PRESERVATION AND STRATIGRAPHIC DISTRIBUTION OF PIGMENTS IN MINNESOTA LAKE SEDIMENTS

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ABSTRACT

Cores of recent sediments from four Minnesota lakes were studied to determine the pigment content at different levels. The lakes represented are: 1) Upper Red Lake, a shallow-water remnant of glacial lake Agassiz in which the sediment is predominantly clastic; 2) Lake Itasca, a eutrophic lake surrounded by a forested recessional moraine; the sediment is predominantly copropelic marl; 3) Long Lake, a deep, oligotrophic lake near Lake Itasca with a similar surrounding environment and smaller watershed; the sediment is copropelic, argillaceous marl; and 4) Blue Lake, a shallow-water lake with abundant aquatic plant life located in glacial moraine; the sediment is copropelic, calcareous sapropel.

The absorption spectra of 90 per cent acetone extracts were very nearly alike for all the lakes, and at different levels in the cores. Main peaks characteristically occurred in the range 411-18 mμ and 665-70 mμ. The surface sample from Upper Red Lake showed a peak at 432 mμ indicative of chlorophyll a. Generally, there was a decrease of pigment content with depth with some cores showing the largest change in the top few inches. Lakes with a higher development of shoreline had a higher amount of preserved pigments.

The length of extraction time does not have a significant effect on quantitative determinations of pigments. The absorption spectra of air-dried samples were found to be significantly different from those of moist samples both in height and location of maxima. Paper chromatography indicated the possible presence of α- and β- carotene and echinenone in the sediment from Long Lake.

INTRODUCTION

When a sample of marine or fresh water sediment is treated with an organic solvent such as acetone the extract usually takes on a greenish-yellow color. This color is the result of pigments or products of pigments preserved in the sediment. These pigments in recent, unconsolidated sediments can be divided into two main groups; the derivatives of chlorophyll and the carotenoids.

The study of these types of compounds in sediments has been stimulated by their possible connection with the origin of petroleum. It has also been suggested by Vallentyne (1955) that the change in pigment content in a vertical core of sediment might be indicative of the past biological history of a lake. Another suggestion by Klenova and Jastebova (1938) is that it might be possible to determine past changes in the gas regimens of water bodies by analyzing sediment cores for pigments.

The main factors which operate to change pigments once they are removed from their natural occurrence are heat, light and oxygen. Pigments are preserved in sediments because of the low temperatures, the lack of light, and reducing conditions which are usually found at the bottom of a lake, all of which reduce the rate of decomposition of organic material.

The true plant chlorophylls are relatively unstable under laboratory conditions after they have been extracted by solvents. They have not been found in sediments, except for the possible

occurrence in small amounts in the surface layer of shallow water sediments. What have been found are compounds which have been variously referred to as sedimentary chlorophyll, chlorophyll degradation products, (SCDP; introduced by Vallentyne, 1957a), pheophytin or simply as green pigments. These are all believed to be derivatives of the common plant chlorophylls. They have absorption spectra similar to those of known plant chlorophylls with absorption maxima located at 665-80 mμ (red portion of the spectrum) and 410-20 mμ (violet portion of the spectrum). Carotenoids and other organic materials which are also extracted absorb light strongly at wave lengths below 550 mμ, but cause only low absorbance above 600 mμ.

The exact structure of these chlorophyll-like pigments has not been determined. It is believed to be intermediate between that of the plant chlorophylls and that of porphyrin compounds in crude oils, bituminous shales and fossil organic deposits.

Trask and Wu (1930) reported chlorophyll-like and carotenoid pigments in marine sediments, but were unable to identify them. Wells and Erickson (1937) identified what they called chlorophyll in marine sediments off the coast of Virginia. Prior to the early 1940's, most analysis was done without the use of spectrophotometry or chromatography. Fox and Anderson (1941) found chlorophyll-like pigments in marine sediments which they subdivided into three fractions by using different solvents.

The oldest reported material containing chlorophyll-like pigments and carotenoids is interglacial gyttja from Denmark

described by Andersen and Gundersen (1955) which is estimated to be 100,000 years old. By paper chromatography they found five fractions of chlorophyll derivatives all of which had similar absorption maxima characteristic of plant chlorophylls.

Vallentyne (1955) found three green fractions in a 90 per cent acetone extract of lake sediment by paper chromatography (developer: petroleum ether). Each fraction had absorption maxima at 412 and 667 mμ. S. R. Brown (unpublished reference mentioned by Vallentyne, 1957a) showed these three fractions to be mixtures, and isolated four different pigments with maxima at 411 and 670 mμ, 425 and 660 mμ, 440 and 662 mμ, and 464 and 656 mμ (solvent: acetone). Gorham (1959) reports chlorophyll derivatives to be present in surface woodland soils. Hodgson et al. (1960) found chlorophyll derivatives in sediments from a shallow, fresh-water lake in Alberta, Canada. They found a gradual decrease in quantity with depth. Gorham (1960) studied sediments from five lakes in England, and found chlorophyll derivatives in them.

While the chlorophyll derivatives in sediments are apparently different in structure from plant chlorophylls, the carotenoids appear to be the unchanged products of the organisms which produced them. Vallentyne (1956) states there is no difference between marine and fresh water sedimentary carotenoids other than that expected from the different types of plant and animal remains that become incorporated in the sediments.

Fox (1937) found carotenoids including α- and β- carotene in a core of marine sediment from a water depth of 6000 feet. Fox and

Anderson (1941) again identified these two carotenes in marine sediments plus six others similar to known carotenoids. Fox, et al. (1944) identified various carotenoids in marine sediments and fresh water sediments from a California lake. They also gave estimations on the amount of pigments contained in sea water.

Andersen and Gundersen (1955) separated four yellow carotenoid zones by paper chromatography in interglacial gyttja from Denmark, and two red zones one of which had a carotenoid-like spectrum. Carotenoids were reported by Vallentyne (1956) in New England and Canadian lake sediments. The oldest samples were dated at 11,000 years. Vallentyne (1956b) found several carotenoids in a 20,000 year old sediment sample from Searles Lake, California. According to Vallentyne (1960, p. 98) a total of 20 carotenoids have been reported from fresh water and marine sediments, but only six of these have been rigorously identified. These are α- carotene, β - carotene, echinenone, rhodoviolascin, lutein and myxoxanthophyll.

Fox (1937) suggested that carotenoids might be synthesized within sediments by microorganisms. This would imply that the carotenoids in sediments are not necessarily as old as the deposit which contains them.

Vallentyne and Craston (1957) state that phytoplankton are the main contributors of pigments to fresh water sediments while rooted aquatic plants contribute only a small amount. An exception to this may occur in shallow lakes clogged with rooted vegetation. They consider the amount contributed by terrestrial plants to be neglible. In support of this Manning and Juday (1941) found that photosynthesis

carried on by higher plants probably represents only a small part of the total photosynthetic activity of a lake.

It is generally assumed that the pigments preserved in sediments represent only a fraction of that originally present in the plants which once inhabited the lake. Orr and Grady (1957) estimate that more than 99 per cent of the chlorophylls synthesized by marine plankton are decomposed in the water column and before burial.

ACKNOWLEDGMENTS

The collection and analysis of sediments was begun at the University of Minnesota, Lake Itasca Biological Station in the summer of 1960. The writer wishes to acknowledge a stipend from the National Science Foundation in support of preliminary work on the problem.

The writer is especially indebted to Dr. Frederick M. Swain, Department of Geology, University of Minnesota, for suggesting the problem, and for his continual aid and advice. Thanks are also extended to Dr. James C. Underhill, Department of Zoology, University of Minnesota, for his assistance at the Lake Itasca Biological Station; Mr. John G. Dobie, Minnesota Conservation Department, for maps and information from the files of the Game and Fish Division; Miss Walentina Sokolsky for the drafting of illustrations; and Miss Theodora Melone, Winchell Library of Geology, University of Minnesota, for aid in the research of the literature.

CHEMISTRY OF THE PIGMENTS

CHLOROPHYLLS: The chlorophylls are magnesium-porphyrin compounds, the most common of which are chlorophyll a ($C_{55}H_{72}O_5N_4Mg$) and chlorophyll b ($C_{55}H_{70}O_6N_4Mg$). The basic unit of both chlorophylls is the porphyrin ring system, a structure made up of four simple pyrrole nuclei joined by carbon linkages. The center of the structure is occupied by a single atom of magnesium. The pyrrole nucleus possesses characteristic side chains, or appendages, to one of which is bound the long chain alcohol phytol. The two chlorophylls differ in only one of the appendages, as shown in Fig. 1. Chlorophyll b possesses an aldehyde group (CHO) in the position chlorophyll a has a methyl group (CH_3).

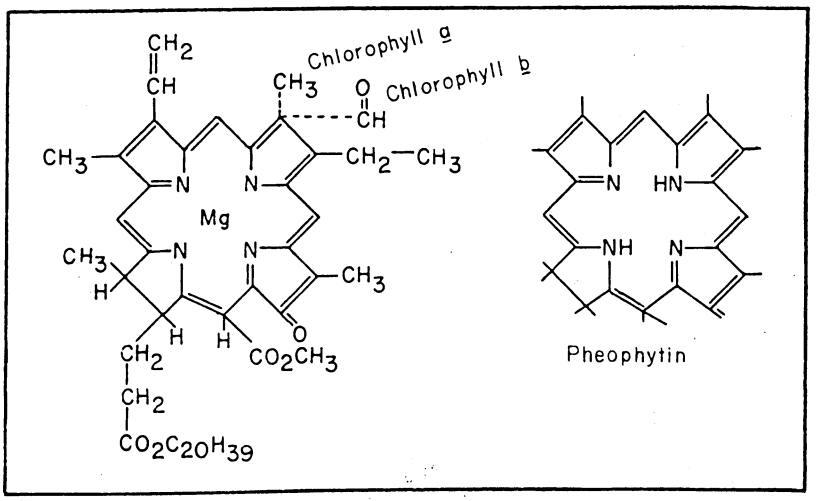


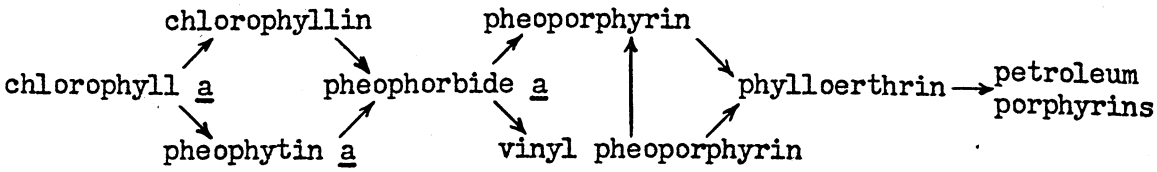
Figure 1. Structure of chlorophylls a and b (differing only as shown in upper right corner of the left figure), and pheophytin (differing from chlorophylls in replacement of Mg by two H).

In the green leaves of higher land plants the ratio of chlorophyll a to b is usually about 2.5. Chlorophyll b is rare in marine plants with the exception of green algae in which there appears to be considerable variation in the total chlorophyll content as well as the ratio of chlorophyll a to b.

In addition to chlorophylls a and b several closely related compounds have been found in nature. Among these are chlorophylls c and d. Their chemical structure has not been established, and the identification is based largely on differences in their absorption spectra.

Chlorophylls a and c are found in brown algae, diatoms and flagellates. Chlorophylls a and d occur in red algae. Bacteriochlorophyll is found in photosynthetic bacteria.

Chlorophyll decomposes into a series of porphyrin compounds. The following sequence shows the probable steps in the decomposition of chlorophyll (Orr et al., 1958, p. 953).



Pheophytin a (Fig. 1) differs from chlorophyll a in the replacement of the magnesium by two hydrogens. Orr et al. (1958) believe this change is caused by the acidic digestive fluids of herbivores feeding on the phytoplankton. Joslyn and MacKinney (1938) found that in 90 per cent acetone there is a 70 per cent conversion of chlorophyll to pheophytin in the first 24 hours.

The conversion of pheophytin to pheophorbide takes place by the hydrolysis of the phytol group, which may occur in the digestive tracts of animals and in the sediments. The change to phylloerythrin occurs in the digestive tracts of animals and by bacterial action under anerobic conditions.

CAROTENOIDS: The carotenoids comprise a group of about 80 different red, orange, yellow and purple pigments whose chemical structure is characterized by a long, straight, unsaturated carbon chain. They are insoluble in water, but are soluable in fats or fat solvents. Included in the group are what are commonly called carotenes and xanthophylls. Carotenoids are present in small amounts in nearly all higher plants and in many microorganisms (e.g., fungi, red and green algae and photosynthetic bacteria). They are also probably present in all animals, but it is not certain whether the pigments are synthesized by the animal or contained in the food eaten by the animal. The yellow pigment carotene was originally isolated from carrots. The structure of many of the carotenoids is still undetermined.

The carotenoids found in nature are considered to be derivatives of the red pigment lycopene, found in tomatoes and many other fruits and flowers as well as microorganisms. Lycopene has the empirical formula $C_{40}H_{56}$, and is a highly unsaturated straight chain hydrocarbon (Fig. 2). Three other important naturally occurring carotenoid hydrocarbons having the same composition are α-, β-,

and α- carotene. One or more of these carotenes is commonly found in all higher plants and in many unicellular organisms.

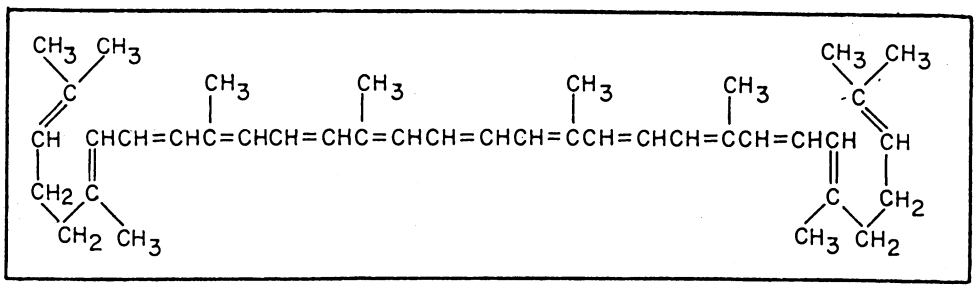


Figure 2. Structure of the carotenoid, lycopene, $C_{40}H_{50}$.

An important derivative of β- carotene is vitamin A ($C_{20}H_{29}OH$) which is essentially an oxidation product of one-half of the β- carotene molecule.

Other groups of pigments which occur in plant life, but which have not been found preserved in sediments, include the water soluble anthoxanthins and anthocyanins.

SPECTROPHOTOMETRY

The chlorophylls and chlorophyll derivatives show strong absorption in the red region of the spectrum (647-700 mμ). Carotenoids and other organic materials which are also extracted with acetone absorb light strongly at wavelengths below 500 mμ, and cause only

low background absorbance above 600 mμ. The absorption maxima of chlorophylls a and b and some of their derivatives in various solvents are listed in the upper half of Table 1, and others are shown in absorption spectra in this section.

Kundt's rule states that the location of maxima depends upon the refractive index of the solvent. As solvents of higher index of refraction are used the location of a maximum should shift towards a longer wavelength.

However, Harris and Zscheile (1943) found that for chlorophyll solutions there were exceptions to this rule. For some solvents with the same index of refraction there was a significant difference in location of maxima. They determined the absorption spectra of chlorophyll a in 13 different solvents and of chlorophyll b in 5 different solvents. The absorption spectra of chlorophylls a and b in acetone are shown in Fig. 3. All absorbance values were multiplied by a factor so that the curves would pass through the same point at 505 mμ, and therefore the curves represent relative absorbance values. For chlorophyll a the greatest variation in wavelength at any maximum for different solvents was 8 mμ, and for chlorophyll b, 26 mμ.

Rabinowitch (1951, pp. 638, 639) has compiled a list of absorption maxima for chlorophylls a and b in different solvents. The location of any particular peak, as determined by different individuals, may vary several millimicrons.

The absorption spectra of chlorophylls a and b, and pheophytins a and b in 80 per cent acetone are shown in Fig. 4 and 5. When the curves of the chlorophylls in 100 per cent acetone (Fig. 3) are

Table 1. Absorption maxima in mμ of cholorphylls and derivatives of chlorophyll and carotenoids (identified from sediments) in various solvents (with refractive index, 20° C.). Values in parenthesis are approximate location of maxima. Source: a) Rabinowitch (1951); b) Lemberg and Falk (1951); c) Smakula (1934); d) Karrer and Jucker (1950); e) Goodwin and Taha (1950); f) Gillam (1935); g) Bonner, et al. (1946); h) Strain (1939); i) Morton and Rosen (1949); j) Orr and Grady (1957).

	petroleum ether	dioxane 1.42	choloroform 1.45	ethanol 1.36	carbon disulfide 1.63
Chlorophyll a $C_{55}H_{72}O_5N_4Mg$					a)674
Chlorophyll b $C_{55}H_{70}O_6N_4Mg$					a)650
Pheophytin a		a)(500-05)(530-35) (605-10)(665-70)	j)(415-20)(505-10) (535-40)(610-15) 668		
Methyl chlorophylline a		a)(585)(620-25) (660-65)			
Methyl pheophorbide a		a)(505)(535)(610) (665-70)			
Phylloerthyrin		b)519 559 584 638			
α-carotene $C_{40}H_{56}$	h)(420)(445)(480) slightly soluble		d)454 485	almost insoluble	c)450 475 506
β-carotene $C_{40}H_{56}$	h)(427)(452)(475)		d)466 497	h)(430)(450-55)(480) almost insoluble	c)455 482 511 d)450 485 520
Echinenone $C_{40}H_{56}O(\pm H_2)$			e)474-76	e)458-60	d)450 488 520 e)494
Lutein $C_{40}H_{56}O_2$			f)275 340 428 453 485	i)(420-25)(447) (475-80)	c)445 476 507
Myxoxanthophyll $C_{40}H_{56}O_7$	insoluble		d)454 484 518	d)445 471 503	insoluble
Rhodoviolascin $C_{42}H_{60}O_2$	almost insoluble		d)476 507 544	d)491 526	d)496 534 574

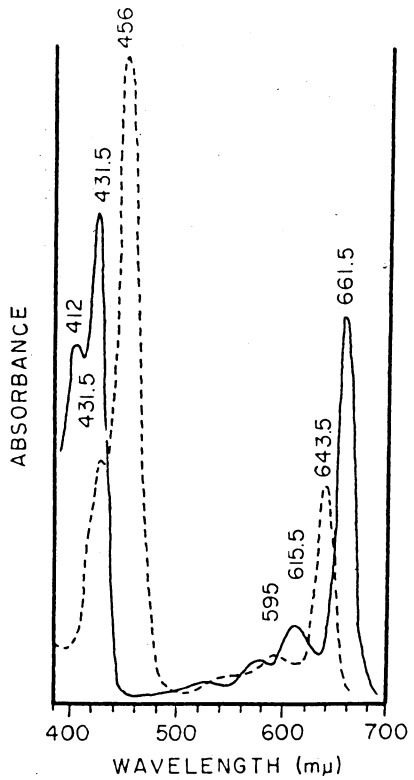


Figure 3. Absorption spectra of chlorophyll a, ——; and b, - - - - -. Solvent: acetone. (Harris and Zscheile, 1943, pp. 519, 520).

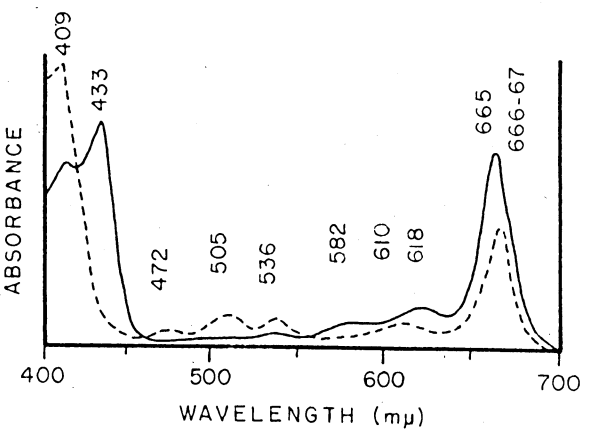


Figure 4. Absorption spectra of chlorophyll a, ——; and pheophytin a, - - - - -. Solvent: 80 per cent acetone. Pheophytin a spectrum determined in presence of oxalic acid. (Vernon, 1960, p. 1146).

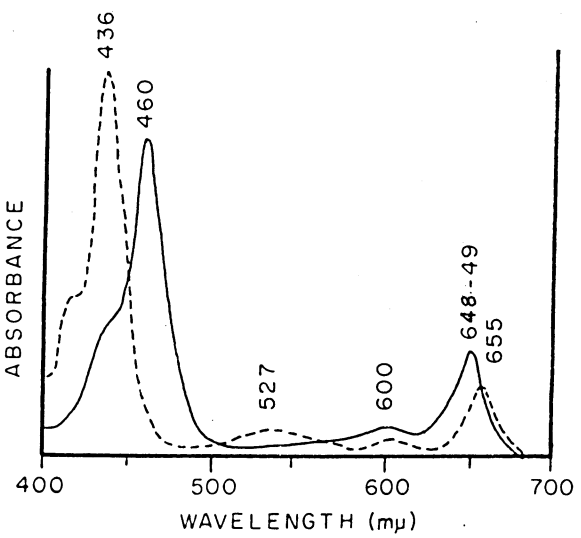


Figure 5 Absorption spectra of chorophyll b; ——, and pheophytin b, - - - - -. Solvent: 80 per cent acetone. (Vernon, 1960, p. 1146).

compared with those in 80 per cent acetone it can be seen, as Vernon (1960) points out, that for the latter the maxima are shifted toward longer wavelengths and are flattened. The spectra of pheophytins a and b are not as sensitive to the water content of the acetone. The spectra of chlorophylls c and d, and pheophytins c and d are shown in Fig. 6 and 7; that of bacteriochlorophyll in Fig. 8.

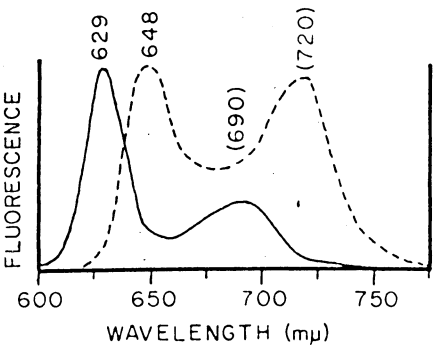
As shown in Fig. 4 and 5 the pheophytins also have rather strong absorption in the red portion of the spectrum. This is also true of the pheophorbides, an example of which is shown in Fig. 9. The presence of magnesium has the effect of increasing the height of the main red absorption band and of weakening the bands in the green. The result is the pure green color of chlorophyll as compared with the dull olive-green of pheophytin. The absorption spectrum of phylloerthrin is shown in Fig. 10.

Six carotenoids have been definitely identified in sediments according to Vallentyne (1960, p. 98). They are listed in the lower half of Table 1 along with their chemical formulas and maxima in various solvents. The absorption spectra of α- and β- carotene are shown in Fig. 11; of echininone in Fig. 12; of lutein in Fig. 13; and of rhodoviolascin in Fig. 14.

COLLECTION AND STORAGE OF SAMPLES

All the cores of lake sediments were obtained with a free fall, Phleger type sampler weighing about 35 pounds. The cores were collected in plastic tubes four feet long and one and three quarters inches in diameter. Cores which could not be worked on within 24 hours were

Figure 6. Fluorescence spectra of chlorophyll c, ——; and pheophytin c, - - - - -. Solvent: ether. Location of peaks in parenthesis are estimated from the curves. (French et al., 1957, pp. 17, 18).



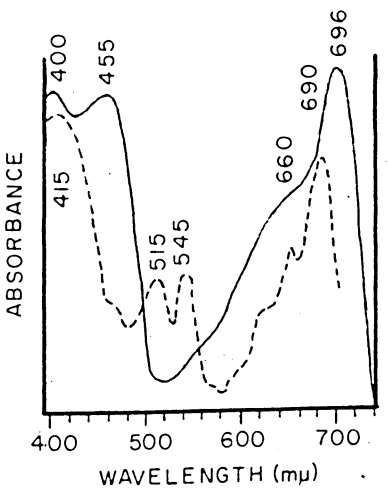
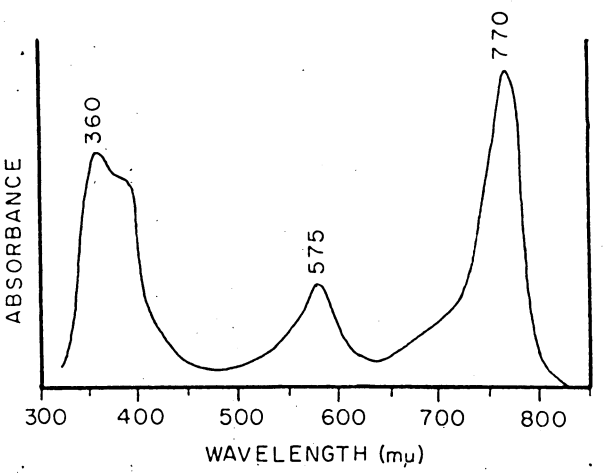


Figure 7. Absorption spectra of chlorophyll d, ——; and pheophytin d, - - - - -. Solvent: methanol. Location of peaks estimated with the exception of the 696 mμ peak of chlorophyll d. (Manning and Strain, 1943, pp. 7, 14).

Figure 8. Absorption spectrum of bacteriochlorophyll. Location of peaks estimated. Solvent: acetone. (Goedheer, 1958, p. 483).



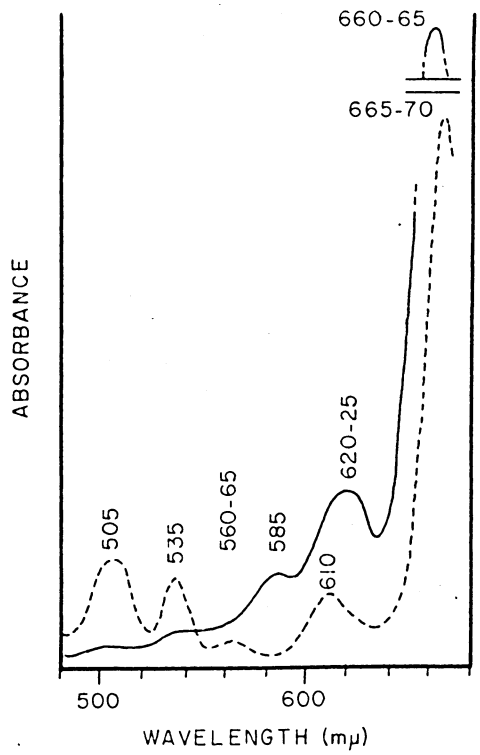
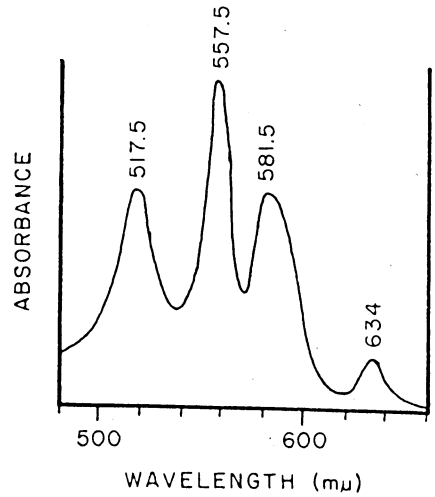


Figure 9. Absorption spectra of methyl chlorophylline a, ——; and methyl pheophorbide a, - - - - -, showing effect of the presence of magnesium (in the chlorophyllin) on the spectrum. Locations of peaks are estimated from the curves. Solvent: dioxane. (Rabinowitch, 1951, p. 628).

Figure 10. Absorption spectrum of phylloerthrin. Solvent: dioxane. (Stern and Wenderlein, 1935, p. 91).



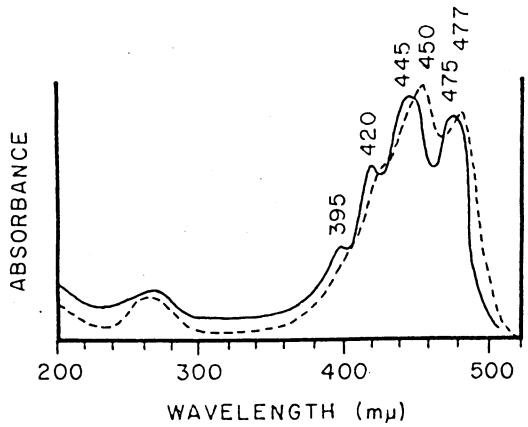


Figure 11. Absorption spectra of α-carotene, ——; and β-carotene, - - - - -. Solvent: hexane. Locations of peaks estimated from the curves. (Karrer and Jucker, 1950, p. 350).

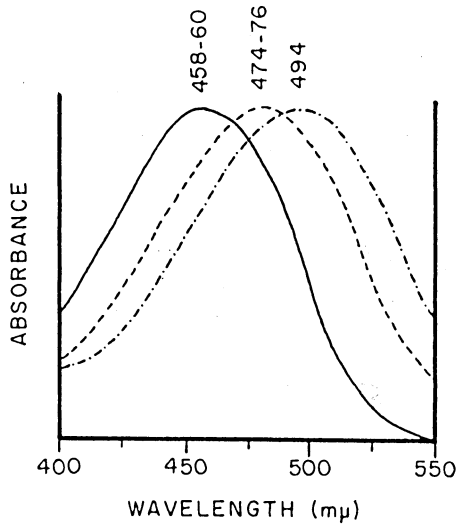


Figure 12. Absorption spectra of echinenone. Solvents: light petroleum (B.P. 40-60°), ———; chloroform, - - - - -; and carbon disulfide, -·-·-·-. (Goodwin and Taha, 1950, p. 245).

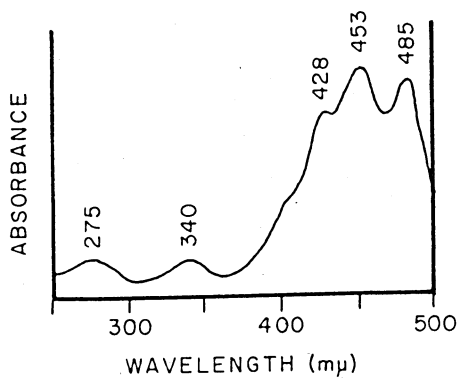


Figure 13. Absorption spectrum of lutein. Solvent: chloroform. (Gillam, 1935, p. 1833).

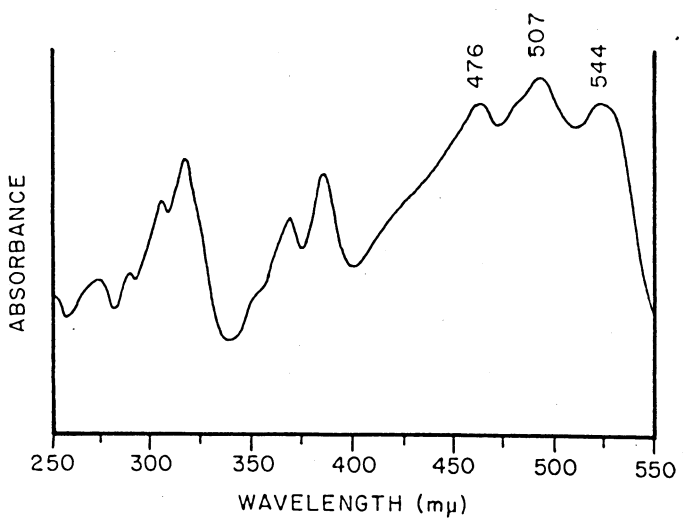


Figure 14. Absorption spectrum of rhodoviolascin. Solvent: hexane. (Karrer and Jucker, 1950, p. 358).

extruded onto aluminum foil and frozen until ready for study. The frozen cores were studied within 11 months after collection.

Vallentyne (1955, p. 307) tested the possibility of post collection change on the quantitative determination of sedimentary chlorophyll. One of the ways in which change might be expected to occur is by oxidation. A sample which was initially reduced was thoroughly oxidized for two days by continuously bubbling air through a mechanically stirred suspension. The quantitative determination of sedimentary chlorophyll was not changed. Vallentyne also noted that sediments stored for periods of up to one year at room temperature showed no change in sedimentary chlorophyll content. Orr and Grady (1957, p. 267) found that storage for a year at 5° C. did not give a detectable change in pigment content.

LABORATORY PROCEDURE

The laboratory procedure of extracting pigments is similar to that used by Vallentyne (1955). Two sets of beakers (30 and 100 ml.) are used. Each beaker is weighed to the nearest milligram, and all subsequent weighing is done to the nearest milligram. The core is sampled at a given interval; generally a three inch interval is used. A portion of the sediment (8-10 gms.) from one interval is placed on a cover glass and homogenized with a spatula. About half is placed in a 100 ml. beaker, and the other half is placed in a 30 ml. beaker. The same procedure is followed the length of the core. Each of the beakers with the sample is weighed to determine the weight of the sample.

The set of 30 ml. beakers is placed in a drying oven at 105° C. for 24 hours. Each beaker with its dried sample is weighed, and the water content determined. By this procedure the dry weight of the sample from which the pigments are to be extracted (second set of beakers) can be determined without actually drying the sample.

Rabinowitch (1945, p. 383) states that the only efficient way to extract pigments from cells is with aqueous organic solvents. The water disintegrates the proteinaceous fraction of the chloroplast structure. Once separated from the cell structure the pigments become easily soluble in an organic solvent.

The solvent used for extracting the pigments is 90 parts acetone to 10 parts distilled water. About 60 ml. of the solvent is poured on each of the samples, and stirred with a glass rod. The beakers are covered with aluminum foil to prevent evaporation. The beakers are placed in a refrigerator, and allowed to stand 10-12 hours with occasional stirring.

At the end of this time the extract is pipetted off, and collected in a test tube. Another quantity of 90 per cent acetone is added to the sample, it is stirred, and placed in the refrigerator again. The same procedure is followed with each beaker using a separate test tube to collect the extract from each sample. After letting the sample stand another 10-12 hours with occasional stirring the extract is pipetted off and collected in the corresponding test tube. This procedure is continued until the addition of solvent to the sample produces no coloration. The volume of extract collected from each sample is recorded.

The absorption spectrum of each sample is determined in the range of 350-800 mμ with a Beckman Model DU quartz spectrophotometer, using 90 per cent acetone in the blank cell. The absorbance values are recorded at intervals of 10 mμ or closer to determine the maxima.

The absorbance at any particular wave length of the absorption spectrum usually represents a mixture of pigments. Vallentyne (1960) states that since we do not know the exact identities or the relative amount and extinction coefficients of certain compounds it is impossible to obtain an accurate weight estimate from spectrophotometric data. If more were known about the identity of the pigments present, quantitative formulas could be derived such as those determined by Richards and Thompson (1952) and Vernon (1960).

At least two alternative methods can be used. The first, and the one on which the data in this report are based, is similar to that suggested by Vallentyne (1955) who bases the quantitative measurement of pigments on absorbance values. He defines one sedimentary chlorophyll unit as giving a density reading of 0.100 in 10 ml. of solvent (90 per cent acetone with 0.5 per cent dimethylaniline). The density reading is taken at the maximum in the red portion of the spectrum. The measurement of sedimentary chlorophyll is given by the formula:

$$\frac{\text{Density reading} \times \text{Volumne of extract (ml.)}}{\text{Dry weight of sample (gms.)}}$$

This formula can be applied to other maxima in the same way to determine relative concentration of pigments.

The second alternative, used by Orr and Grady (1957) converts absorbance values to pigment weight (ppm) by arbitrarily assuming

an absorptivity constant. They use a different procedure for extracting pigments than Vallentyne (1955). The main difference is the length of time involved. Vallentyne allowed the sample to stand overnight with occasional stirring and then placed a fresh quantity of solvent on it. Orr and Grady used a more rapid procedure in which the solvent is placed on the sample, stirred continuously for 5-8 minutes, then replaced with fresh solvent.

One sample was tested to see if the length of extraction time has any effect on the quantitative results. A longer extraction time was used on one part of a homogeneous sample letting the 90 per cent acetone stand on the sediment (with occasional stirring) for 7-10 hours before replacing it with fresh solvent. A more rapid extraction was used on the second part of the sample. In this case 90 per cent acetone was poured on the sediment, and it was continuously stirred for two minutes. It was then allowed to settle several minutes before pipetting off the solvent and replacing it with fresh solvent.

For each sample the pigment content was determined by using the 670 mμ and 413 mμ maxima as shown in Table 2. The faster extraction gave slightly higher results (about 4 per cent greater for the 670 mμ maximum and 1 per cent greater for the 413 mμ maximum). The absorption spectra of both samples had identical maxima. The faster extraction required a smaller volume of solvent.

TABLE 2

	Absorbance at 670 mμ	X	Volumne of extract (ml.)	÷	Dry wt. of sample (gms.)	=	Sedimentary chlorophyll units/gm. of dry wt.
(1)	0.114		395		1.938		23.2
(2)	0.176		293		2.144		24.1
	Absorbance at 413 mμ	**X**	**Volumne of extract (ml.)**	**÷**	**Dry wt. of sample (gms.)**	**=**	**Carotenoid units units/gm. of dry wt.**
(1)	0.469		395		1.938		95.6
(2)	0.749		293		2.144		102.4

Table 2. Data showing difference in results of quantitative determination of pigments by (1) a slow extraction procedure, and (2) a more rapid extraction procedure. Sample from Lake Itasca.

Vallentyne (1955, p. 307) states that the drying of sediments at room temperature does not seriously affect the sedimentary chlorophyll content provided the dried sample is moistened several hours before extraction. If the sample was not moistened prior to extraction he found the values to be lower. Vallentyne (1956, p. 257) found that 20 per cent of the carotenoids were destroyed if a dried sample was used.

Three different samples were tested to determine if drying would affect the quantitative determination of pigments. In each case a homogenized sample was divided into three parts; (1) the original moist sample, (2) an air dried portion which was premoistened before extraction, and (3) an air dried portion which was not moistened prior to extraction.

Pigments in the original moist sample were extracted in the manner previously described. The air dried portion was divided into three parts. A small amount of water was added to the first part

and allowed to stand 3-8 hours. Acetone was added to produce an equivalent of 90 per cent acetone, and thereafter 90 per cent acetone was used to replace that pipetted off. Ninety per cent acetone was added directly to the second part of the sample. The third part was oven dried to determine the moisture content. The results of these tests are shown in Table 3.

TABLE 3

	Sedimentary chlorophyll units (665-70 mμ peak)	Change from original sample (per cent)	Carotenoid units (414-17 mμ peak)	Change from original sample (per cent)	Ratio
A (1)	8.0		59.7		7.5
(2)	10.9	+ 36.	63.0	+ 6.	5.8
(3)	11.9	+ 49.	63.2	+ 6.	5.3
B (1)	7.8		61.9		7.9
(2)	9.2	+ 18.	59.3	- 4.	6.4
(3)	10.0	+ 28.	55.8	- 10.	6.2
C (1)	23.2		95.6		4.1
(2)	23.9	+ 3.	93.2	- 3.	3.9
(3)	20.2	- 13.	77.8	- 19.	3.9

Table 3. Results of experiment to determine the difference in the quantitative determination of pigments in a homogeneous sample: (1) for an original moist sample; (2) for an air dried sample, premoistened prior to extraction; and (3) for an air dried sample, unmoistened prior to extraction. Sample A: Long Lake, station 1, 0-4 inches; B: Long Lake, station 2, 1-2 inches; C: Lake Itasca, station 2, 2-6 inches. Ratio in the column at the right refers to the ratio between the absorbance at the 414-17 mμ peak to that at the 665-70 mμ peak.

The dried sample pigment values (Table 3) showed a significant difference from the original samples, but the values did not follow a definite trend as can be seen by the percentage change figures (both negative and positive). Comparing the values of the original samples with those of the air dried samples it was found there was always a

greater difference between the original sample (1) and the dried, unmoistened sample (3) then there was between the original sample (1) and the dried, premoistened sample (2).

The ratio of absorbance at the 414-17 mμ peak to that at the 665-70 mμ peak was greater for the original samples (1) than for the air dried samples (2) and (3). This would seem to indicate the carotenoids (represented by the lower wave length peaks) undergo more of a change as the result of drying than does the sedimentary chlorophyll (represented by the higher wave length peaks).

The absorption spectra for the air dried samples (premoistened and unmoistened prior to extraction) were almost identical, but they differed from that of the original sample (Fig. 15). There was a slight shift of 1-3 mμ towards a shorter wave length at the 417 mμ peak for the air dried samples. The peak at 447-49 mμ in the original sample showed only as a shoulder in the air dried samples. The peak at 665-70 mμ could not be determined closely enough to decide whether any difference existed.

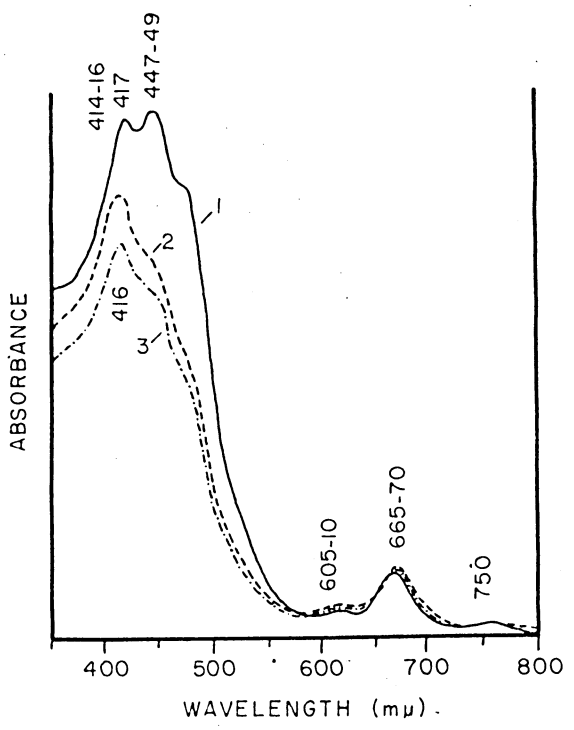


Figure 15. Absorption spectra of a homogeneous sample showing a comparison between: (1) an original moist sample, ———; (2) an air dried sample, premoistened prior to extraction, - - - - -; and (3) an air dried sample, unmoistened prior to extraction, ·-·-·-·-·. Solvent: 90 per cent acetone. Curves adjusted to cross at 575 mμ. Sample from Long Lake, station 1.

It might be pointed out that a moist sample to which solvent is added contains water which in effect dilutes the 90 per cent acetone. For instance, a sample weighing six gm. with a water content of 80 per cent contains five gms. of water. If the total volume of 90 per cent acetone used is 150 ml. the additional water in the sample results in 87 per cent acetone. The change in location of maxima with the addition of water to acetone is discussed in the section on spectrophotometry. The small percentage change of the water content of the acetone is probably responsible for the difference in the location of the 414-17 mμ peak in the dried and the moist samples (Fig. 15).

DESCRIPTION OF LAKES

LAKE ITASCA, CLEARWATER COUNTY: Lake Itasca (Fig. 16) is located in the southeastern corner of Clearwater County within the boundaries of Itasca State Park. It is considered to form the headwaters of the Mississippi River which drains the north end at an elevation of about 1450 feet. The lake is in a recessional moraine formed by the Mankato Wadena lobe of the Wisconsin glacial age. Zumberge (1952) classifies it as having an ice block origin.

It is a hard-water, eutrophic lake with a surface area of 1060 acres. Approximately 55 per cent of this area is less than 15 feet deep. The maximum depth is 43 feet with a median depth of 14 feet. The lake level is relatively constant, and there is poor thermal stratification. The watershed consists of about 80 per cent forest (spruce, balsam fir and aspen) with the remainder being municipal. Temperature, light intensity and other data are shown in Table 4.

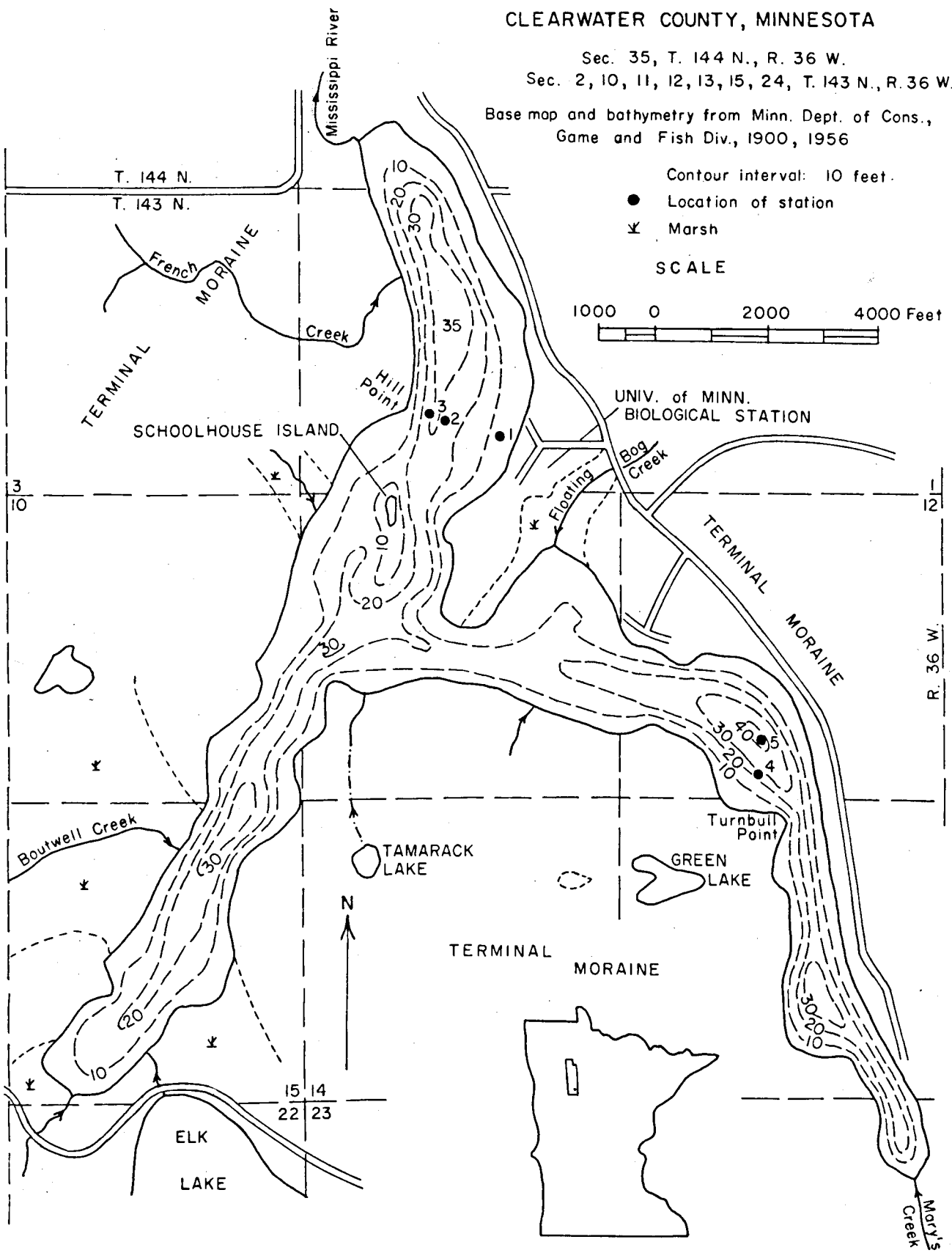


Figure 16. Lake Itasca, Clearwater County, Minnesota.

Table 5 lists the plankton as reported by the limnology class at the Biological Station, and Table 6 lists the aquatic plants as reported by a Minnesota Fisheries Lake Survey.

TABLE 4

Water depth (feet)	Temperature (degrees C.)	Light intensity (per cent of surface light)	pH	Alkalinity (ppm)	Dissolved oxygen (ppm)
surface	20.4	100	8.0-8.7	125	8.0
2	20.4	86			
4	20.4	69			
6	20.4	52			
10	19.1	27			
20	16.9	6		135	
25	14.3	3			4.6
30	10.7	2		132	
38	7.8	0	7.8		0.0

Table 4. Limnological data, Lake Itasca, station 4. All data except dissolved oxygen determined by the limnology class at Lake Itasca Biological Station, 2 p.m., June 16, 1960. Oxygen determinations by Minnesota Fisheries Lake Survey, July 10, 1960.

TABLE 5

Phytoplankton;	
Dinobryon spp.	grass-green algae
Microcystis spp.	blue-green algae
Peridinium spp.	dinoflagellate
Tabellaria spp.	diatom
Zooplankton;	
Asplancha spp.	rotifer
Bosmina longirostris	cladoceran
Ceratium hirundinella	protozoan
Coleps spp.	ciliophoran
Cyclops vernalis	copepod
Daphnia spp.	cladoceran
Diaptomus spp.	copepod
Eucyclops spp.	"
Kellicottia longispina	rotifer
Keratella cochearis	"
Lecane spp.	"
Polyarthra spp.	"
Volvox spp.	protozoan
also: ostracods	

Table 5. Plankton survey of Lake Itasca by the limnology class, Lake Itasca Biological Station, July 1960.

TABLE 6

Plant	Common name	Abundance
Ceratophyllum demersum	Coontail	very common
Chara vulgaris	Muskgrass	" "
Heteranthera spp.	Mud plantain	common
Lemna trisulca	Duckweed	very common
Myriophyllum exalbescens	Water milfoil	common
Najas spp.	Bushy pondweed	"
Nuphar spp.	Yellow water lily	"
Polygonum natans	Smartweed	uncommon
Potamogeton spp.	Pondweed	very common
Sagittaria latifolia	Arrowhead, Duck potato	common
Scirpus acutus	Bulrush	"
Typha latifolia	Cattail	"
Ziania spp.	Wild rice	very common

Table 6. Aquatic plant survey of Lake Itasca by Minnesota Fisheries Lake Survey, July 12, 1960.

LONG LAKE, CLEARWATER COUNTY: Long Lake (Fig. 17) is a small, oligotropic, spring-fed lake located in the southern part of Clearwater County about 10 miles northwest of Lake Itasca. It, too, is in the recessional moraine of the Mankato Wadena Lobe, and probably has an ice block origin. The lake is about one and one-half miles long and has a maximum width of about 1100 feet. The surface area is 164 acres, and the surface elevation is about 1525 feet. The water depth increases rapidly along the sides, but deepens more gradually at each end. The maximum depth of over 80 feet is less than 500 feet from shore. The watershed is relatively small because the sides of the valley rise rapidly above the lake. The vegetation is mainly a mixed hardwood-conifer forest.

A Minnesota Fisheries Lake Survey (September 1951) reported the thermocline limits as 30-40 feet. Temperature data collected by the

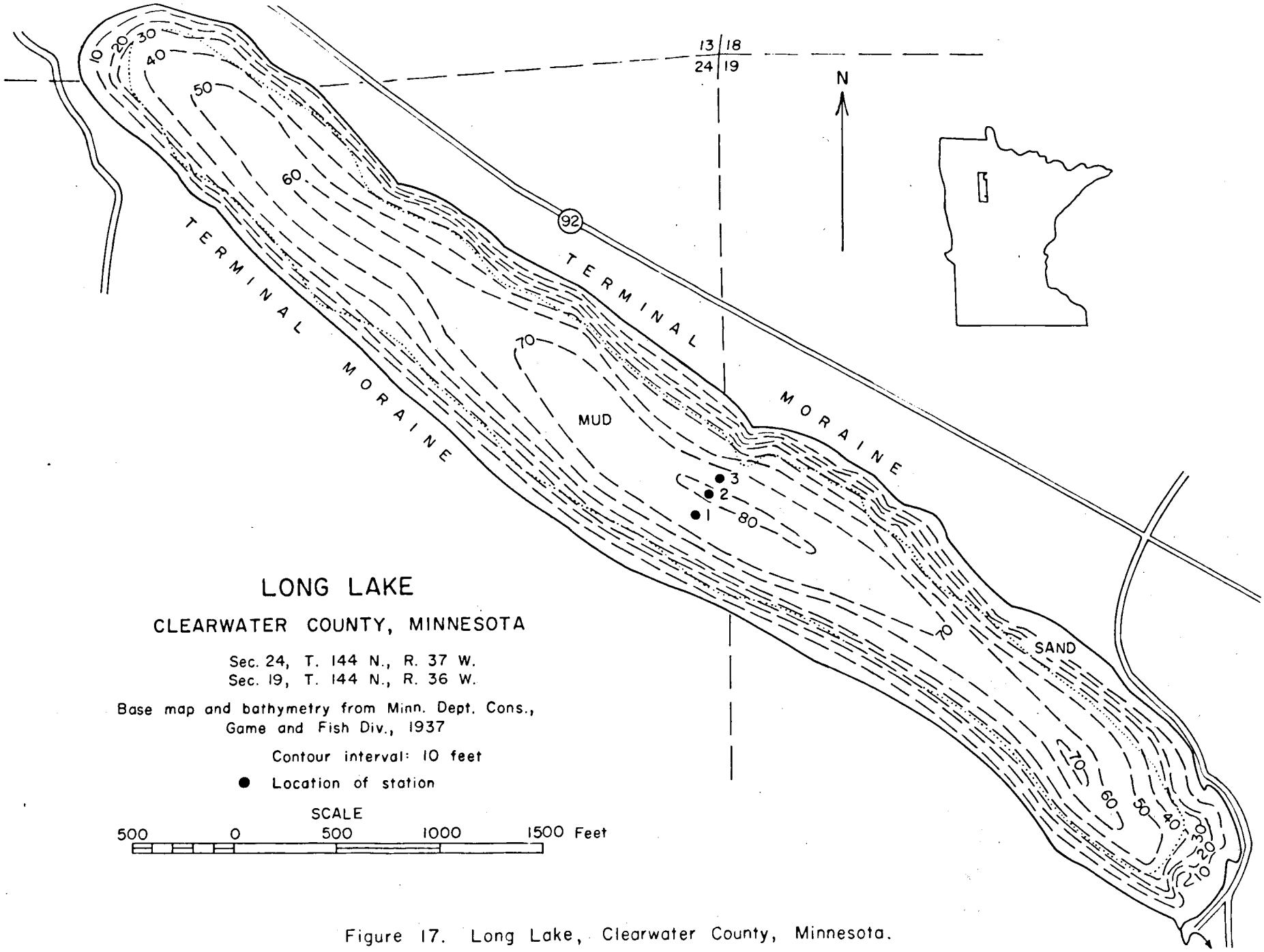


Figure 17. Long Lake, Clearwater County, Minnesota.

limnology class at the Lake Itasca Biological Station showed a sharp change in temperature at 26 feet. Table 7 lists limnological data for Long Lake.

TABLE 7

Water depth (feet)	Temperature (degrees C.)	Light intensity (per cent of surface light)	pH	Alkalinity (ppm)
0	18.2	100	8.5	171
5	18.3	65		
10	18.2	50		
15	17.8	41		
20	17.4	34		
30	10.4	22	8.3	170
40	6.6	15		
50	5.7	8	8.0	172
60	5.4			
80	5.2			

Table 7. Long Lake, station 1, all data collected by the limnology class at Lake Itasca Biological Station, 11 a.m., June 20, 1960.

Aquatic plant and plankton surveys were made by the same limnology class. Table 8 lists the plankton and Table 9 lists the aquatic plants. Because the lake basin deepens rapidly the amount of aquatic plants is relatively small. The deepest water in which plants were found was 25 feet.

TABLE 8

Phytoplankton:	
Asterionella spp.	diatom
Fragilaria spp.	"
Sphaerocystis spp.	grass-green algae
Zooplankton:	
Ceratium hirundinella	protozoan
Cyclops spp.	copepod
Daphnia spp.	cladoceran
Diaptomus spp.	copepod
Kellicottia longispina	rotifer
Polycystis spp.	flatworm
Bottom sample:	
Oligochaeta	worms
Tendipes spp.	midge larvae

Table 8. Plankton and bottom sample survey of Long Lake made by the limnology class at Lake Itasca Biological Station, July 1960.

TABLE 9

Plant	Common name	Abundance
Brasenia schreberi	Water shield	uncommon
Ceratophyllum demersum	Coontail	"
Chara vulgaris	Stonewort, Muskgrass	very common
Elodea canadensis	Waterweed	common
Myriophyllum spp.	Water milfoil	"
Nuphar variegatum	Yellow water lily	uncommon
Potamogeton spp.	Pondweed	common
Sagittaria graminea	Arrowhead, Duck potato	"
Scirpus acutus	Bulrush	uncommon
Typha latifolia	Cattail	"
Utricularia vulgaris	Bladderwort	"

Table 9. Aquatic plants of Long Lake. Survey made by the limnology class at Lake Itasca Biological Station, July 1960.

BLUE LAKE, ISANTI COUNTY: Blue Lake (Fig. 18) is a hard water lake located in the southwestern part of Isanti County. It has a surface area of 309 acres with an average depth of less than 20 feet, and a maximum depth of a little over 30 feet. The basin occupies an elongate ice-block depression in an old drainage course in red

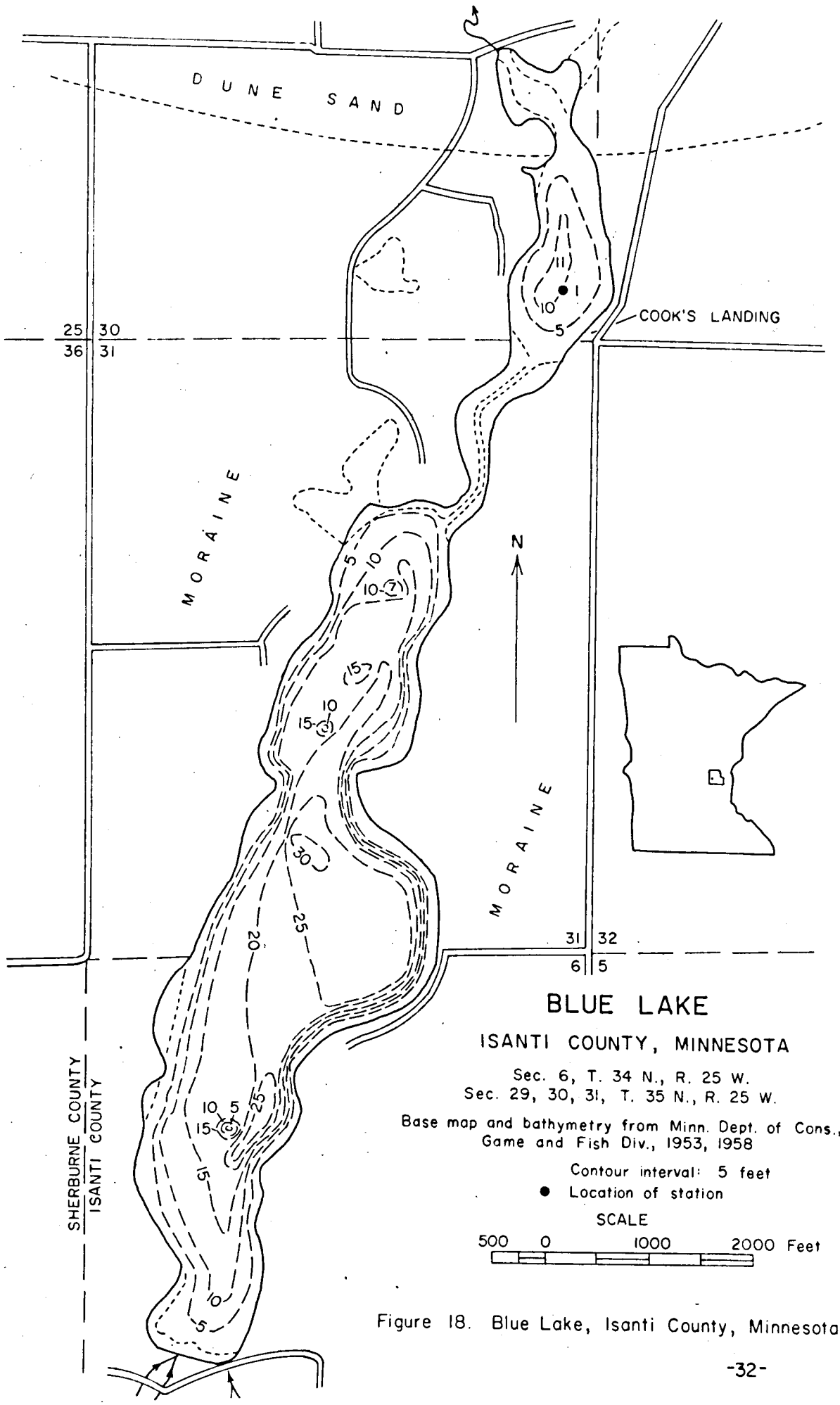


Figure 18. Blue Lake, Isanti County, Minnesota.

gravel and till of the Superior ice lobe. The lake is about two and one-half miles long and less than one-third of a mile wide. The surface elevation is about 975 feet. The watershed consists mainly of agricultural land and forested pasture, and is a gently undulating glacial moraine. Swain (1961, p. 522) classifies the lake as being in a eutrophic-alkalitrophic condition. He found the bottom samples to be in a reduced state (negative Eh values). Alkalinity was 113 ppm according to a Minnesota Lake Survey Report (Department of Conservation) in August 1949. The oxygen range was 0.0-7.9 ppm, and the pH of the surface water was 7.8-8.4.

Aquatic vegetation is fairly abundant, especially in the shallow north arm of the lake. The results of an aquatic plant survey are shown in Table 10. The shallowness of the lake precludes the presence of a good thermocline.

TABLE 10

Plant	Common name	Abundance
Ceratophyllum demursum	Coontail	very common
Elodea canadensis	Waterweed	locally common
Lemna trisulca	Duckweed	uncommon
Myriophyllum exalbescens	Water milfoil	very common
Nuphar spp.	Yellow water lily	locally common
Nymphaea odorata	White pond lily	" "
Polygonum natans	Smartweed	rare
Potamogeton spp.	Pondweed	common
Sagittaria latifolia	Arrowhead, Duck potato	rare
Scirpus validus	Bulrush	"
Typha latifolia	Cattail	common
Vallisneria americana	Wild celery, Tape grass	"
Also algae (Anabaena and Polycystis) and abundant diatoms and desmids.		

Table 10. Aquatic plant survey of Blue Lake, September 1960. (by R. B. Kaul, University of Minnesota, unpublished).

UPPER RED LAKE, BELTRAMI COUNTY: Upper Red Lake (Fig. 19) is one of the largest lakes lying entirely in Minnesota, and if adjoining Lower Red Lake is included it would be the largest with 275,000 acres. It is located 30 miles south of the Canadian border, and has a surface elevation of 1175 feet. The surface area is 118,870 acres. The bottom profile is nearly symmetrical with the greatest depth being a little more than 20 feet, and the mean depth about 8 feet. The basin is classified by Zumberge (1952) as being a remnant of Glacial Lake Agassiz. Surrounding soils are bog, sand and glacial till, but the shore line is composed mainly of sand. The lake is moderately alkaline with a total alkalinity of 120-135 ppm (Smith et al., 1961). Heavy winds which circulate the water cause the water temperature to follow the mean air temperature rather closely. The summer water temperature is about 62-72° F., and no thermocline is present. Table 11 lists zooplankton and insects found in the lake.

TABLE 11

Zooplankton:			
Alona	cladoceran	Epischura	copepod
Bosmina	"	Eurycercus	cladoceran
Chydorus	"	Holopedium	"
Cyclops	copepod	Hyalella	amphipod
Daphnia	caldoceran	Leptodora	cladoceran
Diaphanosoma	"	Sida	"
Diaptomus	copepod		

also: Chironomid, coleoptera, dragonfly and mayfly larvae; Corixidae; and Hydracarina.

Table 11. List of zooplankton and insects found in stomachs of yellow perch in Red Lakes (Pycha and Smith, 1954).

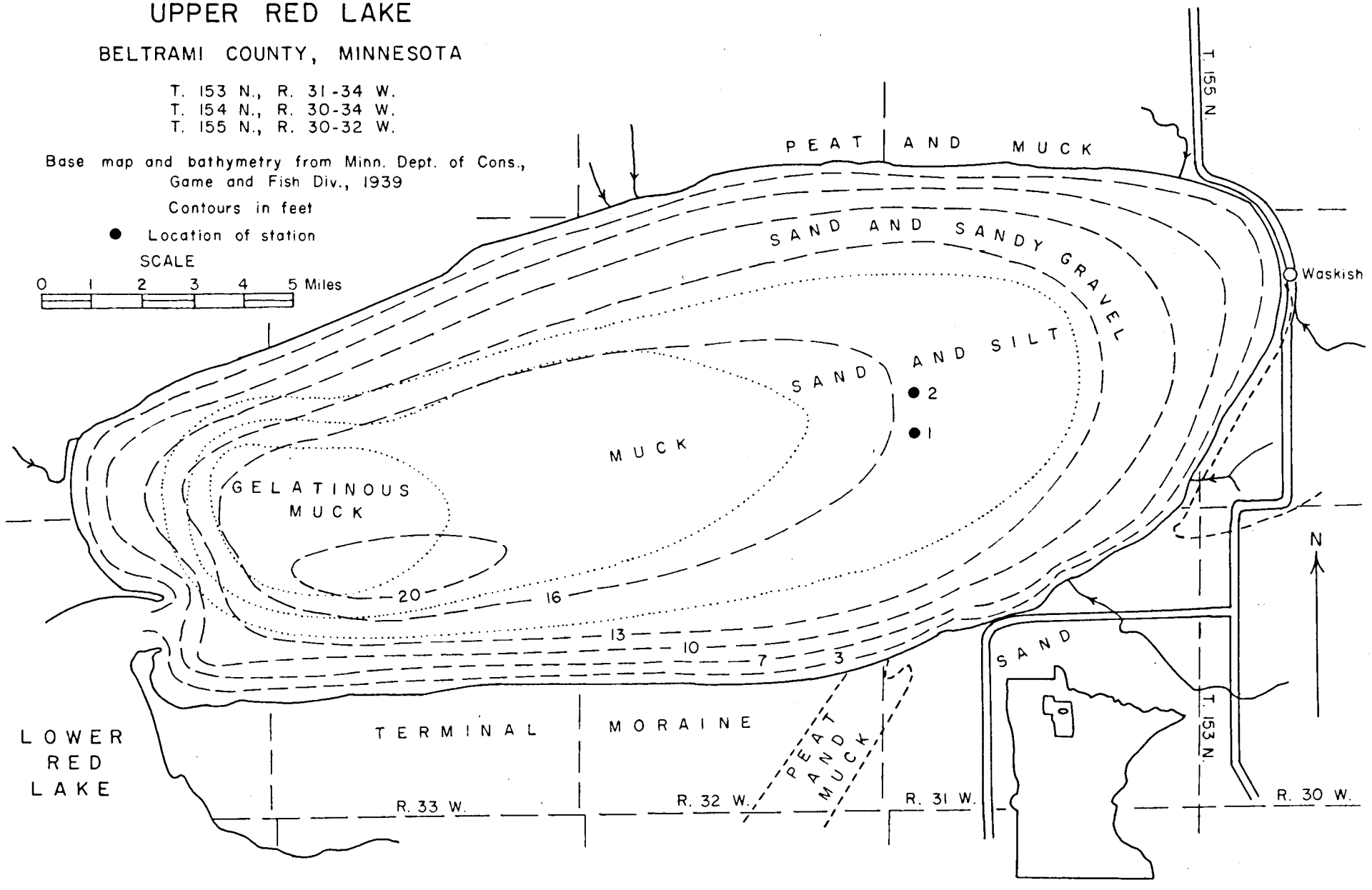


Figure 19. Upper Red Lake, Beltrami County, Minnesota.

RESULTS

Figure 20 shows a typical absorption spectrum of an acetone extract of sediment from Lake Itasca. The absorption spectra for all the other samples in the three cores from Lake Itasca have almost identical peaks as the one shown. The absorption spectra are characterized by: a large peak at 411-16 mμ; a small peak or shoulder at 445-49 mμ; a shoulder at 470-80 mμ; a small peak at 600-15 mμ; a large peak at 665-70 mμ; and a small peak at 745-55 mμ.

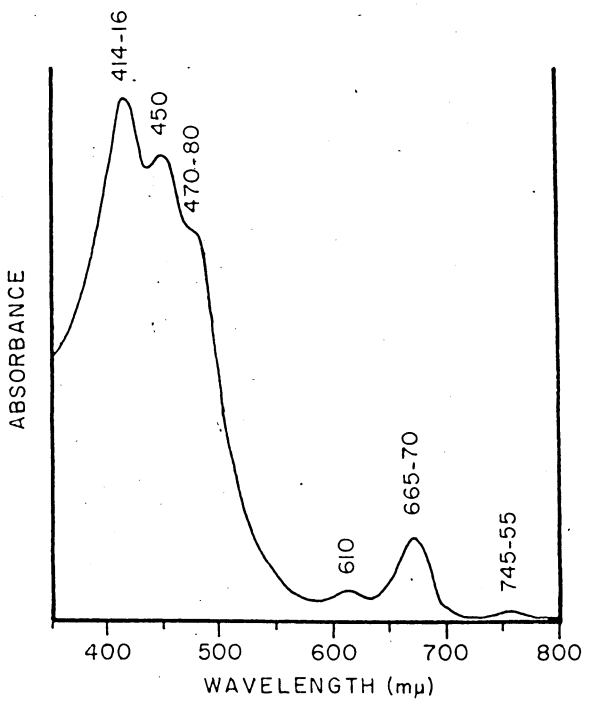


Figure 20. Typical absorption spectrum of 90 per cent acetone extract of sediment from Lake Itasca, station 4, 12 inches below top of core.

The absorption spectra for the Blue Lake samples (one core) are very similar to the one shown for Lake Itasca. They are characterized by: a large peak at 411-14 mμ; a slight shoulder at 445-50 mμ; a shoulder at 465-75 mμ; a small peak at 610-20 mμ; a large peak at 665-70 mμ; and a small peak at 745-55 mμ.

The absorption spectra for the Long Lake samples (one core) are also almost identical to that shown from Lake Itasca. They have the following characteristics: a large peak at 414-18 mμ; a small peak at 444-50 mμ; a shoulder at 470-80 mμ; a small peak at 600-15 mμ; a large peak at 665-70 mμ; and a small peak at 745-55 mμ.

The Upper Red Lake absorption spectra (one core) are also very similar to the one shown from Lake Itasca with the exception of the surface sample. The surface sediment differed in having a peak at 432 mμ instead of peaks in the range of 415-20 mμ and 445-50 mμ. This is the approximate wave length at which chlorophyll a shows maximal absorption. The peak is probably the result of living plant life on the sediment surface. Vallentyne and Craston (1957, p. 36) found a surface sediment sample from a Connecticut lake with this same peak.

The absorbance for the Upper Red Lake samples was determined up to 1050 mμ instead of 800 mμ as on the other samples. The characteristics of the absorption spectra are: a large peak at 410-16 mμ; shoulders at 440-50 mμ; 470-80 mμ; and 605-30 mμ; a large peak at 660-70 mμ; no peak near 750 mμ because the absorbance was too low; and a broad peak at 970-1000 mμ.

In all the samples except those from Upper Red Lake there was a small peak near 750 mμ. Vallentyne and Craston (1957, p. 41) believe this peak indicates the presence of bacteriochlorophyll-like matter.

The absorption spectra maxima determined in these cores as shown in Fig. 20 are very similar to those reported by other investigations (e.g., Vallentyne, 1955; Hodgson, et al., 1960; Gorham, 1961).

Figures 21 and 22 show the results of determining the amount of sedimentary chlorophyll and carotenoid substances in six cores from four Minnesota lakes. The data for these curves are included in Table 12. In all the cores there is a general decrease of pigment content with depth of sediment. The curves for the sedimentary chlorophyll and "carotenoids" are essentially parallel. This result can be expected because the amount of each group of pigments for a particular depth in the core is determined from the same extract on a continuous absorption spectrum. In other words, the relative values for these two determinations should be similar for any particular sample within a core.

In Lake Itasca the pigment content is relatively low in the shallow water core at station 1 (Fig. 21, A, water depth 5.1 feet). It is almost twice as high at station 3 (Fig, 21, B. water depth 30.4 feet) where the water is much deeper. At station 4, (Fig. 21, C, water depth 24.6 feet) although the water depth is less than station 3, the pigment content is highest of the three cores, especially in the upper part. The upper part of this core is a calcareous copropel as compared with the generally copropelic marl in the core at station 3. From the surface down to 18 inches the pigment content decreases at about the same rate for these two cores, or about one unit of sedimentary chlorophyll per inch. One explanation of the higher amount of preserved pigments at station 3 is that the bay in the east part of the lake forms a collection area for organic material brought in by wave action from the prevailing northwesterly winds.

The Blue Lake core shows a fairly high amount of pigments, but there is a rapid decrease with depth (Fig. 22, A). The lithology of

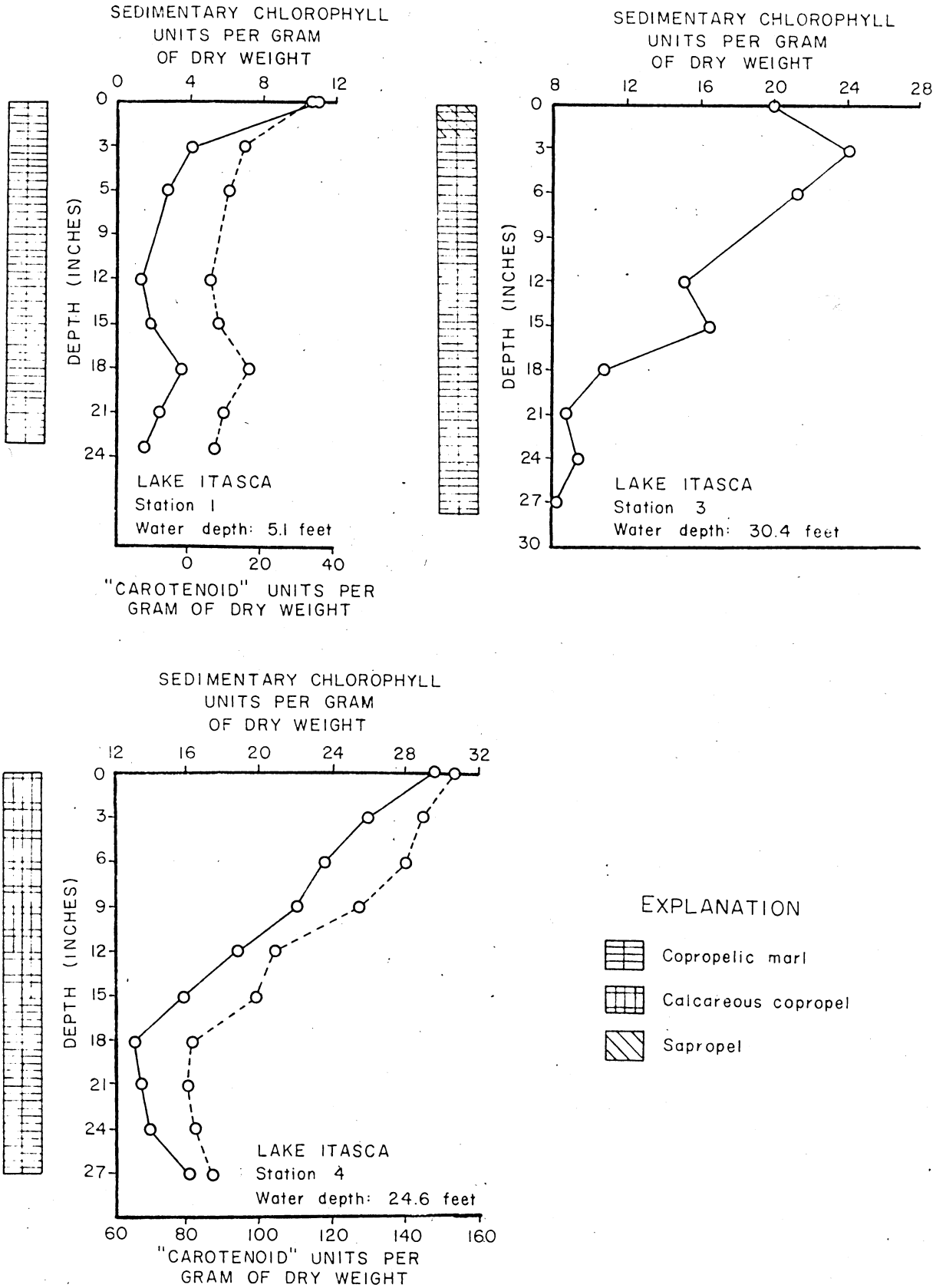


Figure 21. Plots of sedimentary chlorophyll, ——; and "carotenoids", - - - -; Lake Itasca.

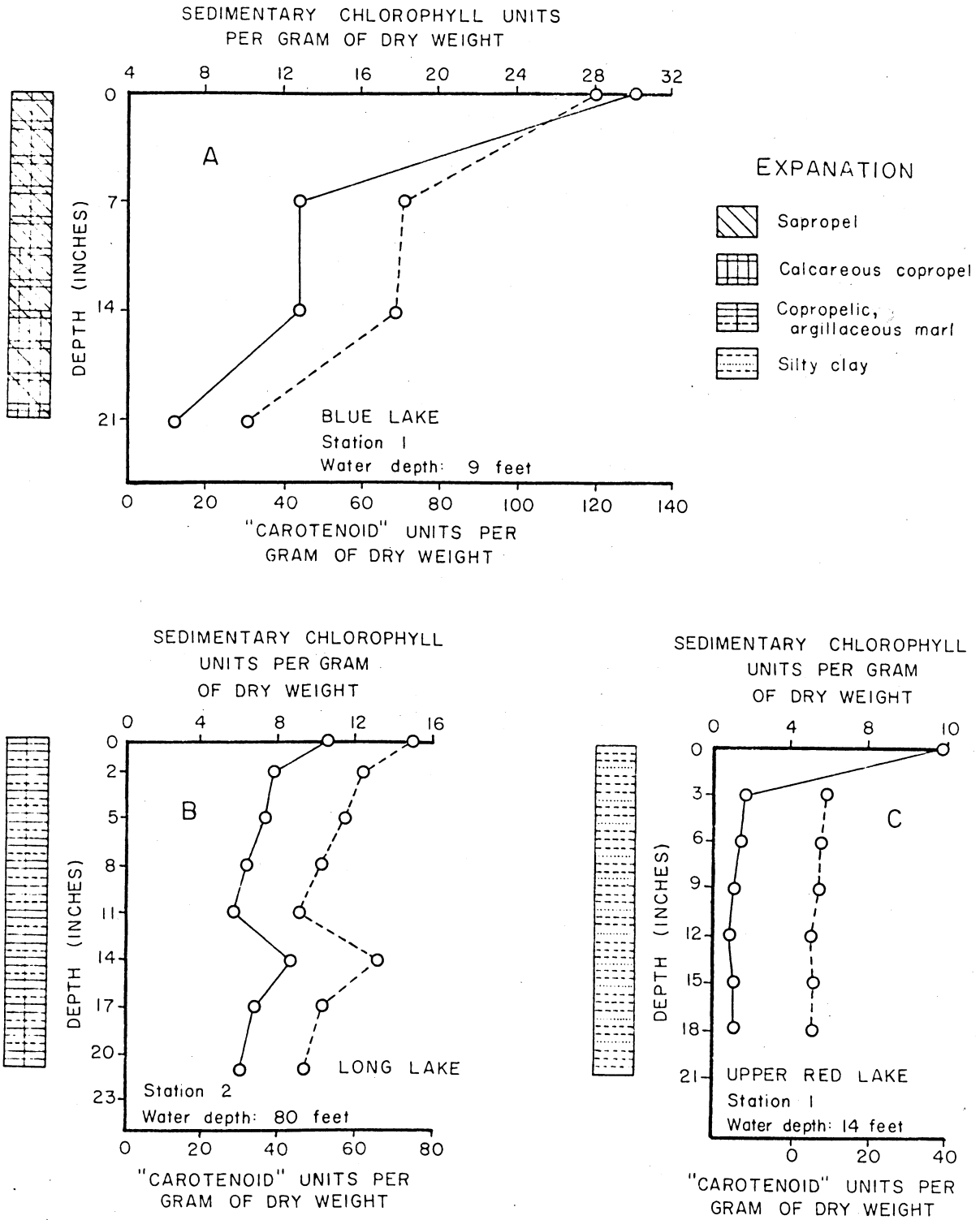


Figure 22. Plots of sedimentary chlorophyll, ———; and "carotenoids", - - - - -; A: Blue Lake; B: Long Lake; C: Upper Red Lake.

TABLE 12. Moisture and pigment content data

	Depth (inches)	Moisture (per cent)	Chlorophyll units per gm. of dry weight	Carotenoid units per gm. of dry weight	Ratio of absorbance at 413-14 mμ to 665-70 mμ	Fluorescence of extract
LAKE ITASCA Station 1	surface	65.2	10.9	33.3	3.0	deep red
	3	62.9	4.0	14.8	3.9	light orange-red becoming orange-yellow downward
	6	65.7	2.6	10.1	3.8	
	9	61.9	n.d.	n.d.	n.d.	
	12	61.1	1.4	5.8	4.1	
	15	62.0	1.9	7.6	4.0	
	18	61.8	3.7	16.1	4.4	
	21	61.9	2.2	9.8	4.3	
	23	48.9	1.7	7.2	4.2	
					average: 4.0	
LAKE ITASCA Station 3	surface	88.6	20.0			
	3	88.9	24.1			
	6	88.2	21.2			
	9	n.d.	n.d.			
	12	85.2	15.2	n.d.	n.d.	n.d.
	15	84.3	16.5			
	18	83.2	10.9			
	21	80.3	8.8			
	24	78.0	9.4			
	27	76.0	8.2			
LAKE ITASCA Station 4	surface	81.8	28.8	153.1	5.3	orange-red becoming lighter downward
	3	80.4	25.8	144.8	5.6	
	6	82.2	23.6	139.3	5.9	
	9	79.0	22.0	127.1	5.8	
	12	78.9	18.8	114.1	6.0	
	15	78.6	15.9	99.0	6.2	
	18	75.4	13.2	81.5	6.2	
	21	75.3	13.5	80.4	6.0	
	24	76.0	14.1	82.4	5.9	
	27	76.8	16.2	87.3	5.4	
					average: 5.8	

Table 12. Continued

	Depth (inches)	Moisture (per cent)	Chlorophyll units per gm. of dry weight	Carotenoid units per gm. of dry weight	Ratio of absorbance at 413-14 mμ to 665-70 mμ
BLUE	surface	88.7	30.1	120.1	4.1
LAKE	7	87.2	12.8	71.1	5.3
Station 1	14	84.4	12.7	68.6	5.3
	21	81.0	6.4	30.1	4.8
					average: 4.9
LONG	surface	65.7	10.5	74.3	7.0
LAKE	2	66.2	7.8	61.9	7.9
Station 2	5	70.4	7.4	57.1	7.6
	8	66.1	6.3	51.1	8.2
	11	69.1	5.7	45.2	8.0
	14	66.9	8.7	65.1	7.5
	17	65.7	6.7	51.1	7.5
	21	70.6	6.1	46.5	7.6
					average: 7.7
UPPER	surface	93.0	11.7	n.d.	n.d.
RED	3	83.9	1.5	8.7	5.9
LAKE	6	81.7	1.3	7.8	6.2
Station 1	9	80.5	1.0	6.9	7.2
	12	74.4	0.7	4.3	6.4
	15	79.6	0.9	4.9	5.5
	18	83.2	1.0	5.0	5.1
					average: 6.1

the core may partially explain the change with depth. The material changes from a calcareous, copropelic sapropel to a calcareous, sapropelic copropel downward. The surface sample was relatively high in pigment content, and the top several inches contained abundant plant remains some of which were green. The shallow water (9 feet) environment is not ideal for the preservation of pigments. The water temperature is as high as 16° C. at the bottom of the lake in August; dissolved oxygen was found in the bottom waters; and a fairly high percentage of surface light probably reaches the bottom.

The results from the Long Lake core (Fig. 22, B) might seem to be anomalous when compared with pigment data from the other lakes. The core is a copropelic, argillaceous marl throughout. Conditions for good preservation (low temperature, lack of light and oxygen) are present in the 80 feet of water, but the quantity of pigments found in the core is somewhat lower than in cores from other lakes in shallower water. The probable reason is that the lake has lower productivity of phytoplankton and aquatic vegetation than Lake Itasca or Blue Lake.

The Upper Red Lake core shows a very low pigment content (Fig. 22, C), and little change with depth. The only exception is the surface sample which has a relatively high sedimentary chlorophyll value. The absorption spectrum for this sample has a peak at 432 mμ which indicates the presence of chlorophyll a. The silty clay lithology is probably a factor in the low quantity of pigments preserved. The shallowness of the water (14 feet) would be another factor in poor preservation.

According to Welch (1952, p. 23), other things being equal, the greater the length of shoreline a lake has the greater will be its

productivity. One way in which this can be expressed is by the development of the shoreline. This is defined as the ratio of the length of shoreline to the length of the circumference of a circle of area equal to that of the lake. The length of shoreline and the development of the shoreline of these four lakes is:

Lake Itasca	24,750 yds.	3.08
Blue Lake	10,700 "	2.47
Long Lake	5,505 "	1.74
Upper Red Lake	106,280 "	1.25

If it can be assumed that the productivity is proportional to the development of the shoreline some correlation can be seen between this factor and the amount of pigments preserved.

Welch (1952, p. 140) also suggests that a lake basin which is steep sided (such as Long Lake) will have lower productivity because of the removal of decomposed material to the deep part of the lake where it is inaccessible as food to other organisms.

With a few exceptions the moisture content of the cores decreased rather uniformly downward (Table 12).

The ratio of the absorbance at the 413-14 mμ peak to that at the 665-70 mμ peak is said by Vallentyne and Craston (1957, p. 37) to be determined by two factors: (1) the amount of carotenoids relative to the sedimentary chlorophyll, and (2) varying proportions of different kinds of sedimentary chlorophyll. They found the ratio varied from 3.1-7.8 for 9 different lakes.

Table 12 shows the values of this ratio for each sample from five different cores, and the average for each core. For all the samples it varied from 3.0-8.2.

Vallentyne and Craston (1957) state that sedimentary chlorophyll has been isolated with absorption peaks at 411, 425, 440 and 464 mμ in acetone. This would indicate that the absorbance in the vicinity of 410-15 mμ cannot be strictly used for a quantitative value of carotenoids.

CHROMATOGRAPHY: The extracts of the samples from the different levels (excluding the surface sample) in the core at station 2 from Long Lake were combined and evaporated to dryness. In order to facilitate the evaporation the liquid was heated to a maximum of 50° C. The dried residue was redissolved in 4 ml. of carbon disulfide. Part of this was applied with a lambda pipette to the base of a 5 inch wide strip of Whatman No. 1 filter paper in a series of spots one-eighth inch apart. The paper was hung in an ascending chromatographic chamber and presaturated in an atmosphere of petroleum ether for 3 hours. The base of the paper was then immersed in petroleum ether and developed for 8 hours. Eight distinct bands developed as shown in Fig. 23. These bands were cut apart, redissolved in carbon disulfide, and the absorption spectrum of each was determined in the visible range of 350-700 mμ. These absorption spectra are shown in Fig. 24 with peaks as indicated. The bands of the chromatogram become less concentrated away from the origin because the absorbance progressively decreased from band 1 to band 7.

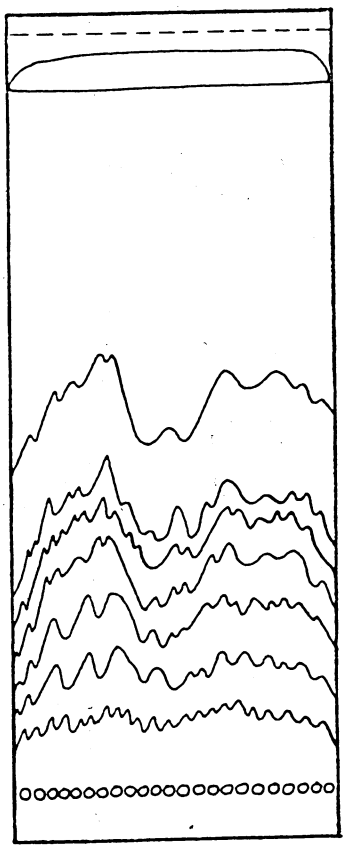


Figure 23. Ascending chromatogram of combined extracts of samples from the core at station 2, Long Lake. Developer: petroleum ether.

Carbon disulfide was used as a solvent because much of the published information of the absorption spectra of carotenoids refers to this solvent, especially in the book by Karrer and Jucker (1950). The absorption spectra in the ultra-violet range was not determined. Karrer and Jucker (1950, p. 53) state that the relationships between the constitution and spectral properties of the carotenoids are more complicated in the ultra-violet than the visible range, and it is not yet possible to predict their absorption spectra from the structure.

While it is questionable whether there was good separation on the chromatogram some of the maxima correspond very closely to published maxima. Several derivatives of carotenoids which have similar maxima are listed in Table 13, and of the carotenoids

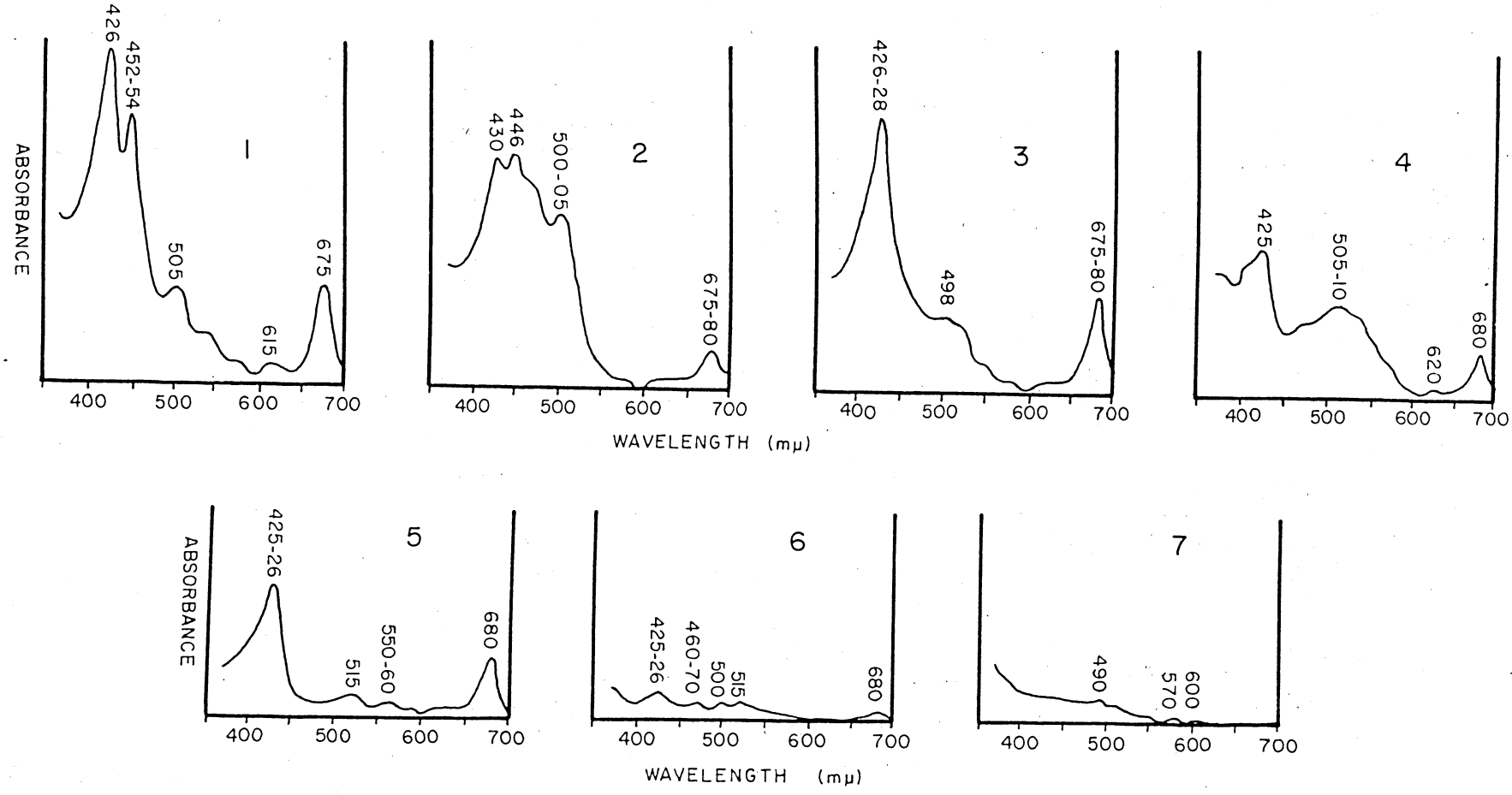


Figure 24. Absorption spectra of different bands of the chromatogram (Fig. 23) from Long Lake. Peaks as indicated. Solvent: carbon disulfide.

previously found in sediments (Table 1) several have maxima close to those of the chromatogram. The maximum at 425-30 mμ which appears in most bands of the chromatogram is common to the carotenoids listed in Table 13. The maximum at 542-54 mμ of band 1 of the chromatogram is also common to these same carotenoids, and also to α- and β- carotene and echinenone. The peak at 505-10 mμ in bands 1, 2 and 4 is common to α- carotene and lutein. The next prominent peak is at 675-80 mμ which is characteristic of a chlorophyll-like substance.

TABLE 13

Carotenoid	Formula	Maxima (mu)
dihydro- β- carotenone (derivative of β- carotene)	$C_{40}H_{58}O_4$	426 455
aurochrome (derivative of β- carotene)	$C_{40}H_{56}O_2$	428 457
Auroxanthin	$C_{40}H_{56}O_4$	423 454
Apo-3-norbixinal methyl ester (derivative of bixin)	$C_{18}H_{22}O_3$	427 455
dihydronorbixin (derivative of bixin)	$C_{24}H_{30}O_4$	428 454
dihydrobixin (derivative of bixin)	$C_{25}H_{32}O_4$	428 454

Table 13. Absorption maxima of carotenoids which have peaks corresponding to bands of the chromatogram (Fig. 24). Solvent: carbon disulfide. Karrer and Jucker (1950).

Andersen and Gundersen (1955) used paper chromatography on ether extracts of interglacial gyttja from Denmark. The bands which developed on the chromatogram were redissolved in acetone and the absorption spectra determined. Some of the peaks are close to those of the Long Lake chromatogram, the difference of 8-10 mμ could be attributed to the different solvents used. However, they do not identify any of these pigments, and state that the final identification would require

the isolation of compounds in a solid state which is possible only with large scale separation on absorption columns.

POST EXTRACTION CHANGES: Tests were made on two 90 per cent acetone extracts to determine the rate at which a change takes place when they are left exposed to normal daylight. The extracts were stored in corked test tubes in the laboratory with no shielding from daylight. The absorbance was determined with a Beckman spectrophotometer at the two main peaks of 412 mμ and 665 mμ. Figure 25 shows the plots of the absorbance over a 70 day interval for one of the samples. Table 14 shows the percentage change in absorbance for both samples. The absorbance decreased more rapidly the first week, and then decreased at a progressively slower rate.

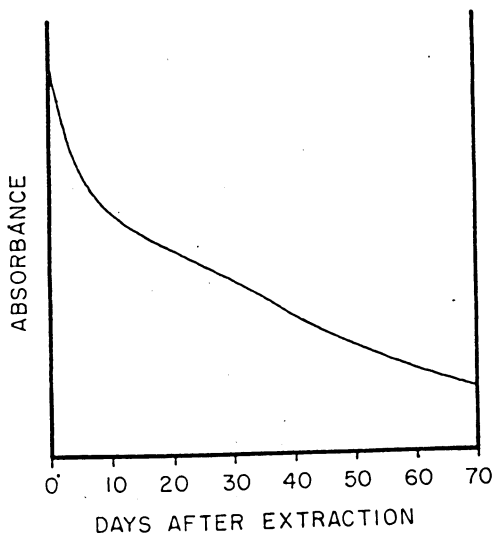


Figure 25. Graph showing decrease in absorbance with time in a 90 per cent acetone extract stored in daylight. The curve represents the 665 mμ peak. The curve for the 412 mμ peak is almost identical.

TABLE 14

	Number of days after extraction	Percentage decrease from original sample	
		412 mμ peak	665 mμ peak
(1)	7	29	27
	30	50	44
	60	63	60
(2)	1	3	8
	7	13	19
	30	48	39
	60	67	60

Table 14. Results of tests to determine the decrease in absorbance of a 90 per cent acetone extract when stored at normal laboratory conditions in daylight. Sample (1) Lake Itasca, station 4, 12 inches below top of core. Sample (2) Long Lake, station 1, 1-4 inches.

Extracts stored in a refrigerator show very little change in absorbance up to several days after extraction. When stored for longer periods, however, the absorbance begins to decrease. In one sample the absorption spectrum was determined 20 weeks after extraction. The 413 mμ peak decreased 5 per cent, and the 670 mμ peak decreased 8 per cent. This is about the same change as takes place in one day when a sample is not refrigerated.

DISCUSSION

When an attempt is made to compare the results of pigment determinations in samples from these four Minnesota lakes with the published data from other sources some difficulties are encountered. This is because other published data are frequently based on laboratory procedures which are different from those used by the writer. Variability results from the use of different solvents, different extraction methods, etc. The location of peaks in the absorption spectra depends upon the solvent used as was pointed out in the section on spectrophotometry. Vallentyne and Craston (1957) used 90 per cent acetone to which 0.2-0.5 per cent dimethylaniline had been added. Vallentyne (1955) found that the value of the sedimentary chlorophyll is about 10 per cent greater when 0.5 per cent dimethylaniline is used than when 90 per cent acetone is used. Orr and Grady (1957) extracted marine sediments with acetone, and concentrated the pigments by transfer to chloroform for spectroscopic analysis.

Vallentyne (1955) expressed the sedimentary chlorophyll in units per gram of dry weight of sediment, but in succeeding publications the value is expressed in units per gram of ignitable matter (Vallentyne and Craston, 1957). The amount of ignitable material can be determined by ashing the dried sample over a Bunsen burner. No attempt was made by the writer to determine the amount of ignitable material in any of the samples. Orr *et al.* (1958) express the pheophytin content in ppm. For comparison, one of Vallentyne's SCDP (sedimentary chlorophyll degradation products) units is approximately equivalent to 15 ppm of pheophytin *a*.

There are many factors which will affect the quantity of pigments or products of pigments which are preserved in sediments. Some of the more important ones are listed here.

(1) Plankton productivity. This might be considered to be the most important factor since phytoplankton are believed to be the main contributing source, and therefore the quantity of pigments preserved may be directly proportional to the quantity produced. Vallentyne (1956, p. 261) concluded that the relative proportion of carotenoids preserved in sediments differs little from the proportions originally deposited. Orr et al. (1958), however, concluded from their study of marine sediments that the quantity of pigments preserved depends more upon the decomposition during transport through the water and exposure on the bottom before burial than it does on productivity.

(2) Depth of water. The greater the depth of water through which the organic material must settle before reaching the bottom the longer time it will be exposed to decomposition. Orr and Grady (1957, p. 269) estimated that 99 per cent of the chlorophylls synthesized by plankton is decomposed in the water column and prior to burial.

(3) Light, temperature and oxygen. Since light, high temperature and oxygen tend to break down pigments we would expect a greater amount of decomposition to take place in shallow water. Kleerekoper (1953) found that the bulk of decomposition of sinking detritus occurs in the epilimnion. Vallentyne (1955) points out that the cold reducing medium of lake sediments offers ideal conditions for the preservation of organic compounds. Reducing conditions in the sediments tend to inhibit the decomposition of pigments, but decomposition is not completely stopped.

(4) Sedimentation rate. As is true of the preservation of any fossil the more quickly organic matter is buried the more likely it will be preserved.

(5) Post depositional synthesis. There is a possibility that some carotenoids may be synthesized by microorganisms in the sediment. Bacteria have been found to decrease logarithmically with depth dropping to almost zero at one foot (Henrici and McCoy, 1938). Zobell (1945) states that bacteria are functional in marine sediments to depths as great as 25 feet. Vallentyne (1956, p. 259) believes the amount of post depositional synthesis of carotenoids would be small when compared with that derived directly from dead phytoplankton.

(6) pH of the sediments. Hodgson and Hitchon (1959) carried out experiments to determine the variability in preservation with change in pH of sediments. They found that the pH was a major factor. At high pH values chlorophyll was extensively destroyed, but as the acidity was increased there was better preservation of pigments with a corresponding increase in pheophytin preservation.

The pH of the sediment within two cores was determined by the writer; one from Lake Itasca (station 5) and one from Long Lake (station 3) (see Appendix). The pH in the Lake Itasca core varied from 6.8-7.2, and in the Long Lake core from 7.2-7.7. There was no significant change in pH with depth. The slightly higher alkalinity of the Long Lake sediment may be a factor in the lower quantity of pigment preserved as compared with the Lake Itasca sediment.

Orr, et al. (1958, p. 951) found that the most significant feature of depth variations of pheophytin is that most of the change (usually a decrease) occurs in the top few inches of the sediments. G. W. Hodgson

(written communication; Alberta Research Council, Edmonton, Alberta, Canada) found that the major part of the transformation of pigments takes place in a very short period of time; the amount required for the deposition of a few inches of sediments. This finding seems to be in accord with the results from several of the cores studied by the writer where the greatest quantitative change appears between the surface and 2-3 inches.

In all the cores studied by the writer it was found that the amount of pigments generally decreased with depth (Fig. 13 and 14) with a levelling off farther down in the cores. If the majority of the transformation and breakdown of pigments occurs near the mud-water contact as believed, and we assume that the rate of change did not vary for the time interval represented by a core, then the amount of pigments preserved can be said to indicate the relative amount of source material available. Under this assumption the lakes studied have had an increasing amount of source material available during the interval represented by the sediment.

When the results of the core studies of the four lakes are compared they can be approximately arranged in order of increasing amount of pigments as:

Upper Red Lake---Long Lake---Blue Lake---Lake Itasca.

This succession can be correlated with the classification of productivity of the lakes from oligotrophic to eutrophic-alkalitrophic to eutrophic.

According to some classifications oligotrophic lakes have a low quantity of plankton while eutrophic lakes are rich in plankton.

If the main source of pigments in the sediments is from phytoplankton, then the same order of lakes (Upper Red Lake to Lake Itasca) can be used for productivity.

CONCLUSIONS

1. Although there are many variables involved in the amount of pigments preserved in sediments there appears to be a correlation with the productivity of the lake. The productivity, in turn, is dependent upon physical and biological factors. One of the physical factors is the development of the shoreline. Of the four lakes studied it was found that those with a higher development of shoreline had a higher amount of preserved pigments.

2. In several of the cores the greatest decrease in pigments was found in the top few inches, indicating the greatest change occurs soon after deposition.

3. The amount of pigments generally decreased with depth of sediment. This can be interpreted as indicating an increasing amount of source material up to the present time.

4. Absorption spectra of 90 per cent extracts at different levels in all cores were very similar with the exception of a surface sample from Upper Red Lake which showed a peak at 432 mμ which is indicative of chlorophyll a.

5. The length of time (minutes vs. hours) the solvent is allowed to stand on a sediment sample does not significantly affect the absorption spectra or the quantitative determination of pigments. A short extraction time would be satisfactory and more convenient.

6. The absorption spectra for air-dried samples as compared with moist samples were found to be significantly different both in the location and height of the peaks, especially in the visible range below 500 mμ.

7. Refrigerated extracts do not show significant change in absorption spectra with time. However, samples exposed to normal daylight in the laboratory show a relatively large change with time.

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APPENDIX

Description of Cores

LAKE ITASCA, Station 1

Location: North arm of the lake about 250 feet westnorthwest of the dock next to the Lakeside Lab, University of Minnesota Biological Station (Fig. 16).

Water Depth: 5.1 feet.

Length of Core: 23.3 inches.

Lithology: Copropelic marl; small percentage of medium to fine quartz grains, subangular to angular.

Color: Surface to 21 inches is light olive gray (wet), yellowish-gray (dry); 21 inches to bottom is olive gray (wet), light olive gray (dry).

Fossils: Numerous ostracods, pelecypods and plant fragments throughout the core; plant seed cases and charophyte oogonia common. Ostracod genera include Candona, Cypria, Limnocythere, Darwinula, Cyclocypris and Paracandona. Pelecypod genera include Sphaerium and Pisidium. Gastropod genera include Gyraulus, Valvata, Planorbula and Physa.

Carbonate Determination:

Depth (inches)	Carbonate (percentage)
2	90
8	92
14	94
20	86

Remarks: Also see Table 12.

LAKE ITASCA, Station 2

Location: North arm of the lake about 750 feet from shore east-southeast of Hill Point (Fig. 16).

Water Depth: 25.5 feet.

Length of Core: 27.8 inches.

Lithology: Copropelic marl, slightly sapropelic near the top.

Color: Olive gray (wet), light olive gray (dry).

LAKE ITASCA, Station 2 (continued)

Carbonate Determination:

Depth (inches)	Carbonate (percentage)
surface	80
8	80
17	80
25	78

LAKE ITASCA, Station 3

Location: North arm of the lake about 500 feet from shore east-southeast of Hill Point (Fig. 16).

Water Depth: 30.4 feet.

Lenght of Core: 27 inches.

Lithology: Copropelic marl, slightly sapropelic from 0-2 inches.

Color: Olive gray (wet), light olive gray (dry).

Remarks: Also see Table 12.

LAKE ITASCA, Station 4

Location: Southeast arm of the lake about 750 feet from shore north of Turnbull Point (Fig. 16).

Water Depth: 24.6 feet.

Length of Core: 27 inches.

Lithology: Very calcareous copropel in the upper part becoming a very copropelic marl in the lower part of the core.

Color: Olive gray (wet), medium gray (dry).

Remarks: Also see Table 12.

LAKE ITASCA, Station 5

Location: Southeast arm of the lake about 1200 feet from shore north of Turnbull Point (Fig. 16).

Water Depth: 42.6 feet.

Length of Core: 26 inches.

Lithology: Very calcareous copropel from the surface to 12 inches, becoming a very copropelic marl to the bottom of the core.

Color: Olive gray (wet), medium gray (dry).

Fossils: Woody and plant fragments, sponge spicules and cladoceran ephippia common. Ostracod genus: Candona.

Carbonate and pH Determination:

Depth (inches)	pH	Carbonate (percentage)
surface	7.2	42
3	7.1	
5	7.0	
7	6.9	46
9	6.9	
11	6.9	
13	6.9	51
15	6.8	
17	6.8	
19	7.0	70
21	6.9	
23	6.9	
25	6.9	73

LONG LAKE, Station 1

Location: Approximate center of the lake length about 300 feet from the southwest shore (Fig. 17).

Water Depth: 75 feet.

Length of Core: 16.5 inches.

Lithology: Copropelic, argillaceous marl.

Color: Olive gray (wet), light olive gray (dry).

Carbonate Determination:

Depth (inches)	Carbonate (percentage)
surface	63
8	76
16	78

LONG LAKE, Station 2

Location: Approximate center of the lake length about 400 feet from shore.

Water Depth: 80 feet (Fig. 17).

Length of Core: 22 inches.

Lithology: Copropelic, argillaceous marl.

Color: Light olive gray with some olive gray streaks; dark gray to grayish-black woody fragments common (wet); yellowish-gray (dry).

Fossils: Diatoms very common, cladoceran ephippia and woody plant material common, some ostracods. Ostracod genera include Candona, Limnocythere and Entocythere.

Remarks: Apparent varving in upper part of the core; approximately 3 layers per 0.1 inch. Also see Table 12.

LONG LAKE, Station 3

Location: Approximate center of the lake length about 400 feet from the northeast shore (Fig. 17).

Water Depth: 75 feet.

Length of Core: 28 inches.

Lithology: Copropelic, argillaceous marl.

Color: Olive gray (wet), light olive gray (dry).

Moisture and pH: (distilled water of pH 7.3 added to sample prior to pH determination).

Depth (inches)	Moisture (percentage)	pH
surface	80.3	7.7
4	70.8	7.5
7	n.d.	7.3
10	73.1	7.3
11	n.d.	7.5
12	n.d.	7.7
13	75.4	7.7
14	n.d.	7.5
15	n.d.	7.4
16	74.4	7.2
19	67.2	7.5
22	68.7	7.6
25	68.3	7.6
28	65.3	7.7

BLUE LAKE, Station 1

Location: North bay of the lake about 400 feet northwest of Cook's Landing (Fig. 18).

Water Depth: 9 feet.

Length of Core: 21 inches.

Lithology: Calcareous, copropelic sapropel 0-14 inches, becoming a calcareous, sapropelic copropel 14 inches-bottom. Green plant fragments 0-5 inches.

Color: Brownish-black 0-14 inches, becoming gray-brown 14 inches-bottom (wet); light gray (dry).

Fossils: Diatoms and sponge spicules very common, cladoceran ephippia and carapaces. Ostracod genera include Cypridopsis and Cypria. Plant fragments abundant at top, decreasing downward.

Remarks: Also see Table 12.

UPPER RED LAKE, Station 1

Location: Center of the lake about 8 miles west of the town of Waskish (Fig. 19).

Water Depth: 14 feet.

Length of Core: 21 inches.

Lithology: Silty clay.

Color: Very dark gray (wet), gray (dry).

Sieve analysis:

Screen	Top	(percentage)	Bottom
28 mesh	0.0		0.0
100 mesh	5.0		1.8
Pan	95.0		98.2

Fossils: Cladoceran ephippia, some plant remains and diatoms. Ostracod genera include Candona (very common in lower part of core) and Limnocythere. Gastropod genera include Valvata and Pisidium.

Remarks: Also see Table 12.

UPPER RED LAKE, Station 2

Location: Center of the lake about one mile north of station 2 (Fig. 19).

Water Depth: 14 feet.

Length of Core: 26 inches.

Lithology: Silty Clay.

Color: Very dark gray (wet), gray (dry).

pH Determination:

Depth (inches)	pH
water	7.6
surface	7.2
13	7.4
26	7.6

Amino acids: (F. M. Swain, unpublished)

	Quantity in parts per 10,000		
	Top	Middle	Bottom
Cystine	5.98	1.75	-
Lystine	.70	.84	.40
Histidine, arginine	.83	-	-
Serine, glycine, aspartic acid	3.01	2.69	1.86
Glutamic, threonine	2.32	1.94	.95
Alanine	3.37	3.47	1.95
Tyroxine	.59	.47	.03
Valine	.66	.75	2.98
Phenylalanine	.37	.24	.22
Leucine--isoleucine	1.48	1.22	.81

All color determinations based on Rock Color Chart, 1948, National Research Council, Washington, D. C.

Carbonate determination made by adding hydrochloric acid to a dried sample and weighing the dried residue.