $D_{630}$. The distance between these lines is quite arbitrary. The $D_{665}$ line is then drawn parallel and at an arbitrary distance to the right of the first two lines. On an 8 × 10 piece of paper the distance between $D_{645}$ and $D_{630}$ can conveniently be about three inches, the distance between $D_{630}$ and $D_{665}$ about four inches. The $D_{645}$ line should be subdivided from zero to about 0.7 unit in optical density, zero being placed at the top. The spacing of the subdivisions should be great enough to assure reading accurately to about .002 unit. The $D_{630}$ line is now graduated similarly with corresponding divisions located on a line perpendicular to the divisions on $D_{645}$. To allow for both positive and negative signs that occur in the equation, the direction from bottom to top of the page is considered positive. The $D_{665}$ line is now scaled with zero at the bottom on a common perpendicular with the 0.7 mark of the $D_{645}$ and $D_{630}$ lines, the 0.7 at the top in line with the zero mark of the latter lines.

The equation $C_a$ (mg/L) $= 15.6D_{665} - 2.0D_{645} - 0.8D_{630}$ is now divided into two parts. The last two components are set equal to a factor called $Q$. Thus, $-1.0D_{645} - 0.8D_{630} = Q$ and $15.6D_{665} + Q = C_a$ (mg/L)

The scale moduli for $D_{645}$ and $D_{630}$ are 1/2.0 and 1/0.8 respectively. The $Q$ line is placed between $D_{645}$ and $D_{630}$ at a location determined by the ratio of these scale moduli. In this case the ratio is 1/2.0 divided by 1/0.8 or 0.8/2.0. The position of $Q$ is conveniently determined by assuming that the number of units of distance between $D_{645}$ and $D_{630}$ is 2.8 units and by locating $Q$ 0.8 units from $D_{645}$ and 2.0 units from $D_{630}$. To simplify further calculations, a substitution is made by referring to the scale moduli with letters and subscripts as follows:

$$M_{645} = 1/2.0;\ M_{630} = 1/.08;\ \text{and}\ M_{665} = 1/15.6 \quad .$$

$Q$ is now located on the nomogram, but a scale modulus for it, $Mq$, must now be determined. $Mq$ gives the ratio between the size of the divisions of $Q$ and those of $D_{665}$, $D_{645}$ and $D_{630}$. However, $Q$ need not be graduated, and $Mq$ is merely used later for determining the placement of the chlorophyll $a$ line. $Mq$ is found in the following manner:

$$\frac{M_{645} \times M_{630}}{M_{645} + M_{630}} = Mq = \frac{1/2.0 \times 1/0.8}{1/2.0 + 1/0.8} = \frac{1}{2.8} \quad .$$

The chlorophyll $a$ line is now positioned. The ratio of $Mq/M_{665}$ or 1/2.8/1/15.6 places the $C_a$ line 15.6 units from $Q$ and 2.8 units from $D_{665}$, if the distance between $Q$ and $D_{665}$ is assumed to be 18.4 units. The final calculation is the scale modulus for chlorophyll $a$. It is determined in much the same manner as for $Mq$. Thus,

$$M_{Ca} = \frac{Mq \times M_{665}}{Mq + M_{665}} = \frac{1/2.8 \times 1/15.6}{1/2.8 + 1/15.6} = \frac{1}{18.4} \quad .$$

This means that each unit on the $C_a$ scale is 1/18.4 times as large as the units on the optical density scales. Since the optical density scales are in tenths, the scale modulus, $M_{Ca}$, may be multiplied by ten to convert the chlorophyll units from tenths to units. Thus, 1(mg/L) is equal in length to 1/1.84 times the length devoted to 0.1 optical density. Graduation of the chlorophyll $a$ line is accomplished by solving the nomogram to determine the zero point on the chlorophyll $a$ line and then marking off the successive units along the scale.

The nomogram is used in the following manner. A straight edge is employed to line up the values of $D_{645}$ and $D_{630}$ as determined by the analysis; the intersection of this edge with $Q$ is then noted. The

straight edge is then moved to join the intersection on $Q$ with the value obtained for $D_{665}$. This line now crosses the chlorophyll *a* line, and the value of chlorophyll *a* (in mg/L) is read directly.

All other nomograms in this series are constructed in the same manner, using appropriate scale moduli. It should be noted that the equations for the carotenoids necessitate solving first for the $D_{res480}$ and $D_{res510}$. These equations involve four variables instead of the three as in the case of chlorophyll; otherwise, the same procedure is followed. Chlorophyll *a* and chlorophyll *b* combine to designate an intersection on the $Q$ line; $Q$ and chlorophyll *c* combine to designate a $W$ line intersection and $W$ and $D_{480}$ or $D_{510}$ combine to designate the intersecting $D_{res}$ lines. The solving of these diagrams consists of the same stepwise procedure described for chlorophyll *a*.

*Errors and Range of Values.* The amount of error introduced by using the nomographs depends largely upon the accuracies to which

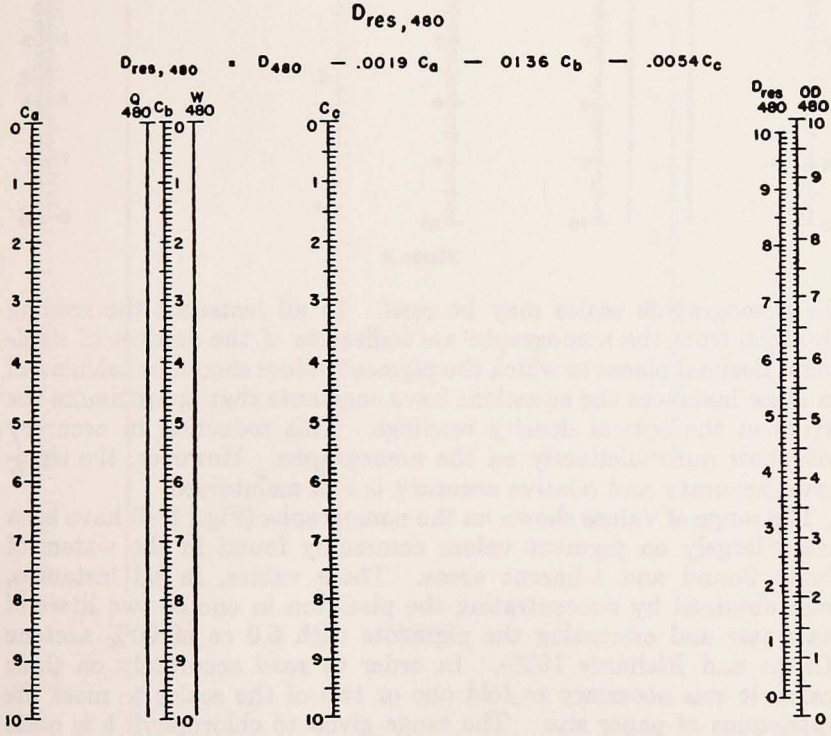


Figure 1

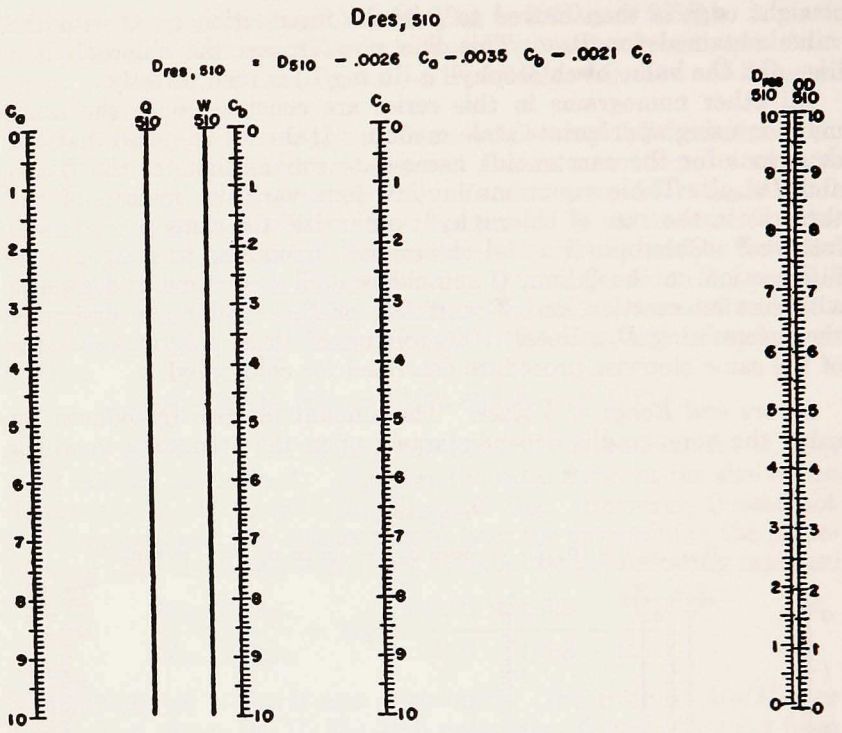


Figure 2

the nomographic scales may be read. In all instances the reading obtained from the nomographs are indicative of the number of significant decimal places to which the pigment values should be calculated. In some instances the equations have constants that approximate the errors in the optical density readings. This reduction in accuracy will show quite distinctly on the nomographs. However, the significant accuracy and relative accuracy is still maintained.

The range of values shown on the nomographs (Figs. 1–6) have been based largely on pigment values commonly found in the waters of Puget Sound and adjacent areas. These values, in all instances, were obtained by concentrating the plankton in one or two liters of sea water and extracting the pigments with 5.0 cc of 90% acetone (Creitz and Richards 1955). In order to read accurately on these scales, it was necessary to fold one or two of the scales to meet the restrictions of paper size. The range given to chlorophyll *b* is quite arbitrary, since this pigment was seldom found in large amounts in

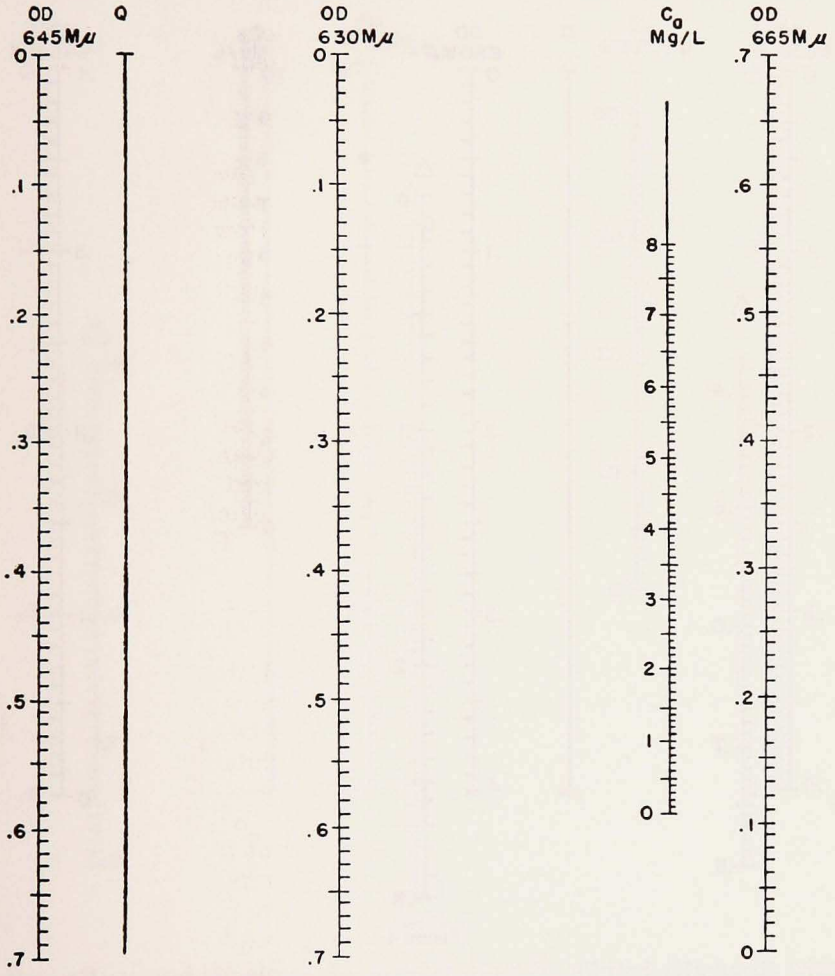
CHLOROPHYLL a
$C_a$ (Mg /L) = 15.6 $D_{665}$ − 2.0 $D_{645}$ − 0.8 $D_{630}$
OD 645Mμ
Q
OD 630Mμ
$C_a$ Mg/L
OD 665Mμ
0
.1
.2
.3
.4
.5
.6
.7
8
7
6
5
4
3
2
1
0

Figure 3

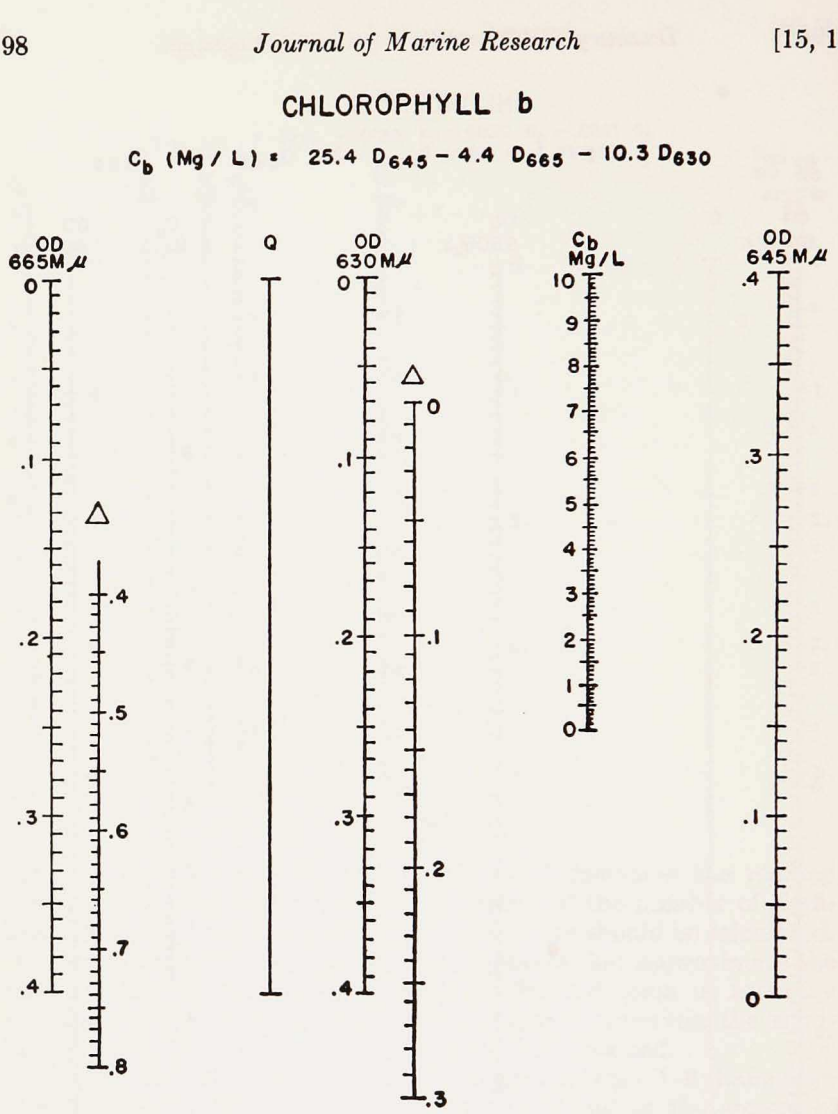


Figure 4

marine plankton. The range of values shown for the animal (astacian) carotenoids may too low, since some zooplankters are not caught in standard hydrographic sampling bottles. No optical density values are extended farther than 1.0, since an extract with a concentration giving an optical density reading higher than 0.90 will not follow

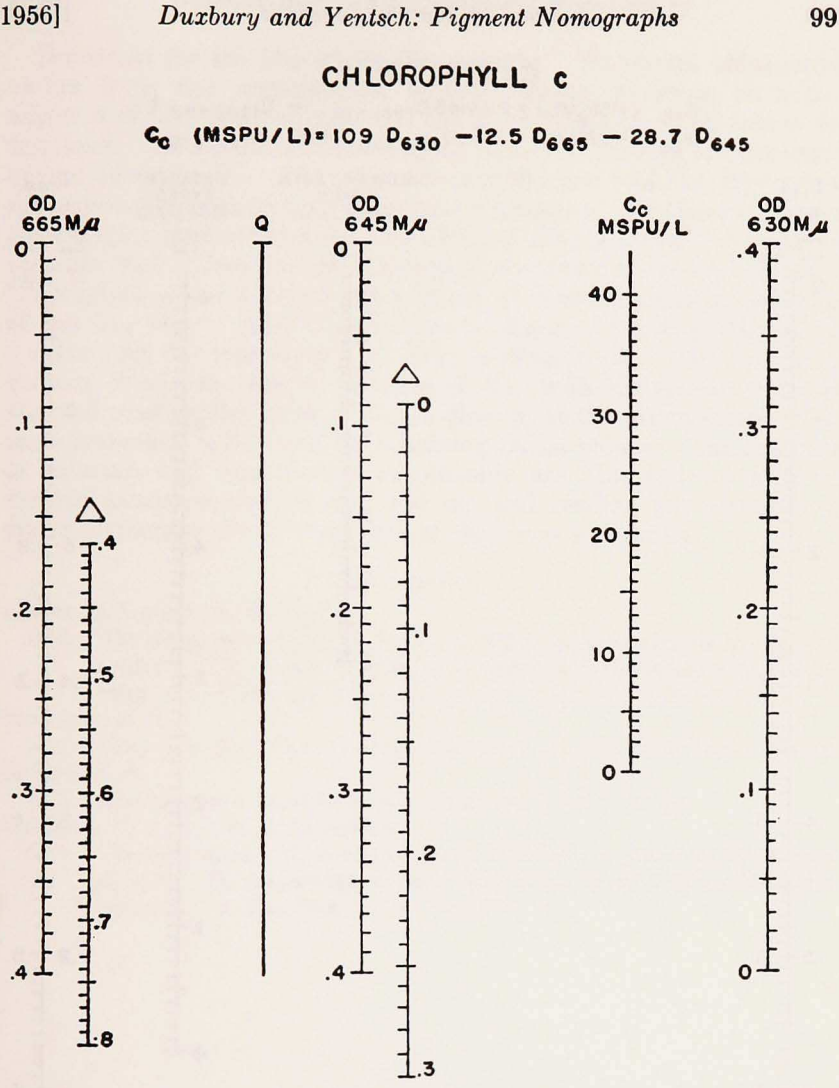


Figure 5

Beer's Law and therefore the extract must be diluted (Richards with Thompson, 1952). Greater color sensitivity may be obtained by using longer light paths. The plankton pigment nomographs, following the original equations, are based on optical density values using a 1 cm light path. When longer light paths are employed, either the optical density readings or the pigment values may be corrected accordingly.

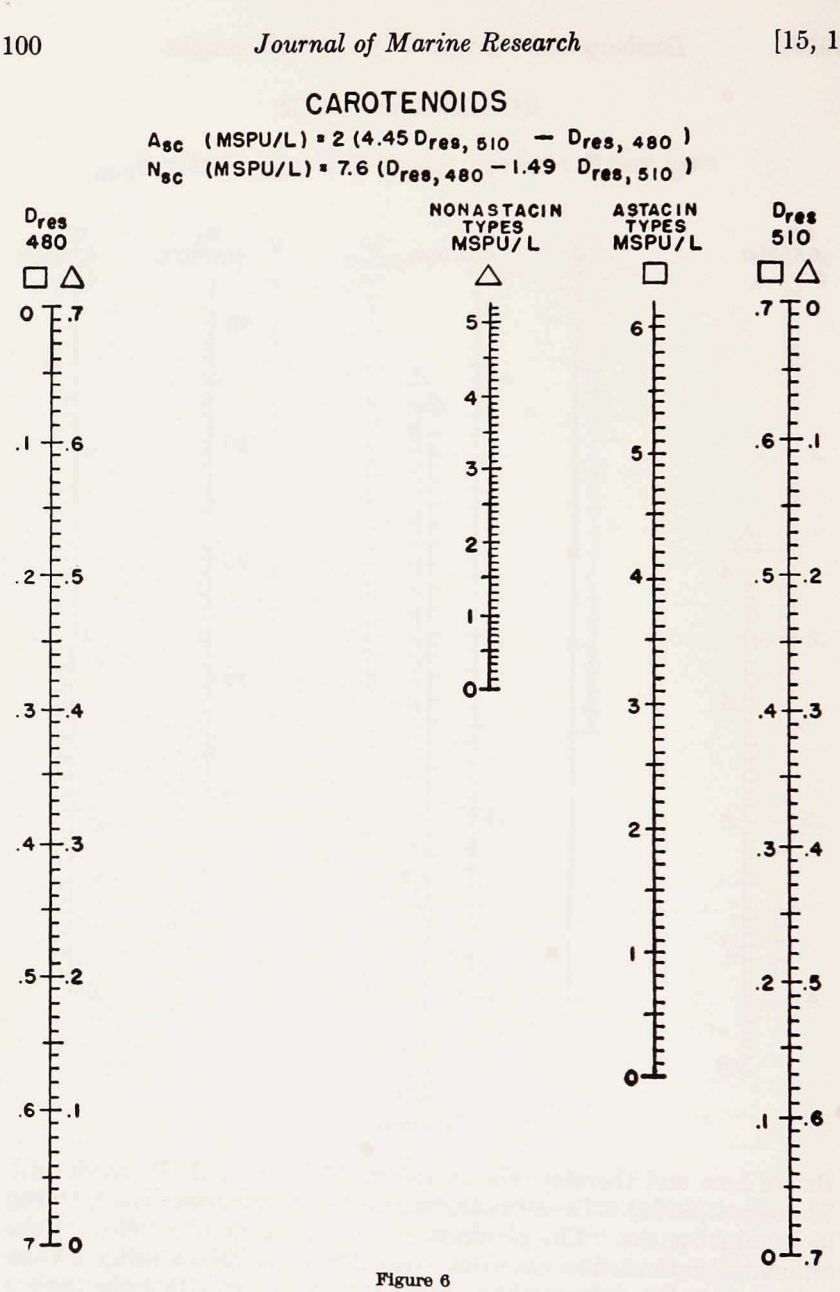
CAROTENOIDS
$A_{sc}$ (MSPU/L) = 2 (4.45 $D_{res, 510}$ − $D_{res, 480}$ )
$N_{sc}$ (MSPU/L) = 7.6 ($D_{res, 480}$ − 1.49 $D_{res, 510}$ )
$D_{res}$ 480
NONASTACIN TYPES MSPU/L
ASTACIN TYPES MSPU/L
$D_{res}$ 510
0 .7
.1 .6
.2 .5
.3 .4
.4 .3
.5 .2
.6 .1
7 0
0 1 2 3 4 5
0 1 2 3 4 5 6
.7 0
.6 .1
.5 .2
.4 .3
.3 .4
.2 .5
.1 .6
0 .7

Figure 6

*Directions for the Use of the Nomographs.* To obtain chlorophyll values from the nomographs, optical density readings on scales adjacent of line $Q$ are aligned and the intersection on the $Q$ line is determined. This point is then aligned with the reading for the third optical density scale. The intersection of this line with the chlorophyll scale gives the value of the chlorophyll in question. For the carotenoid nomographs, residual absorbencies 510 and 480 mμ must be considered together with values for the chlorophyll observed in the same sample. Chlorophyll *a* and *b* values are aligned to determine the intersection of the $Q_{510}$ scale. This intersection is aligned with the chlorophyll *c* value and the intersection on $W_{510}$ is determined. The final step consists of aligning the intersection of $W_{510}$ with the correct value of $D_{510}$ and reading the value of $D_{res510}$ directly at the intersection. The same procedure is followed for obtaining $D_{res480}$ values. The amounts of astacian and nonastacian carotenoids are obtained by aligning residual absorbencies 510 and 480 mμ and reading the amounts of either pigment at the intersection of the respective scales.

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