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The concentrations of the photosynthetic pigments, chlorophyll a and c exhibit a diel periodicity in situ off of the Oregon coast. At depths to 15 meters, and occasionally 25 meters, the maximum concentrations of these pigments occur during the early evening to the middle of the night with minimum concentrations usually in the late afternoon. At 50 meters, the maximum concentration usually occurs during the day and the minimum concentration at night.

The ubiquitous marine diatom Skeletonema costatum was used to study this phenomenon in the laboratory. Cultures of this diatom were grown under three light intensities and spectral qualities simulating surface, 10, and 50 meter light conditions, and three photoperiods of 9, 12, and 16 hours. At the higher light intensities, and photoperiods of 12 and 16 hours, the cellular concentration of chlorophyll a and c showed a maximum several hours into the dark period,

then a decline into the middle of the light period followed by an increase again into the dark period.

At lower light intensities, such as at the simulated 50 meter depth, the opposite occurred, i. e., the maximum concentration of these pigments occurred in the light period and minimum concentration in the dark period. Experiments were performed which showed bleaching to be the cause of the minimum concentration in the light period. Decline of chlorophyll that occurred in the dark period was delayed by the addition of an external carbon source in the form of glucose and pyruvic acid. It appears that chlorophyll synthesis can occur in the dark in Skeletonema, and perhaps other diatoms, and that an energy-yielding substrate is necessary to maintain chlorophyll in the dark.

The diel periodicity of photosynthetic pigments in situ at sea is seen to be controlled by light, not grazing, which would cause the chlorophyll minimum to occur at night, not during the day as seen. The chlorophyll concentration of cells in nature appears to be a short-term adjustment rather than a long-term adaptation. The ecological significance of these diel changes in chlorophyll content in response to light is that of keeping a constant photosynthetic rate. At lower light intensities, chlorophyll is synthesized and assimilation increased due to a higher concentration of this catalyst. At higher light intensities, bleaching occurs, decreasing the concentration of

chlorophyll. This may prevent the build-up of harmful substances at higher light intensities.

The chlorophyll method of estimating primary production at sea requires correction factors for the diel periodicity of chlorophyll.

The Ecological Significance of the Diel Periodicity of Photosynthetic Pigments in Marine Phytoplankton

by

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THE ECOLOGICAL SIGNIFICANCE OF THE DIEL PERIODICITY OF PHOTOSYNTHETIC PIGMENTS IN MARINE PHYTOPLANKTON

INTRODUCTION

Marine phytoplankton are members of the algae, usually microscopic diatoms and dinoflagellates, suspended in the water column with very limited motility. These organisms are the basis of all life in the oceans due to their ability to produce organic materials by photosynthesis. The term "net production", the net rate of autosynthesis of the organic constituents of plant material in water over the 24-hour day, is commonly used by biological oceanographers to denote the rate at which the photosynthetic process is carried on by these organisms. This can be determined by measuring changes in oxygen evolution in light bottles containing water samples from the position in the water column at which such a measurement is desired. Another technique measures the rate of carbon-14 uptake by these organisms. This latter method, as first described by Steeman Nielsen (1952), is the most commonly used method today. Results obtained from this method are most frequently expressed in the unit of milligrams carbon fixed per cubic meter of water per hour, with dimensions of $ML^{-3}T^{-1}$.

Biological oceanographers also measure the standing stock of phytoplankton. This is defined by Strickland (1960) as "the instantaneous value of the amount of living plant material present in water",

and is measured in the dimensions of ML^{-3}. Methods of measuring standing stock include direct counting of cells, measuring weight or volume of cells, determining carbon content of cells, and analysis of other cellular constituents such as nitrogen, phosphorous, and specific organic compounds. A common way of estimating standing stock is by pigment analysis. In this method, the photosynthetic pigments such as chlorophyll a, b, and c, and plant carotenoids are determined by filtering the phytoplankton from a given volume of water, extracting the pigments in an appropriate organic solvent, and subsequently determining them spectrophotometrically. As chlorophyll a is the main "catalyst" in photosynthesis, standing stocks, as estimated by the pigment method, are usually expressed in terms of milligrams chlorophyll a per cubic meter of water (Strickland 1960).

Such chlorophyll a values can be related to primary production by multiplying the pigment value by the "assimilation ratio." This number has been defined by Gessner (1949) as the ratio of photosynthesis (CO_2 uptake) to chlorophyll. Ketchum et al. (1958) have more specifically defined assimilation number as the number of grams of carbon taken up per gram of chlorophyll a per unit time, usually per hour. McAllister, Shaw, and Strickland (1964) found that at low light intensity, primary production correlated better with the sum of chlorophyll a and c than with chlorophyll a alone. Ryther

and Yentsch (1957) have used chlorophyll and light data to estimate phytoplankton primary production *in situ*.

Both primary production rates and standing stocks of phytoplankton vary seasonally and spatially. Factors such as light, water temperature, salinity, nutrients, and grazing by herbivores cause these variations. Recently, diel changes (those changes taking place over the 24-hour day) in both primary production and standing stock have been demonstrated in the oceans (Doty and Oguri 1957, Yentsch and Ryther 1957, Shimada 1958, Yentsch and Scagel 1958, Menzel and Vaccaro 1961, Lorenzen 1963, McAllister 1963, El-Sayed and Mandell 1965, and Wood and Corcoran 1966). These diel changes complicate the use of the pigment method of measuring the standing stock and primary production measurements by the assimilation ratio method. It was the purpose of this investigation to determine the factors controlling diel changes of chlorophyll *a* and *c* and the ecological significance of such changes.

Doty and Oguri (1957) noticed a 5. 7-fold diel variation in phytoplankton photosynthesis. They found that maximum carbon fixation occurred a few hours before mid-day, and minimum activity at 1900 local time. They concluded that concordant chlorophyll variations may be present. Doty (1959) found that such photosynthetic variations were a function of latitude, with the greatest variations occurring at the lowest latitudes. For example, a ten-fold diel

variation in photosynthesis occurred at 4-5° South, a two- to three-fold variation at 18° North, a two-fold variation at 41°, and a 20 percent variation occurred at 56° North.

Shimada (1958) measured photosynthetic activity and chlorophyll a concentration over a 46-hour period in the eastern North Pacific and found a diel variation in photosynthesis which correlated with the chlorophyll content of the water. Highest chlorophyll values were found at 0600 local time, with lowest values at 1800 increasing to a peak at 1000 the next morning. He found an assimilation ratio of 4. 24 at a light intensity of 1000 foot-candles.

Yentsch and Scagel (1958) investigated the diel periodicity of photosynthetic pigments in East Sound, Washington. They studied the variation of chlorophyll a at depths of 0, 3, 5, 10, 15, and 25 meters, made concurrent cell counts, and found a diel variation of chlorophyll a down to a depth of ten meters. At the surface and three meters, chlorophyll a was found to be five times higher at midnight than at mid-day. At five meters, the variation was not as great, and at 10, 15, and 25 meters depth, almost constant values of the pigment were seen. At times, the pigment changes were associated with changes in cell numbers, but most of the time such pigment changes were too great in magnitude to be explained by changes in cell numbers alone.

Chlorophyll c also showed such a diel periodicity, but not in phase with chlorophyll a. The authors felt that light was the causative factor of diel pigment variations and stated that such a rhythm was influenced by light duration and intensity, previous light history, stability of the water column, temperature, cell age, and nutrition. However, they showed no evidence to support their statements.

Yentsch and Ryther (1957) studied diel variations of chlorophyll a in the Atlantic off the northeastern United States. They found pigment maxima to occur at 0800 and minimum values at midnight. These in situ variations were too great to be explained by cell division or death alone. Ryther, Menzel, and Vaccaro (1961) found a nocturnal decrease of chlorophyll a with a subsequent daytime increase in the Sargasso Sea. Lorenzen (1963) studied diel chlorophyll variation in an estuary of Long Island Sound and found a ten-fold variation in chlorophyll a concentration. The highest values were found at noon, and the lowest values in the middle of the night to just before sunset. Photosynthetic potential varied concurrently, although it was not of the same magnitude. El-Sayed and Mandelli (1965) found that a diel periodicity of chlorophyll a occurred in the Weddell Sea and Drake Passage in the Antarctic Ocean. This pigment was highest at 0900 and 2100 and lowest at 1300. The ratio between maximum and minimum values was about two. The cause of diel pigment periodicity was attributed by McAllister (1963) to grazing

of phytoplankton by herbivorous animals. He studied this phenomenon in the North Pacific and found highest values of chlorophyll a at noon when photosynthesis was at a maximum. The lowest values of pigment were found at sunset and sunrise which corresponded with the maximum concentration of zooplankton herbivores. Steeman Nielsen and Jorgensen (1962) had previously believed that grazing was the cause of the low pigment values found in the early afternoon hours. They showed no evidence to support their hypothesis. Wood and Corcoran (1966) studied the diel variation in cell numbers and chlorophyll a in the Tongue of the Ocean and the Guinea and Benguela Currents. They showed no constant correlation between cell numbers and chlorophyll a concentration, but thought that cell numbers always decreased at night due to grazing. They found that highest chlorophyll a concentrations occurred in the late afternoon, and lowest concentrations in the middle of the night.

Ryther and Menzel (1959) reported that phytoplankton could adapt to light intensity and exist as "sun" and "shade" species depending on their position in the water column. Steeman Nielsen and Hansen (1959) reported that light adaptation existed in phytoplankton, and that internal chlorophyll content of temperate surface species should be twice as high as tropical surface species, and half that of typical shade species found at depth. Humphrey (1963) showed Nitzchia closterium had greater chlorophyll when grown at 420

foot-candles than at 680 foot-candles, but found that light intensity had no effect upon the pigment content of Gymnodinium and Skeletonema costatum.

Yentsch and Lee (1966) stated that the chlorophyll content of phytoplankton represents a balance between photo-oxidation (due to high light intensities) and pigment synthesis. They believed that the pigment content of surface phytoplankton decreased in the afternoon, thereby preventing harmful photo-oxidations.

Several studies have been made on the diel periodicity of chlorophyll a in laboratory cultures of algae. Gibor and Meehan (1961) used Chlorella, Stichococcus and Euglena grown under a light intensity 250 foot-candle on a schedule of 12 hours light and 12 hours dark. The latter two species showed decline in chlorophyll a with the onset of the dark period, and a beginning of resynthesis after nine hours in the dark. Yentsch and Reichert (1963) grew Dunaliella at 800 foot-candles to logarithmic stage of growth and then completely darkened the culture. They found that chlorophyll a decreased in the dark. After 100 hours in the dark, exposure to light caused severe pigment bleaching and photosynthesis could not be resumed. Terborgh and Thimann (1964) studied the interaction between day length and light intensity in the chlorophyll content of Acetabularia. They found that plants grown under 8 hour light and 16 hour dark periods had more chlorophyll than those grown under a 16 hour light-8 hour dark

cycle at a range of different light intensities. They concluded that photoperiod regulated the pigment content of this species.

Castenholz (1964) noticed small pigment increases in the dark in the diatom Biddulphia grown at different light-dark periods. But he found that the chlorophyll content of this species was directly proportional to the length of the light period. Edmonds (1965) studied Euglena and found that pigment synthesis occurred only in the light period and abruptly ceased in the dark. In this experiment, the cells were grown at 330 foot-candles with a 14 hour light-10 hour dark period. The chlorophyll concentration in the culture almost doubled during the light period, but reached its starting concentration at the end of the dark period. Jorgensen (1966) studied chlorophyll changes in cultures of Skeletonema costatum grown at 280 foot-candles under 12 hour light-12 hour dark periods. He found that both chlorophyll a and c increased during the light period, but he didn't explain the decrease of pigment in the dark.

Chlorophyll a production in relation to photoperiod in Dunaliella was studied by Eppley and Coatsworth (1966). They found that the synthesis of chlorophyll a occurred primarily during the light period, with initial rates of pigment synthesis nearly proportional to the length of the photoperiod. Net chlorophyll a synthesis ceased after 12-14 hours of light, and declined in the last hours of an 18 hour photoperiod. A renewed burst of synthesis took place in the following

dark period after the 18 hour photoperiod. The light energy used in this experiment was 0.05 langleys per minute. Yentsch (1965) placed Phaeodactylum tricornutum in the dark after 40 hours exposure to light, and found an increase in chlorophyll reaching a maximum after 70 hours of darkness. Then, a decline in chlorophyll took place accompanied by an increase in phaeophytin concentration until no chlorophyll was present after 270 hours.

Thus, diel variations of chlorophyll concentration have been observed in the oceans and in algae grown under laboratory conditions. However, results tend to be conflicting. For example, in the ocean, Yentsch and Scagel (1958), Steeman Nielsen and Jorgensen (1962), and El-Sayed and Mandelli (1965) found that highest chlorophyll values occurred at night. Highest chlorophyll values during the day, and lowest values at night, were reported by Shimada (1958), Yentsch and Ryther (1957), Ryther, Menzel, and Vaccaro (1961), Lorenzen (1963), McAllister (1963), and Wood and Corcoran (1966). In laboratory studies, chlorophyll increases in the dark were seen by Gibor and Meehan (1961), Yentsch and Reichert (1963), Castenholz (1964), and Yentsch (1965). Decreases of chlorophyll in the dark, and increases in the light were seen by Gibor and Meehan (1961), Terborgh and Thimann (1964), Edmonds (1965), Jorgensen (1966), and Eppley and Coatsworth (1966). In order to determine the cause of diel pigment periodicity and explain the discrepancies in the

literature, several cruises were made between July 1965 and January 1967. Experiments were also performed in the laboratory to study the phenomenon under controlled conditions of photoperiod and light intensity.

METHODS

Sampling Procedure at Sea

All sampling at sea was done aboard the Oregon State University Research Vessel, the R. V. YAQUINA. Upon arriving on station, the ship was allowed to drift in order to remain within the same water mass and thus sample the same patch of phytoplankton as closely as possible. During cruises in the late fall and winter, this proved to be fairly difficult due to high winds. Upon drifting four-five miles from station, the ship would proceed back to station at as slow a speed as possible, usually around two knots.

Approximately every two hours while on station, water samples were collected with Van Dorn bottles from depths of 10, 15, 25, and 50 meters. Surface water samples were collected with a plastic bucket. One or two liters of sea water from each depth were vacuum-filtered through a Millipore® AA (0.8 micron pore size) membrane filter. A small amount of saturated magnesium carbonate solution was added to prevent the chlorophyll on the filters from becoming acid and degrading into phaeophytin. The filters were then folded into Whatman #1 filter paper, placed into a dessicator, and stored in the ship's freezer at -7° C or lower.

On some cruises, incoming solar radiation was measured by means of a recording Eppley pyrheliometer. Occasionally the

Simrad® echo sounder was operated to record the depth of the deep scattering layer in an attempt to determine the depth at which grazing herbivores were present.

Growth of Cultures for Laboratory Study

Skeletonema costatum (Grev.) Cleve grown at 200 foot-candles was used for all of the laboratory studies. The medium used to grow this species was prepared by autoclaving membrane-filtered sea water and enriching it. The enrichment is given in Table 1.

Table 1. Composition of Nutrient-Enriched Sea Water.

Major Ions, Added as Stock Solutions	Concentration per Liter
Sodium nitrate	1.76 milligram-atoms
Sodium dihydrogen phosphate	72.5 microgram-atoms
Iron sequestrene	23.2 microgram-atoms iron
Sodium silicate·$9H_2O$	214 microgram-atoms
Sodium bicarbonate	0.2 grams
Thiourea	1.0 grams
Vitamins	
Thiamin HCl	0.2 milligrams
Biotin	1.0 micrograms
Vitamin B_{12}	1.0 micrograms
Trace Metals	
Cupric sulfate·$5H_2O$	0.079 microgram-atoms of the metal
Zinc sulfate·$7H_2O$	0.015 microgram-atoms of the metal
Cobalt chloride·$6H_2O$	0.085 microgram-atoms of the metal
Manganese chloride·$4H_2O$	1.82 microgram-atoms of the metal
Sodium molybdate·$2H_2O$	0.052 microgram-atoms of the metal

For experiments requiring the measurement of pigments every two hours, 12 liters of enriched sea water were prepared and placed into a Pyrex jug. This was inoculated with 100 ml. of Skeletonema culture and placed within a light bath consisting of three circular "delux warm white" fluorescent lamps, delivering a light intensity of 400 foot-candles. During the dark period, the bath was covered with a hood to prevent light from entering. The light bath was kept at $18 \pm 1^{\circ}$ C. Air was bubbled into the culture to keep it well-stirred. Every two hours, samples were drawn from the culture for pigment analysis and cell counts. The pigment samples were prepared as previously described in the section on sampling at sea. Cell counts were made with a Fuchs-Rosenthal counting chamber.

In other laboratory culture experiments done since December 1966, I used a Sherer-Gillet Growth Chamber (Model CEL 34-7) capable of being programmed for light duration and intensity by switching three sets of incandescent and fluorescent lights. The fluorescent lights consisted of 13 lamps, balanced between "Grow-Lux®" and "Daylight" types, as given in Table 2. A five-compartment box was placed inside the growth chamber. Each compartment was covered by a Corning molded, unpolished glass filter chosen to simulate the intensity and quality of light at a given depth in the ocean. Table 2 gives the light types used, energies involved, and filter types.

Table 2. Light Types, Energy, and Filter Type Used in Laboratory Culture Experiments.

Simulated Depth	Corning Filter Number	Radiant Energy, Langleys per Minute		
		Low Light	Medium Light	High Light
surface	none	0.001	0.041	0.050
10 meters	2781	0.0003	0.010	0.015
50 meters	2827	0.0001	0.003	0.004

Types of lights used in various light treatments:

Low light - four 60 watt incandescent lamps
Medium light - six Sylvania 48" daylight type, F48T12-VHO and incandescents
High light - Two Sylvania "Grow-Lux"®, type F48T12-GRO-VHO
Four Sylvania daylight types, F48T12-D-VHO
One GE photoreproduction, type F48PG 17.1 PR
in addition to lights in medium light treatment.

The lights were turned on and off by timers to simulate sunrise, morning, noon, afternoon, and sunset. All experiments were performed at 18° C to duplicate conditions of temperature in the culture room.

In the December 1966 experiment, cells were grown in enriched sea water until logarithmic growth was attained. Then, 800 ml quantities of the culture were placed in one-liter bottles in the appropriate filter compartment. Samples were removed for pigment analysis and cell number determinations. Starting with the January 1967 experiments, cultures were grown in one-gallon polyethylene collapsible water bottles.

Determination of Pigments

All pigment analyses were based upon methods described by Strickland and Parsons (1965). After the cells were collected on the Millipore® filters, they were stored in a dessicator at -20° C in the laboratory. Storage never exceeded one week except for the YALOC-66 cruise, in which the storage time was approximately two months.

Prior to August 1966, the filters were placed into calibrated 12 ml. screw-capped centrifuge tubes. Eight ml. of cold 90 percent acetone was added and the tubes were shaken on a mechanical shaker in the dark at 5° C for 18-20 hours. Since August 1966, the filters have been dissolved in three milliliters of cold 90 percent acetone and homogenized in a Serval Omni-Mixer® at 8-9000 r. p. m. for three minutes. In either case, the dissolved filter and acetone were made up to a volume of exactly 10.0 ml. and centrifuged at 3-4000 r. p. m. in a clinical centrifuge in order to remove cell debris and magnesium carbonate.

The optical density of the clear supernatant was measured in a one-centimeter path-length glass cuvette in a Beckmann model DB spectrophotometer against 90 percent acetone at wavelengths of 750, 665, 645, 630, 510, and 480 millimicrons. Pigments were determined quantitatively by use of the original equations of Richards and Thompson (1952). The original equations were used in order to keep

pigment data consistent with that data obtained in previous cruises, and with other workers using these equations. Extinction coefficients are being revised periodically, but the data can be recalculated using the most up-to-date coefficients at any time.

RESULTS

Cruise Results

The purposes of the *in situ* stations at NH-25 were to study the relationship of primary productivity to environmental conditions and to study factors affecting assimilation ratios. Earlier work was done at this station by Curl and Small (1965) who studied variations of photosynthetic assimilation ratios. However, the assimilation ratio method is based upon knowledge of chlorophyll concentration *in situ*, and the purpose of this particular study is to determine the characteristics of diel chlorophyll variations, the influence of light duration and intensity on these variations, and the effect of grazing on diel periodicity. Laboratory studies were also performed in order to study the diel periodicity of chlorophylls *a* and *c* under controlled light conditions in order to explain what controls the diel chlorophyll variations.

Cruise 6507

The first indication of diel pigment periodicity off the Oregon coast occurred during Cruise 6507 (July 20-21, 1965) at NH-25, 25 miles due west of Newport, Oregon, at 44° 40'N, 124° 40'W. One part of this study was to determine if the depth of maximum chlorophyll varied dielly. Table 3 gives the pigment values found. The

Table 3. Chlorophyll Values, Cruise 6507, July 20-21, 1965, Station NH-25.

Date, Time (PST)	Depth, Meters	Pigment Values, mg/m^3 Chlorophyll a	Chlorophyll c
July 20, 1965			
1850	1	1.6	7.0
	10	3.7	4.4
	15	3.0	2.0
	25	2.9	2.4
	50	1.0	0.3
2100	1	2.6	1.0
	10	7.3	1.4
	15	4.9	0.8
	25	4.9	0.8
	50	1.2	0.2
2300	1	3.8	3.9
	10	7.2	6.7
	15	7.5	5.4
	25	3.8	3.2
	50	0.7	0.0
July 21, 1965			
0100	1	4.1	4.2
	10	8.9	6.6
	15	2.2	1.9
	25	3.0	3.6
	50	.6	1.1
0300	1	5.3	9.8
	10	4.4	4.2
	15	2.6	4.1
	25	3.7	3.2
	50	0.7	2.7
0535	1	4.9	6.5
	10	5.2	4.4
	15	3.0	2.4
	25	3.8	3.3
	50	0.5	0.8
0805	1	3.5	4.2
	10	---	---
	15	3.1	3.0
	25	---	---
	50	1.1	3.1

Table 3. Continued.

Date, Time (PST)	Depth, Meters	Pigment Values, mg/m^3	
		Chlorophyll *a*	Chlorophyll *c*
1025	1	4.0	2.8
	10	3.2	2.8
	15	2.7	2.6
	25	2.3	5.5
	50	0.8	3.3
1500	1	2.9	2.8
	10	4.7	5.4
	15	2.9	3.1
	25	2.3	3.6
	50	0.4	0.9
1700	1	1.3	5.2
	10	2.2	2.2
	15	1.4	2.1
	25	0.7	1.8
	50	0.5	1.6

depth of maximum chlorophyll a was 10 meters (Fig. 1). The concentration of chlorophyll a at this depth varied, the lowest values occurring in the early evening, and the highest values in the middle of the night (Fig. 1).

This phenomenon is more clearly seen when chlorophyll a at each depth is plotted against time (Fig. 2). A diel periodicity of chlorophyll a occurred at all depths, with highest values at night and lowest values in the early afternoon. With decreasing depth, the chlorophyll a peak came earlier in the evening. At the surface, the maximum came at 0300 hours, at 10 meters, 0100 hours, at 15 meters, 2300 hours, and at 25 meters, 2100 hours. At 50 meters, a maximum was noted at 2100 and 0800. The greatest variation was at the surface and at 15 meters, with ratios of maximum to minimum values of chlorophyll a of 3.3 and 3.4, respectively. The minimum ratio was at 25 meters (2.1), and 10 and 50 meter ratios were intermediate, with ratios of 2.8 and 3.0, respectively.

A diel variation of chlorophyll c also occurred, with the surface maximum at 0300, the 10 and 15 meter peaks at 2300, and the 25 and 50 meter maxima at 1025 (Fig. 3). Low values occurred in the late afternoon to early evening. Maximum variation took place at the surface with a ratio of maximum to minimum chlorophyll c of 9.8, while the ratio was 4.8 at 10 meters, 6.7 at 15 meters, 6.9 at 25 meters, and 14.0 at 50 meters (if the 0.0 value of chlorophyll c which occurred

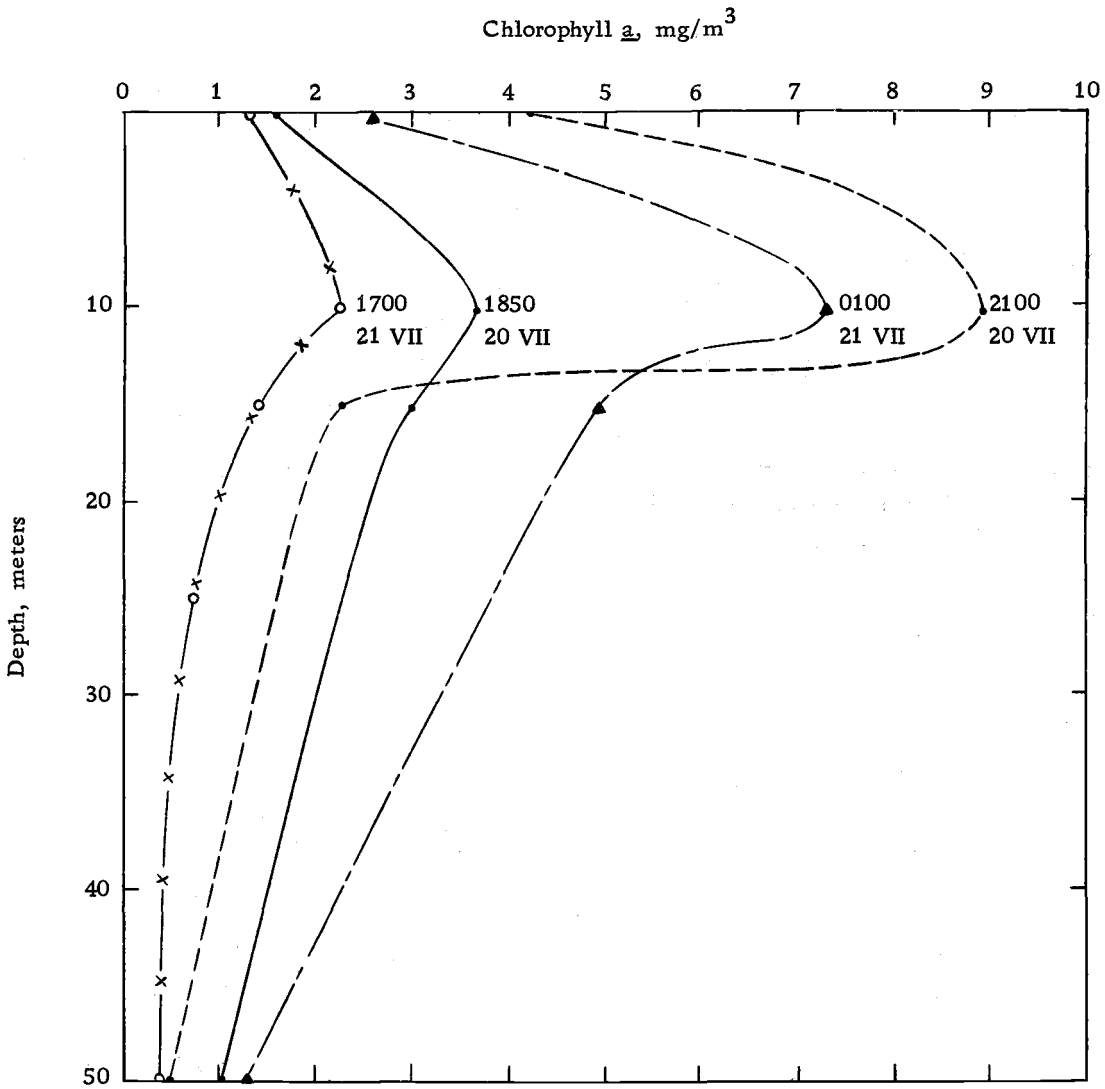


Figure 1. Chlorophyll a vs. depth, Cruise 6507, Station NH-25, 20-21 July 1965.

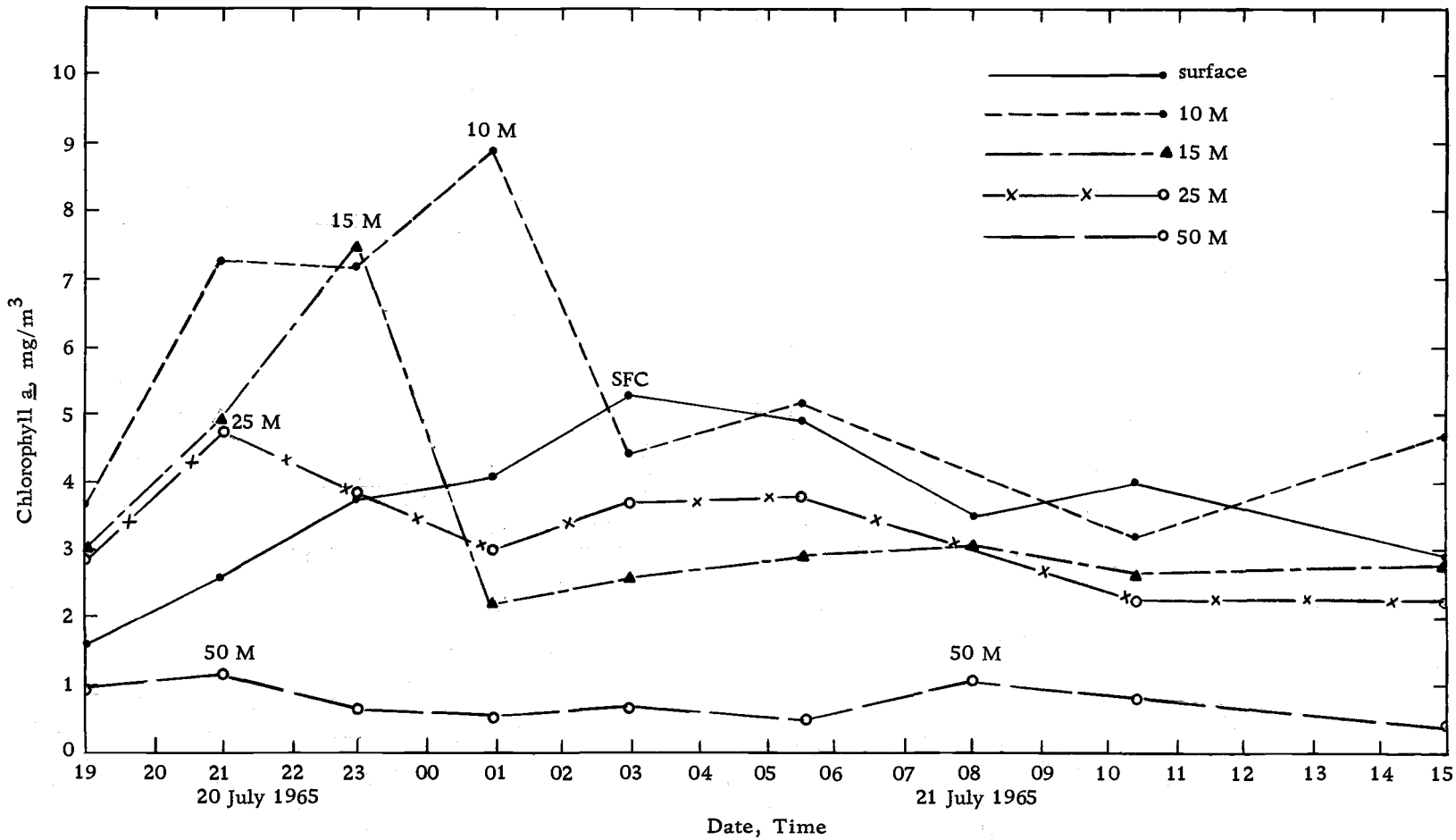


Figure 2. Chlorophyll a, Cruise 6507, Station NH-25, 20-21 July 1965.

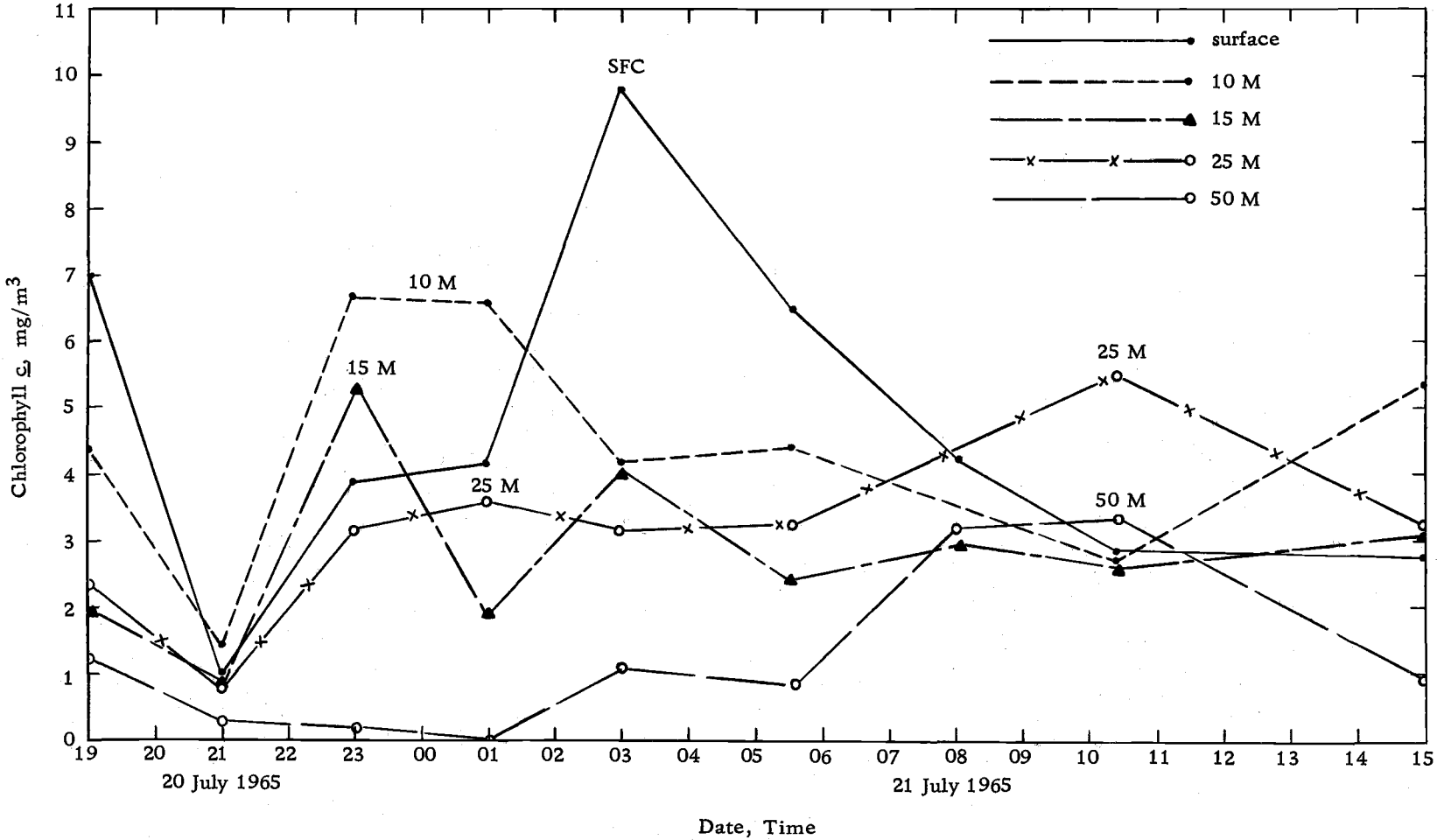


Figure 3. Chlorophyll c, Cruise 6507, Station NH-25, 20-21 July 1965.

at 2300 is ignored). Thus, chlorophyll a and c both show a diel periodicity, although they are not in phase.

Cruise 6509

During cruise 6509, two stations were occupied, Station SB, 16.2 miles due west of Yachats, Oregon, at 44° 21'N, 124° 29'W, and station NH-55, 55 miles due west of Newport, Oregon, at latitude 44° 39'N.

Station SB was occupied from 0300 on September 25 to 2030 on September 26, 1955. On September 25th, the weather was foggy until around noon with solar radiation averaging about 0.05 langleys per minute at 0900, and 0.4 at 1000. At noon the fog lifted and clear skies were present until 1800 when overcast set in. September 26 was clear all day with maximum solar radiation averaging about 1.5 langleys per minute. Table 4 gives the values of chlorophyll a and c determined on this station. A diel periodicity of chlorophyll a was again present, but the data were more variable than on the previous cruise. At the surface maximum chlorophyll a occurred at 0100 on the 26th decreasing to a minimum at 1400 on the 26th, then increasing again. The ratio between the minimum and maximum value was 3.5. At 10 meters, no clear maximum was seen, but lowest values occurred around noon on the 26th. The maximum at 15 meters was at 2300 on the 25th, with the minimum at 1115 on the 26th; the ratio

Table 4. Chlorophyll Values, Cruise 6509, September 25-26, 1965, Station SB.

Date, Time (PST)	Depth, Meters	Pigment Values, mg/m^3 Chlorophyll a	Chlorophyll c
September 25, 1965			
0300	1	13.4	9.0
	10	17.3	9.6
	15	25.5	13.2
	25	28.3	6.1
	50	0.6	0.7
0610	1	16.3	10.0
	10	18.0	9.2
	15	19.1	12.4
	25	23.5	13.4
	50	1.8	1.7
0825	1	16.3	9.7
	10	17.6	11.2
	15	19.9	9.5
	25	20.7	11.0
	50	0.9	1.9
1110	1	14.4	7.8
	10	19.5	13.0
	15	24.7	11.0
	25	9.9	5.5
	50	0.7	0.0
1415	1	9.0	4.9
	10	19.2	12.0
	15	23.8	12.5
	25	12.0	4.4
	50	1.1	0.3
1800	1	9.7	5.9
	10	18.3	10.2
	15	24.0	16.5
	25	23.3	13.7
	50	0.9	0.9
2000	1	8.8	.67
	10	19.0	11.3
	15	23.1	13.1
	25	31.2	17.4
	50	1.2	0.1

Table 4. Continued.

Date, Time (PST)	Depth, Meters	Pigment Values, mg/m^3	
		Chlorophyll a	Chlorophyll c
2300	1	10.8	7.0
	10	17.3	12.7
	15	30.9	37.6
	25	13.0	6.1
	50	1.0	0.6
September 26, 1965			
0100	1	12.2	7.3
	10	13.4	6.0
	15	21.2	0.0
	25	25.7	11.9
	50	1.3	0.0
0305	1	10.5	6.1
	10	13.5	11.3
	15	18.2	0.0
	25	20.8	13.5
	50	1.7	1.4
0600	1	7.2	3.9
	10	9.1	4.2
	15	18.0	13.8
	25	14.3	8.1
	50	1.7	1.1
0830	1	6.6	6.8
	10	12.3	8.5
	15	24.1	14.1
	25	9.8	4.0
	50	0.6	0.7
1115	1	5.4	1.4
	10	6.9	5.3
	15	10.5	6.3
	25	22.0	33.7
	50	1.5	2.0
1410	1	3.5	2.0
	10	10.6	6.8
	15	12.6	7.1
	25	9.1	4.5
	50	1.0	0.7

Table 4. Continued.

Date, Time (PST)	Depth, Meters	Pigment Values, mg/m^3	
		Chlorophyll a	Chlorophyll c
1800	1	----	----
	10	21.3	18.2
	15	14.9	9.0
	25	1.9	3.8
	50	0.7	0.9
2020	1	16.3	9.3
	10	18.4	11.2
	15	16.3	10.1
	25	2.9	2.4
	50	1.0	1.2

between these two values was 2. 9. At 25 meters, several peaks were seen, so a diel periodicity was hard to ascertain. At 50 meters, chlorophyll a values were fairly constant (Fig. 4).

A diel periodicity of chlorophyll c is apparent from the data (Fig. 5). At the surface, maximum chlorophyll c was at 0100 on the 26th, while minimum values were seen at 1115 on the 26th, the ratio of these two being 5. 2. At 10 meters, the maximum chlorophyll c value was at 2300 on the 25th, decreasing to a minimum at 0600 on the 26th, the ratio between these two being 3. 0. At 15 meters, a large periodicity was seen, with 37. 6 mg/m^3 the maximum value at 2300 on the 25th and a minimum value on the 26th at 0100 and 0305 of 0. 0. At 25 meters, the chlorophyll c maximum occurred at 1115 on the morning of the 25th, and the minimum at 1400, the ratio being 7. 5. No periodicity was apparent at 50 meters.

Thus, at station SB a periodicity of both chlorophyll a and c occurred, although they were again not in phase. At 25 meters and 50 meters no periodicity was seen, but maximum values occurred in the middle of the night.

At Station NH-55, chlorophyll samples were taken between 1115 on September 28th, and 0715 on September 30, 1965 (Table 5). The weather on the 28th was overcast all day, with maximum solar radiation averaging 0. 3-0. 4 langleys per minute. On the 29th, the weather was again overcast while on station.

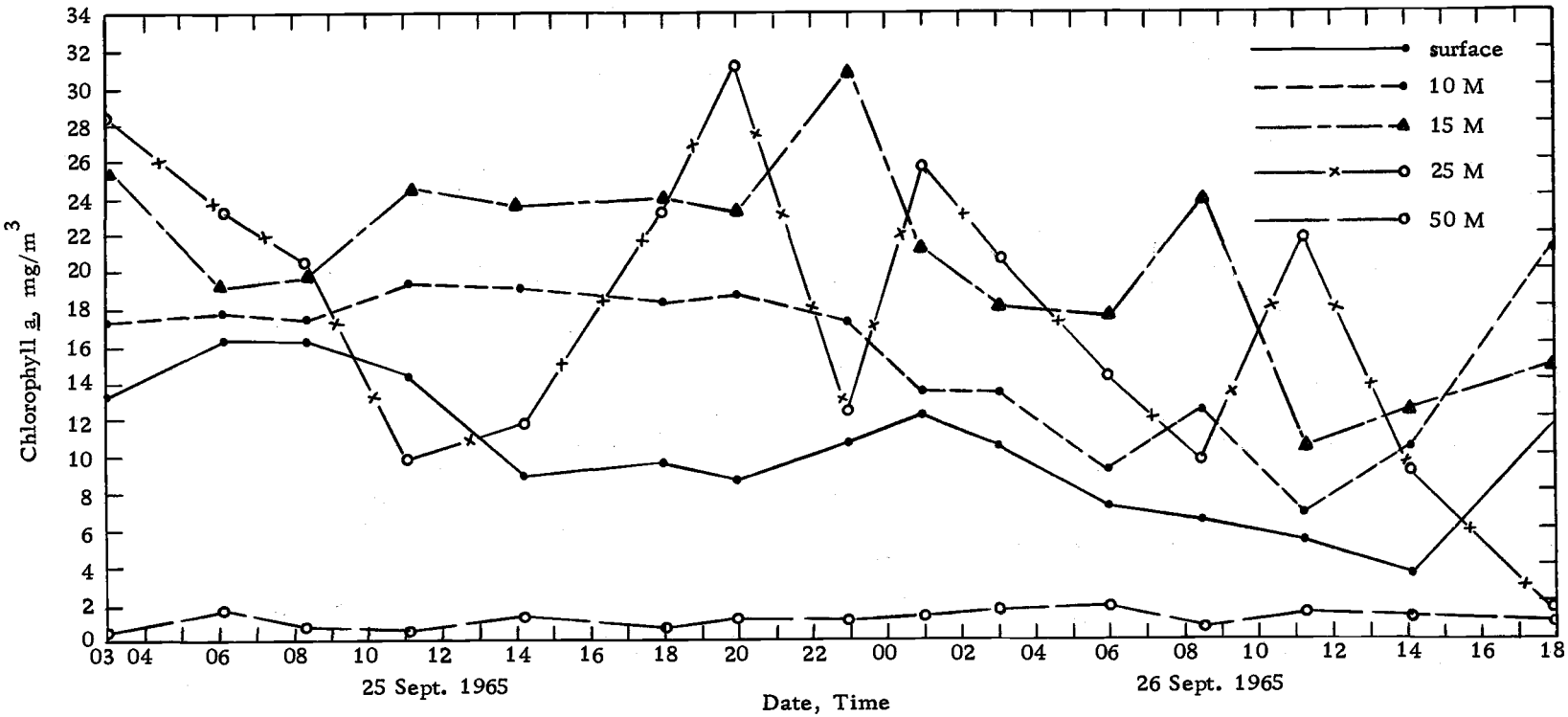


Figure 4. Chlorophyll a, Cruise 6509, Station SB, 25-26 Sept. 1965.

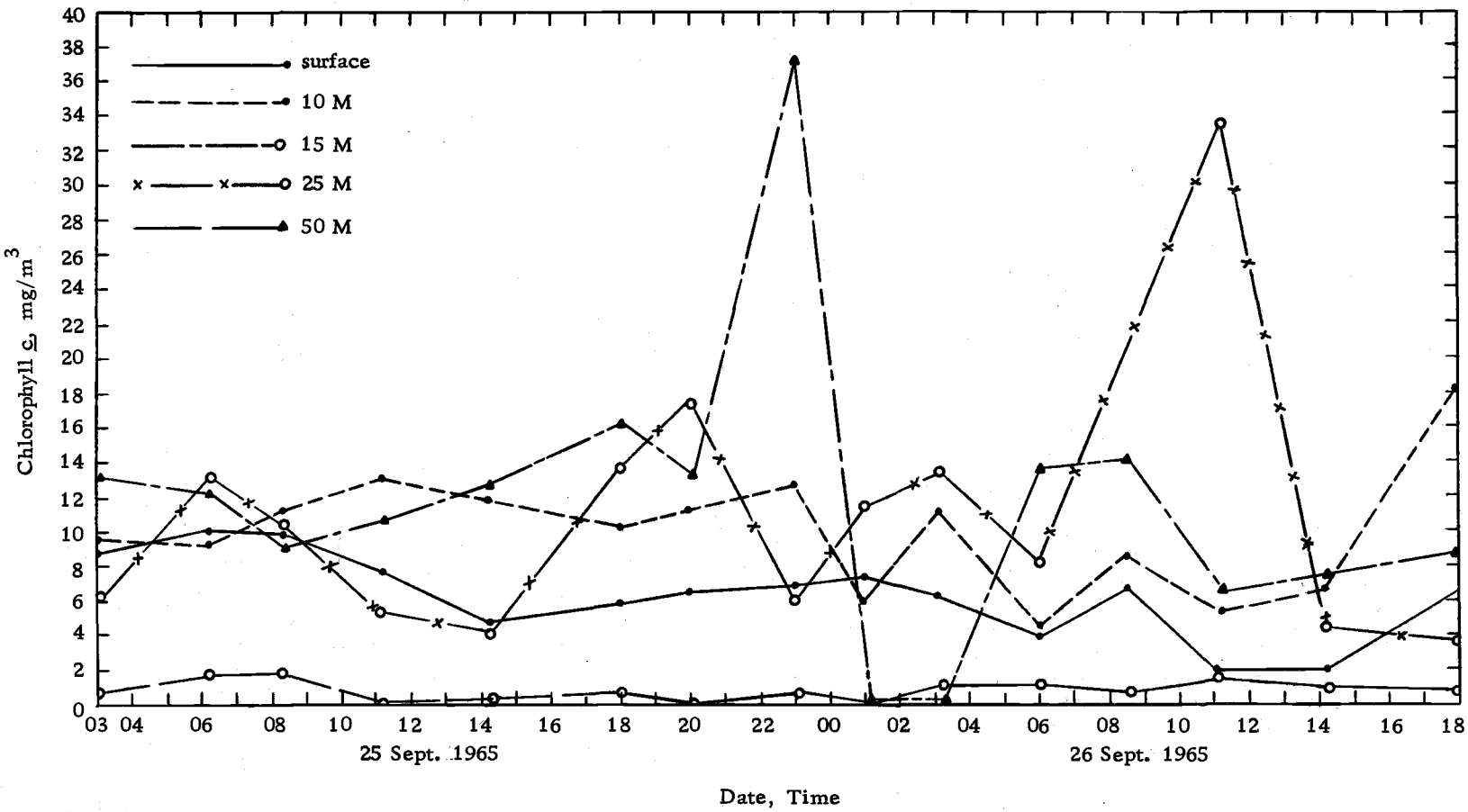


Figure 5. Chlorophyll c, Cruise 6509, Station SB, 25-26 Sept. 1965.

Table 5. Chlorophyll Values, Cruise 6509, September 28-30, 1965, Station NH-55.

Date, Time (PST)	Depth, Meters	Pigment Values, mg/m^3 Chlorophyll a	Chlorophyll c
September 28, 1965			
1115	1	1.0	0.6
	10	1.4	0.2
	15	1.9	2.9
	25	1.2	0.9
	50	0.4	0.8
1403	1	1.3	1.3
	10	1.0	0.0
	15	2.1	1.2
	25	1.1	1.0
	50	1.0	2.0
1625	1	1.3	0.2
	10	1.7	0.5
	15	1.4	0.6
	25	0.8	0.9
	50	1.0	1.1
1905	1	1.3	0.0
	10	1.3	1.6
	15	1.9	2.2
	25	0.8	0.5
	50	0.8	0.0
2125	1	1.2	1.5
	10	1.3	1.4
	15	2.1	1.9
	25	1.0	3.6
	50	1.0	4.1
2335	1	9.7	5.9
	10	1.9	5.5
	15	1.7	4.5
	25	2.0	3.2
	50	0.1	0.0
September 29, 1965			
0240	1	1.5	3.6
	10	2.0	3.8
	15	2.6	4.1
	25	1.2	3.3
	50	0.8	1.2

Table 5. Continued.

Date, Time (PST)	Depth, Meters	Pigment Values, mg/m^3 Chlorophyll a	Chlorophyll c
0445	1	1.9	3.9
	10	1.4	1.2
	15	2.4	0.4
	25	1.0	1.1
	50	0.5	0.8
0815	1	1.4	3.1
	10	1.6	0.0
	15	2.1	1.1
	25	1.1	0.7
	50	0.4	0.0
1218	1	0.8	1.3
	10	1.4	2.1
	15	---	---
	25	1.1	1.9
	50	0.5	1.4
1510	1	0.8	0.8
	10	2.0	0.9
	15	1.7	1.5
	25	0.9	0.3
	50	0.4	0.0
2050	1	1.1	2.2
	10	1.8	1.0
	15	1.2	1.5
	25	---	---
	50	0.4	0.0
2305	1	---	---
	10	1.0	1.1
	15	1.4	2.6
	25	1.4	2.0
	50	0.7	0.3
September 30, 1965			
0115	1	0.9	1.3
	10	2.1	1.5
	15	1.9	1.8
	25	1.0	1.6
	50	0.5	0.4

Table 5. Continued.

Date, Time (PST)	Depth, Meters	Pigment Values, mg/m^3	
		Chlorophyll a	Chlorophyll c
0315	1	1.1	1.7
	10	2.2	2.4
	15	1.5	2.7
	25	0.8	0.0
	50	0.7	1.1
0520	1	1.6	1.3
	10	1.4	1.7
	15	1.0	1.3
	25	1.3	1.4
	50	0.2	0.2
0715	1	1.0	1.0
	10	1.5	0.4
	15	1.4	3.0
	25	0.9	0.8
	50	0.2	0.2

At the surface, the maximum value occurred at 2335 on the 28th (Fig. 6). This value may be in error as it appears to be much greater than any of the other values which were determined. At 10 meters, the chlorophyll a maximum occurred at 1240 on the 29th, and the minimum at 1403 on the 28th, the ratio between these two being 2.0. At 15 meters, the maximum also occurred at 0240 on the 29th, with the minimum at 2050 on the same day, the ratio being 2.2. At 25 meters, a chlorophyll maximum occurred at 2335 on the 28th, with a minimum at 1625 and 1905 on the same day, the ratio being 2.5. At 50 meters, the chlorophyll a maximum occurred at 1403 and 1625, and the minimum at 2335 on the 28th, the ratio being 10.0.

A diel pigment periodicity of chlorophyll c was seen. At the surface, 10, and 15 meters, the maximum value occurred at 2335 on the 28th, and at 25 and 50 meters, the maximum occurred at 2125 on the same day. The minimum values of chlorophyll c at these respective depths were at 1905 on the 28th, at the surface - 1403, at 10 meters - 0445 on the 29th at 15 meters, 0315 on the 30th at 25 meters, and at 2335 on the 28th at a depth at 50 meters. Ratios are not reported for these depths as zero values of chlorophyll c occurred at the surface, 10, 25, and 50 meters (Fig. 7).

Thus at NH-55 at this time of the year, a diel periodicity of chlorophyll a and c was noticed, with maximum values occurring in the middle of the night and minimum values occurring in the later

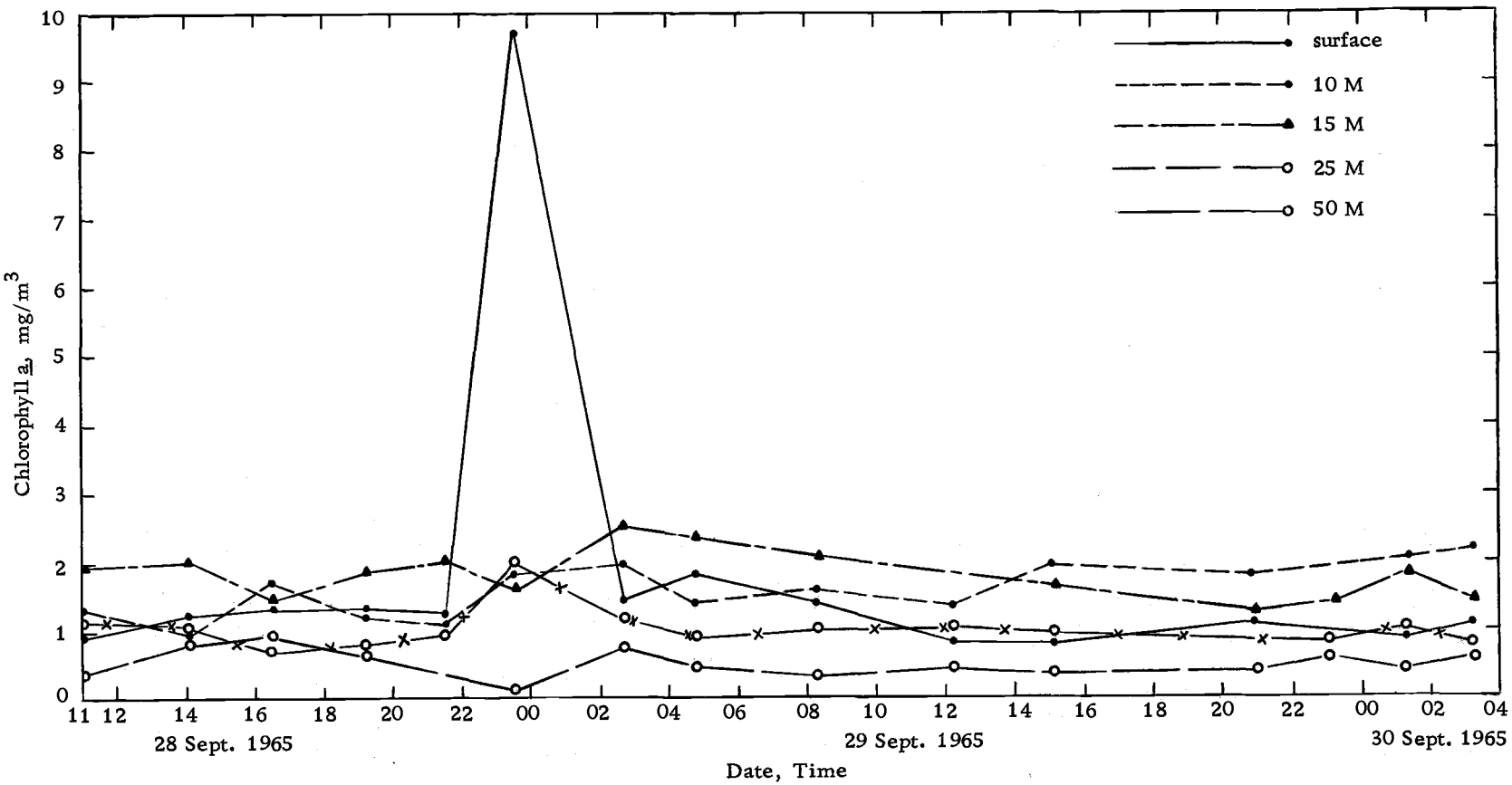


Figure 6. Chlorophyll a, Cruise 6509, Station NH-55, 28-30 Sept. 1965.

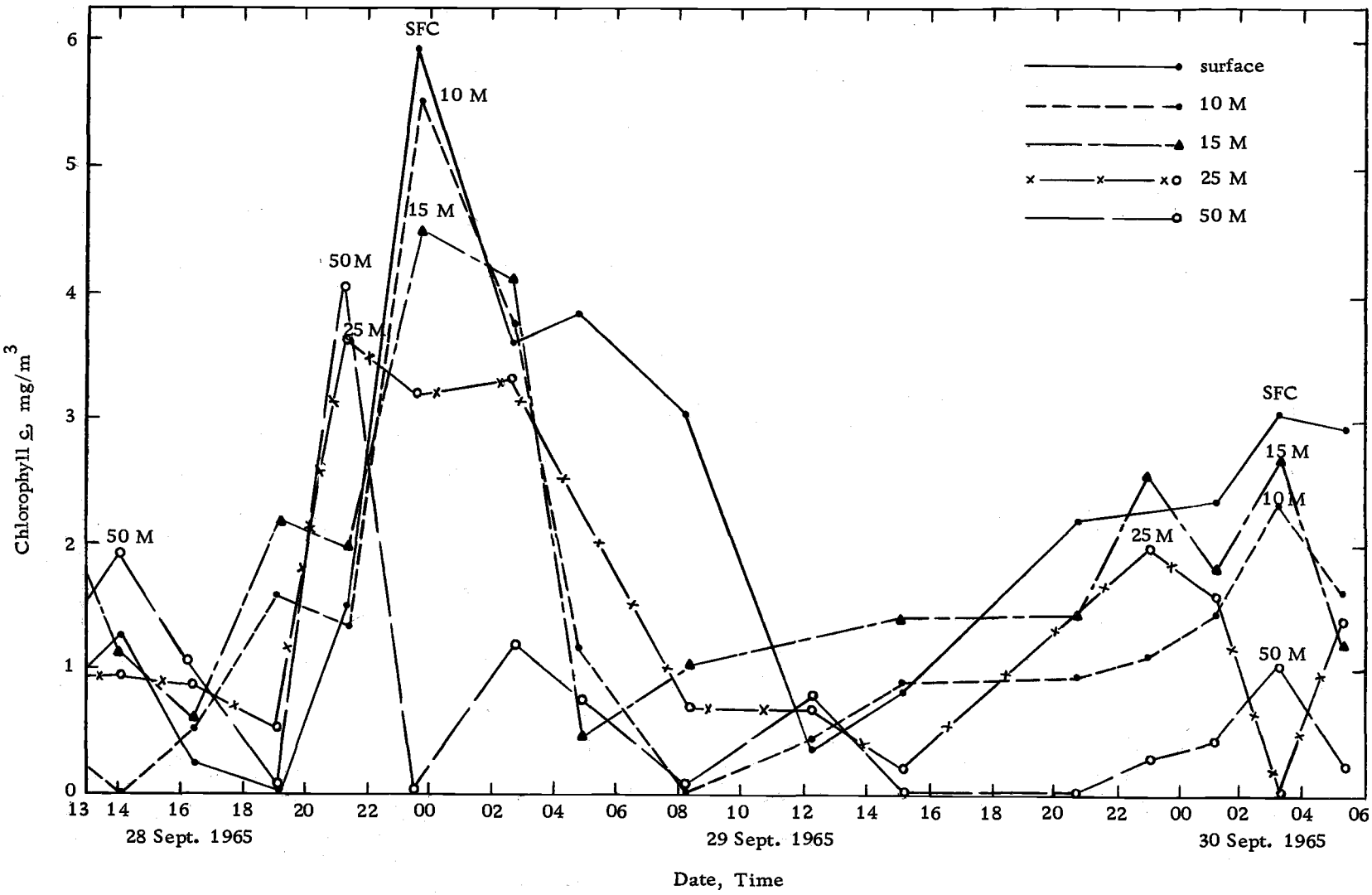


Figure 7. Chlorophyll c, Cruise 6509, Station NH-55, 28-30 Sept. 1965.

afternoon, except for chlorophyll a at 50 meters, the opposite occurred.

Cruise 6511

Cruise 6511 occupied Station NH-25 from 1715 on November 28, to 1115 on November 30, 1965. The weather on station was poor with continually overcast skies and rough seas. Table 6 presents the chlorophyll obtained. No diel periodicity was seen either for chlorophyll a and c (Figs. 8 and 9). Maxima occurred both during the day and night for these two pigments.

Cruise 6602

Cruise 6602 occupied Station NH-25 from 1900 on February 19 to 1300 on February 21, 1966. The weather was overcast on the 19th and 20th, but became sunnier on the 21st. No pyrheliometer data was available for this cruise. Seas were fairly rough while on station.

There was variation in chlorophyll a values at each depth, but a clear diel pattern was not present (Fig. 10, Table 7). For example, at the surface, maxima occur at 2300 on the 19th, 1145 on the 20th, and 0515 on the 21st. At 25 meters, four maxima were present while on station, as at 50 meters.

An extreme variability of chlorophyll c occurred. For example,

Table 6. Chlorophyll Values, Cruise 6511, November 28-30, 1965, Station NH-25.

Date, Time (PST)	Depth, Meters	Pigment Values, mg/m^3 Chlorophyll *a*	Chlorophyll *c*
November 28, 1965			
1715	1	0.7	1.4
	10	0.6	0.9
	15	0.6	1.4
	25	0.8	1.8
	50	0.8	2.5
1930	1	0.6	1.4
	10	0.9	1.1
	15	0.7	1.8
	25	0.6	1.5
	50	0.7	1.3
2130	1	0.6	1.5
	10	0.3	1.3
	15	0.7	1.0
	25	0.7	1.5
	50	0.6	1.0
2330	1	0.8	2.5
	10	0.6	1.1
	15	0.6	1.5
	25	0.5	0.2
	50	0.7	1.1
November 29, 1965			
0130	1	0.7	1.9
	10	0.8	1.8
	15	0.6	1.9
	25	0.8	1.8
	50	0.7	1.1
0330	1	0.7	1.1
	10	0.9	2.0
	15	0.9	2.4
	25	0.6	2.3
	50	0.5	0.8
0700	1	0.9	1.1
	10	0.7	1.4
	15	0.7	1.1
	25	0.6	1.8
	50	0.7	1.3

Table 6. Continued.

Date, Time (PST)	Depth, Meters	Pigment Values, mg/m^3	
		Chlorophyll a	Chlorophyll c
0915	1	0.8	1.0
	10	0.7	1.1
	15	0.8	1.2
	25	0.8	2.1
	50	0.7	1.3
1135	1	0.6	1.5
	10	0.8	1.8
	15	1.1	0.6
	25	0.9	3.2
	50	0.9	4.9
1415	1	1.0	5.8
	10	0.9	4.1
	15	0.9	3.8
	25	0.5	3.8
	50	0.8	2.2
1645	1	0.8	5.9
	10	0.9	4.9
	15	0.8	3.7
	25	0.8	1.8
	50	0.8	5.2
1845	1	0.8	2.2
	10	1.0	4.4
	15	1.2	5.5
	25	0.7	3.5
	50	0.8	3.8
2050	1	1.0	5.6
	10	1.4	7.9
	15	1.0	4.9
	25	0.6	1.5
	50	0.8	3.3
2250	1	1.1	5.0
	10	1.2	5.1
	15	1.1	4.4
	25	1.2	4.7
	50	0.9	3.2

Table 6. Continued.

Date, Time (PST)	Depth, Meters	Pigment Values, mg/m^3 Chlorophyll a	Chlorophyll c
November 30, 1965			
0100	1	1.1	6.1
	10	1.1	5.2
	15	1.1	4.2
	25	0.9	4.2
	50	1.1	5.9
0315	1	1.0	3.7
	10	0.9	5.5
	15	1.1	5.1
	25	1.2	5.8
	50	0.9	2.8
0515	1	1.0	7.8
	10	0.9	5.5
	15	1.1	5.1
	25	1.2	5.8
	50	0.9	2.8
0515	1	1.0	7.8
	10	1.1	5.2
	15	0.9	4.3
	25	1.3	6.3
	50	1.0	4.2
0645	1	1.3	7.2
	10	0.9	3.8
	15	0.9	5.5
	25	0.8	3.8
	50	0.9	4.7
0910	1	1.1	4.8
	10	1.1	3.9
	15	0.9	4.5
	25	0.7	2.6
	50	1.0	3.7
1115	1	1.2	7.1
	10	0.9	3.6
	15	1.0	4.6
	25	1.3	7.1
	50	0.9	4.5

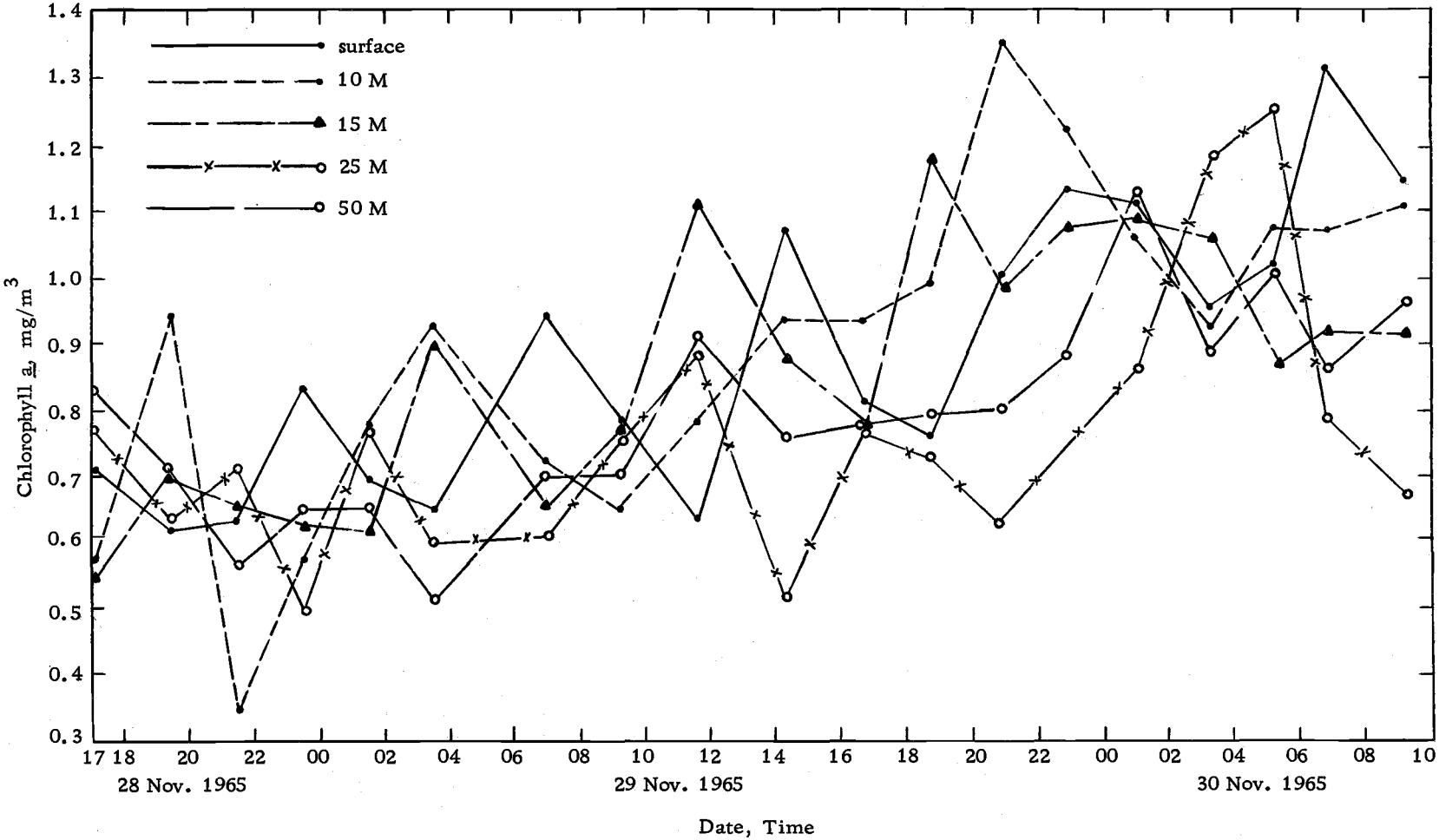


Figure 8. Chlorophyll a, Cruise 6511, Station NH-25, 28-30 Nov. 1965.

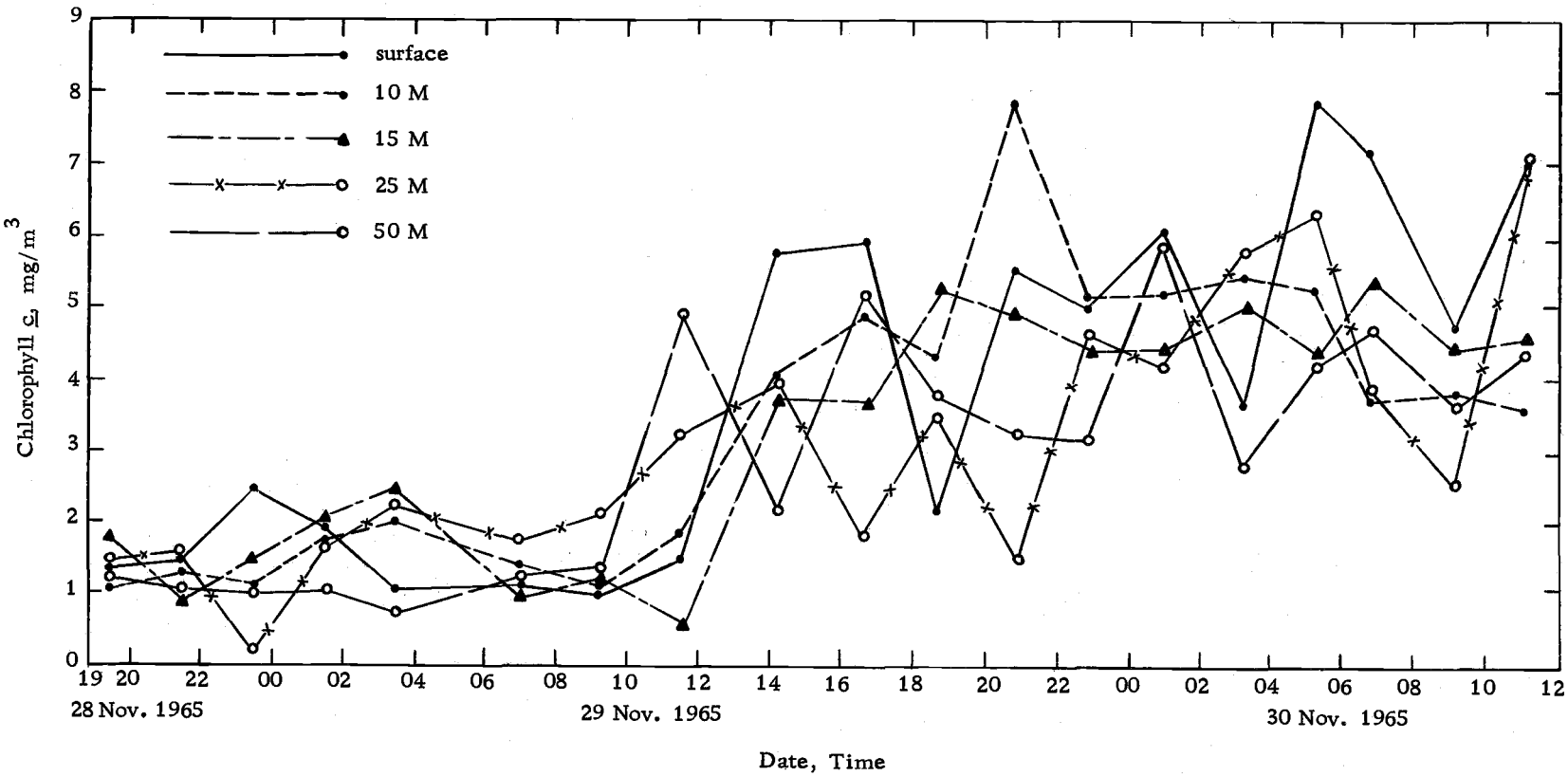


Figure 9. Chlorophyll c, Cruise 6511, Station NH-25, 28-30 Nov. 1965.

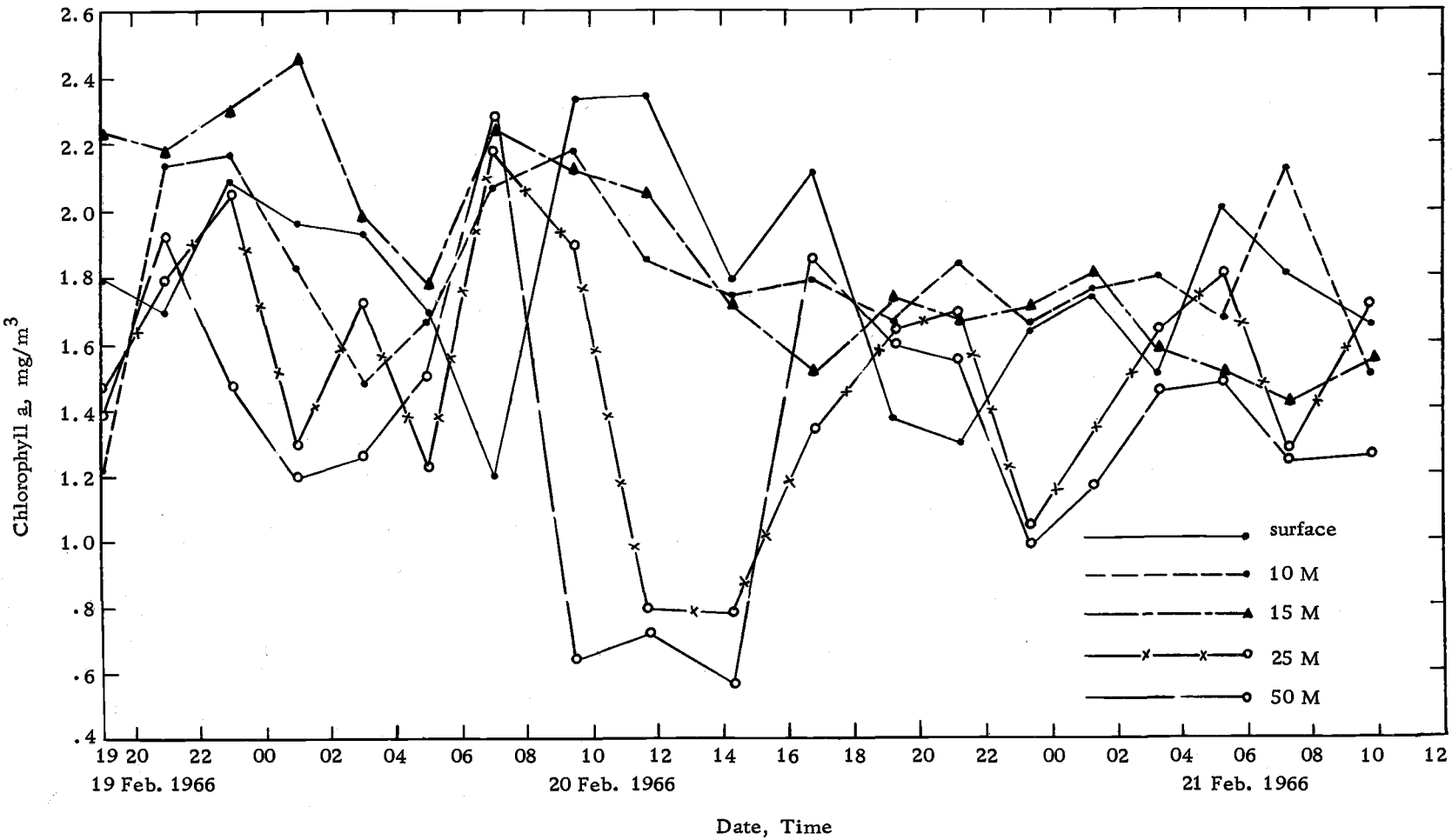


Figure 10. Chlorophyll a, Cruise 6602, Station NH-25, 19-21 Feb. 1966.

Table 7. Chlorophyll Values, Cruise 6602, February 19-21, 1966, Station NH-25.

Date, Time (PST)	Depth, Meters	Pigment Values, mg/m^3 Chlorophyll a	Chlorophyll c
February 19, 1966			
1900	1	1.8	2.3
	10	1.2	2.2
	15	2.2	3.1
	25	1.5	2.6
	50	1.4	2.9
2100	1	1.7	2.8
	10	2.1	3.1
	15	2.2	6.0
	25	1.8	2.7
	50	2.0	2.3
2300	1	2.1	3.6
	10	2.2	2.3
	15	2.3	2.1
	25	2.1	4.0
	50	1.5	3.5
February 20, 1966			
0100	1	2.0	1.6
	10	1.8	5.1
	15	2.5	5.9
	25	1.3	4.9
	50	1.2	2.5
0300	1	1.9	3.8
	10	1.5	3.7
	15	2.0	3.2
	25	1.7	6.1
	50	1.3	2.8
0500	1	1.7	3.2
	10	1.7	4.1
	15	1.8	3.7
	25	1.2	1.1
	50	1.5	2.7
0700	1	1.2	2.6
	10	2.1	2.7
	15	2.2	2.7
	25	2.2	3.1
	50	2.3	2.2

Table 7. Continued.

Date, Time (PST)	Depth, Meters	Pigment Values, mg/m^3	
		Chlorophyll a	Chlorophyll c
0930	1	2.3	1.8
	10	2.2	2.0
	15	2.1	2.8
	25	1.9	1.2
	50	0.6	1.6
1145	1	2.3	1.7
	10	1.9	3.6
	15	2.1	1.7
	25	0.8	1.3
	50	0.7	1.4
1415	1	1.8	2.7
	10	1.7	1.7
	15	1.7	3.2
	25	0.8	1.2
	50	0.6	1.4
1645	1	2.1	4.6
	10	1.8	2.7
	15	1.5	2.2
	25	1.3	4.0
	50	1.9	3.6
1915	1	1.4	1.5
	10	1.7	2.0
	15	1.7	4.5
	25	1.7	1.5
	50	1.6	1.5
2115	1	1.5	3.0
	10	1.8	4.0
	15	1.7	2.0
	25	1.8	5.0
	50	1.5	5.5
2315	1	1.6	3.4
	10	1.6	3.5
	15	1.7	5.7
	25	1.1	2.0
	50	1.0	2.3

Table 7. Continued.

Date, Time (PST)	Depth, Meters	Pigment Values, mg/m^3 Chlorophyll a	Chlorophyll c
February 21, 1966			
0115	1	1.7	4.5
	10	1.8	3.0
	15	1.8	3.5
	25	1.7	6.1
	50	1.2	1.7
0315	1	1.5	1.8
	10	1.8	5.2
	15	1.6	2.1
	25	1.6	1.8
	50	1.5	1.7
0515	1	2.0	3.3
	10	1.7	1.5
	15	1.5	2.2
	25	1.8	2.2
	50	1.5	2.7
0710	1	1.8	2.3
	10	2.1	1.5
	15	1.4	3.2
	25	1.4	1.6
	50	1.2	3.8
0940	1	1.7	4.5
	10	1.5	3.3
	15	1.5	0.8
	25	1.7	2.4
	50	1.3	2.5
1300	1	1.9	3.8
	10	1.6	2.7
	15	1.5	2.7
	25	1.5	1.6
	50	1.3	2.4

at 25 meters, six maxima occurred while on station, while at the surface, five maxima occurred (Fig. 11).

A simple diel periodicity of either chlorophyll a or c did not occur during this particular cruise.

Cruise YALOC-66, Fixed Station "A"

Fixed Station "A" was occupied between 2320 on June 8 and 0345 on June 10, 1966, and was located at 29° 12'N, 161° 30'W. Continuous pyrheliometer data was collected and is presented along with chlorophyll a data (Fig. 13). Table 8 gives chlorophyll a and c data.

A definite diel periodicity of chlorophyll a occurred. On the night of the eighth and ninth, maximum chlorophyll a occurred at the surface at 0800, at 10 meters the maximum occurred at 0420, at 15 meters - 1015, at 25 meters - 0800, and at 50 meters - 0420. During the second night, that of the ninth, all maxima occurred simultaneously at 2235. Of interest are the maxima that occurred at all depths between 1000 and 1600 (Fig. 12). At approximately 1200, a cloud cover came in and incoming radiation dropped from about 1.8 to 0.3 langleys per minute (Fig. 13). At the same time chlorophyll a increased at all depths by as high a factor as 6.0 times (at the surface). When the cloud cover quickly disappeared at approximately 1500, the chlorophyll concentration started to decrease at all

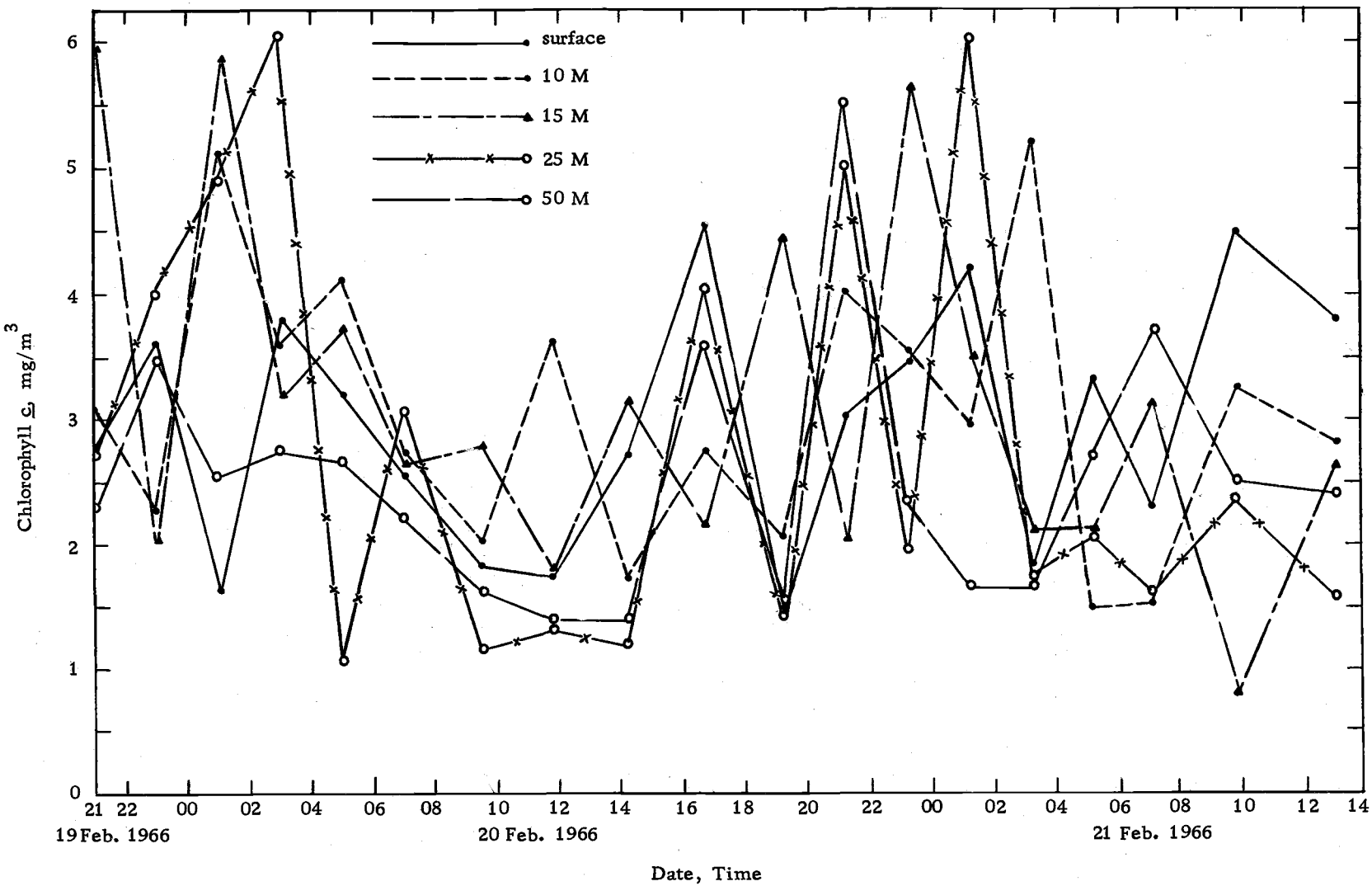


Figure 11. Chlorophyll c, Cruise 6602, Station NH-25, 19-21 Feb. 1966.

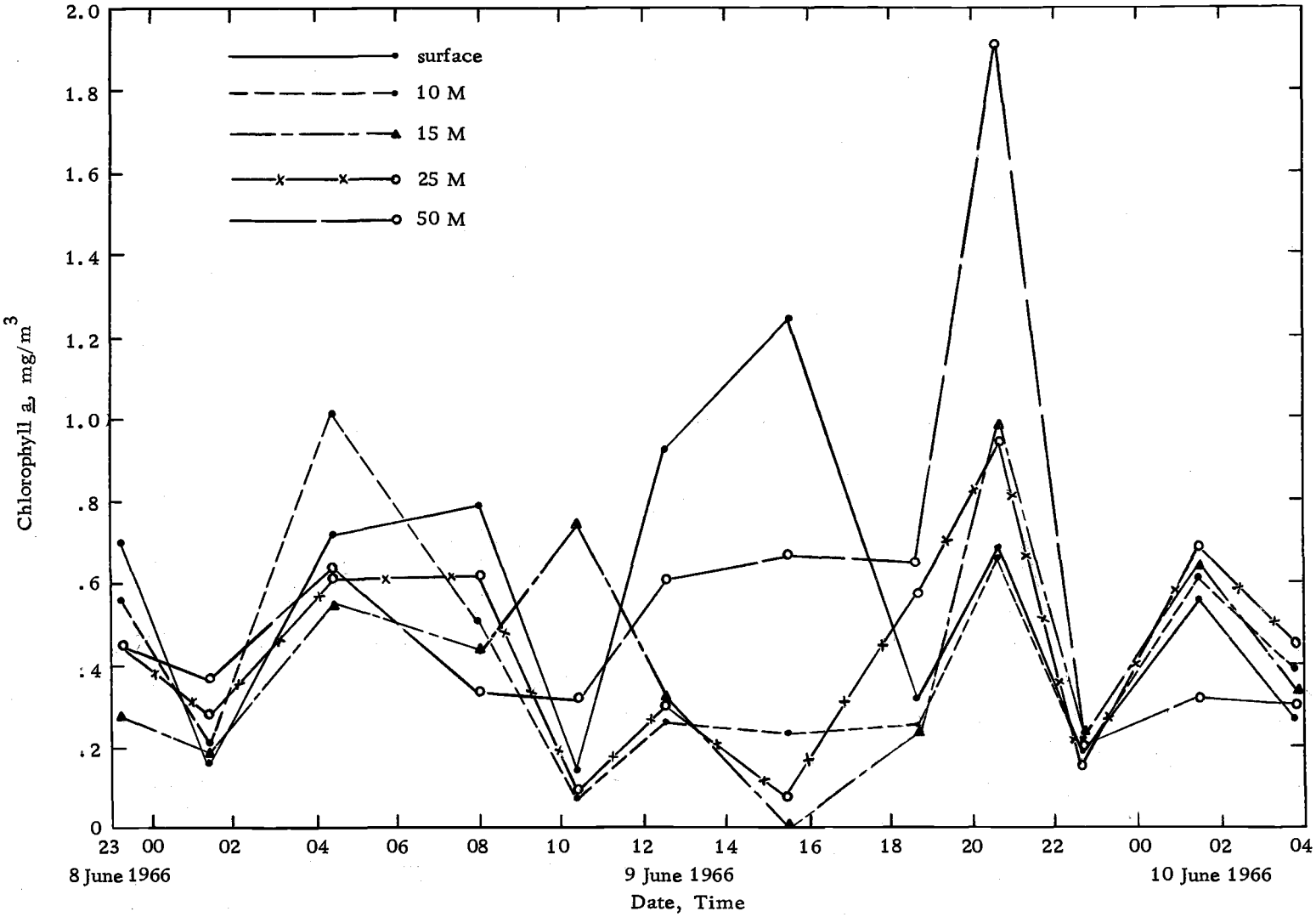


Figure 12. Chlorophyll a, Cruise YALOC-66, Fixed Station "A" (29° 12'N, 161° 30'W), 8-10 June 1966.

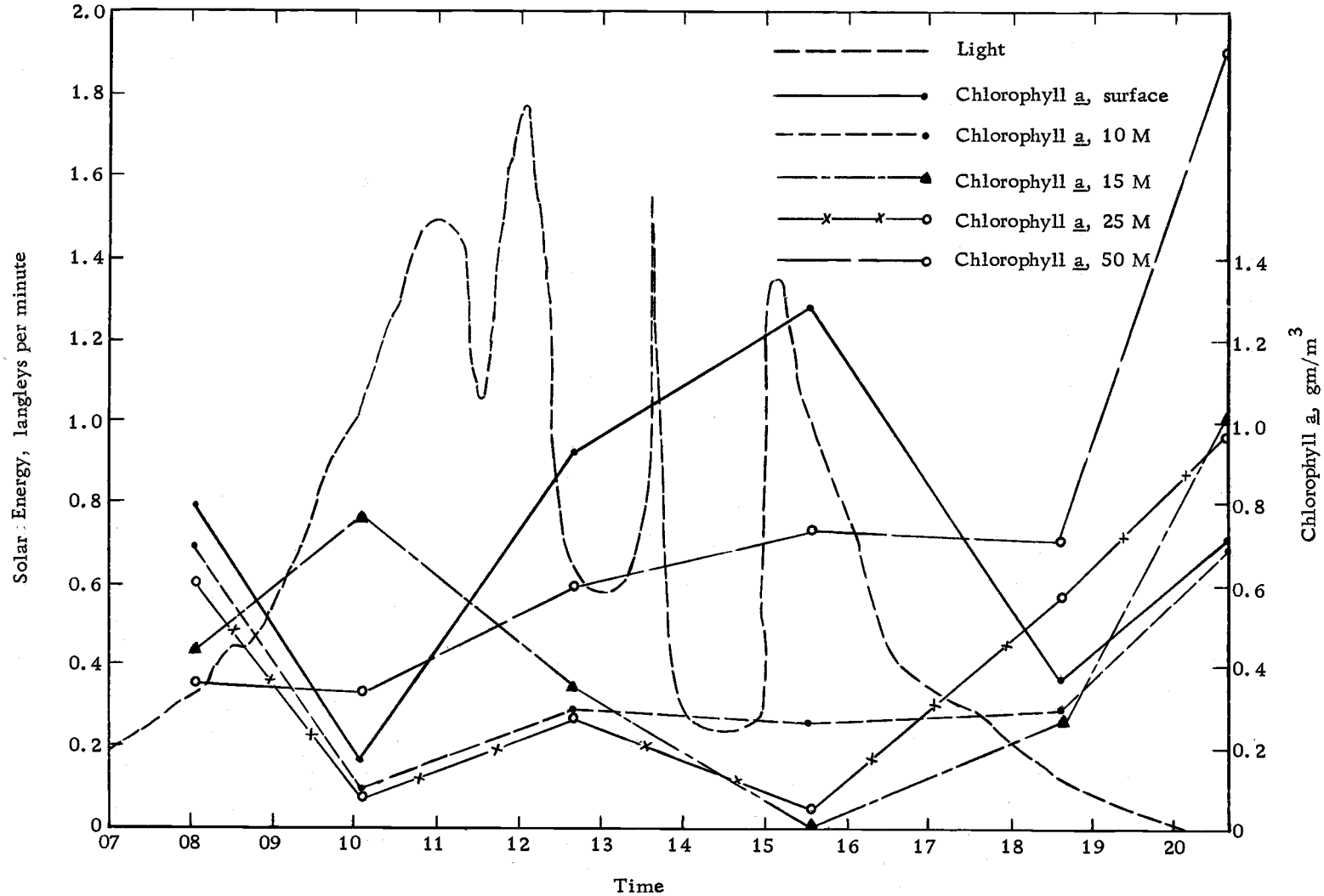


Figure 13. Chlorophyll a vs. incoming solar radiation, Cruise YALOC-66, Fixed Station "A" (29° 12'N, 161° 30'W), 9 June 1966.

Table 8. Chlorophyll Values, Cruise YALOC-66, June 8-10, 1966, Fixed Station "A", 29° 12'N, 161° 30'W.

Date, Time (HST)	Depth, Meters	Pigment Values, mg/m^3 Chlorophyll *a*	Chlorophyll *c*
June 8, 1966			
2320	1	0.7	3.7
	10	0.6	3.5
	15	0.3	0.6
	25	0.5	2.4
	50	0.6	3.1
June 9, 1966			
0120	1	0.2	0.0
	10	0.2	0.6
	15	0.2	1.0
	25	0.3	0.6
	50	0.4	0.4
0420	1	0.7	5.8
	10	1.0	10.1
	15	0.6	3.5
	25	0.6	4.7
	50	0.6	4.9
0800	1	0.8	6.7
	10	0.5	3.1
	15	0.4	2.8
	25	0.6	4.9
	50	0.3	1.3
1015	1	0.2	0.0
	10	0.1	0.0
	15	0.8	5.6
	25	0.1	0.0
	50	0.3	1.7
1230	1	0.9	7.0
	10	0.3	1.4
	15	0.3	1.1
	25	0.3	1.7
	50	0.6	5.3
1530	1	1.2	4.2
	10	0.2	1.8
	15	0.0	0.0
	25	0.1	0.0
	50	0.7	5.7

Table 8. Continued.

Date, Time (HST)	Depth, Meters	Pigment Values, mg/m^3	
		Chlorophyll a	Chlorophyll c
1835	1	0.3	1.7
	10	0.3	1.4
	15	0.3	1.3
	25	0.6	3.1
	50	0.7	3.5
2035	1	0.7	5.2
	10	0.7	5.7
	15	1.0	7.7
	25	1.0	9.9
	50	1.9	10.5
2235	1	0.2	0.5
	10	0.2	0.7
	15	0.2	0.7
	25	0.2	0.0
	50	0.2	1.0
June 10, 1966			
0130	1	0.6	3.5
	10	0.6	4.3
	15	0.6	4.5
	25	0.7	5.2
	50	0.3	1.7
0345	1	0.3	0.4
	10	0.4	1.5
	15	0.3	1.3
	25	0.5	0.0
	50	0.3	0.0

depths, especially at the surface. Then the chlorophyll a concentration began to increase in the evening hours with maxima occuring at all depths at 2235 that evening.

Chlorophyll c showed a similar diel periodicity. Maximum values of this pigment occurred just before sunrise on the 9th, and on that same evening at 2035. The maximum variation occurred at a depth of 10 meters, where the ratio between maximum and minimum chlorophyll c was 16. 8. The chlorophyll c maximum-to-minimum ratios at the other depths were lower. But in general, maximum chlorophyll c values were seen in the night, and minimum values in the late afternoon. Chlorophyll c showed a similar trend to chlorophyll a during the hours in the early afternoon of the 9th when the cloud cover came in (Fig. 14).

During the YALOC-66 Fixed Station "A", a diel periodicity of both chlorophylls was present. However, as will be discussed later, this periodicity appears to have been modified by changing light conditions during the afternoon of June 9th.

Cruise YALOC-66, Fixed Station "C"

Fixed Station "C", 50° 28'N, 176° 14'W was occupied from 0800 on June 22 to 0200 on June 23, 1966. The weather was overcast during the entire time on station with a few brief sunny periods between 1000 and 1200 on the 22nd; here, solar radiation reached 1. 4

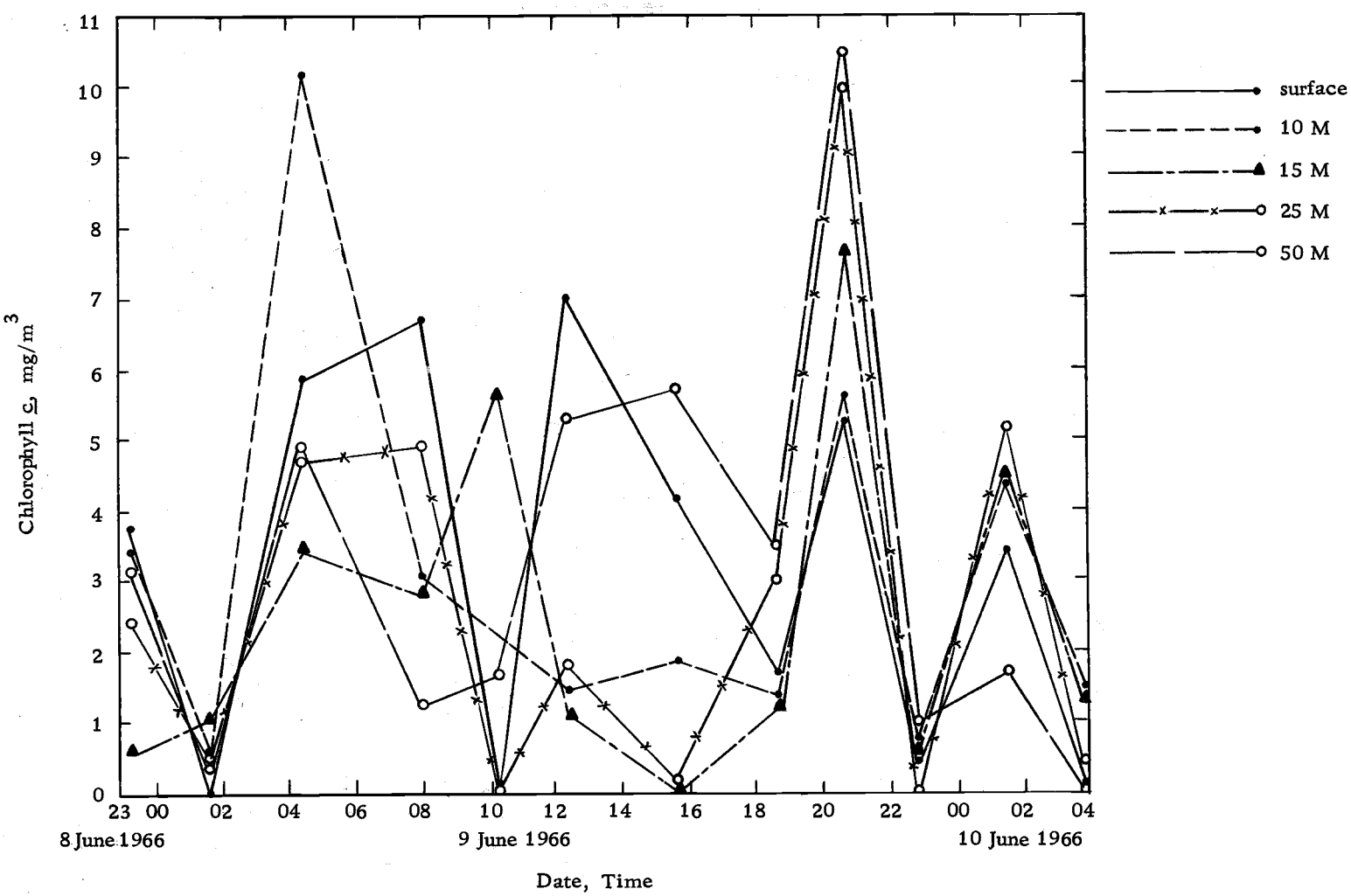


Figure 14. Chlorophyll c, Cruise YALOC-66, Fixed Station "A" (29° 12'N, 161° 30'W), 8-10 June 1966.

langleys per minute. But in general, between 0700 and 1700, solar radiation ranged between 0.3 to 0.4 langleys per minute.

Table 9 presents the chlorophyll a and c data obtained from this station. A diel periodicity of chlorophyll a occurred (Fig. 15). At the surface, maximum chlorophyll occurred at 1900, while the minimum value occurred at 0800 (the ratio of these two values was 2.9). At 10 meters, the maximum occurred at 2100 which was also the time of the chlorophyll a maximum at 15 meters. Minimum values at 10 meters were at 0800, and at 15 meters, 0200. The ratios at maximum-to-minimum chlorophyll a values at these two depths were 3.4, and 2.0 respectively. At 25 meters, the maximum occurred at 1800, and the minimum at 0800, the ratio being 1.4. At 50 meters, the maximum occurred at 1400, and the minimum at 0200, the ratio being 1.7.

A periodicity of chlorophyll c also occurred (Fig. 16). The surface maximum of this pigment occurred at 1900, the maxima for 10, 25, and 50 meters all occurred at 1800, and at 15 meters, the maximum occurred at 1400. Minimums for these depths occurred at 0200, 0200, 1030, 1030, and 0200, respectively. (The respective maximum-to-minimum ratios at these depths were 18.8, 2.3, 4.7, 5.2, and 19.5.)

At Fixed Station "C", a diel periodicity was again seen, but maximum values occurred earlier in the evening than seen at Fixed

Table 9. Chlorophyll Values, Cruise YALOC-66, June 22-23, 1966, Fixed Station "C", 50° 28'N, 176° 14'W.

Date, Time (BST)	Depth, Meters	Pigment Values, mg/m^3 Chlorophyll a	Chlorophyll c
June 22, 1966			
0800	1	0.8	1.2
	10	0.5	0.7
	15	2.3	15.2
	25	0.8	2.1
	50	1.5	3.9
1030	1	0.9	2.8
	10	1.0	6.2
	15	0.9	0.9
	25	0.8	0.6
	50	1.5	1.4
1400	1	1.0	2.6
	10	1.1	2.3
	15	1.4	7.8
	25	1.1	2.3
	50	1.9	5.5
1800	1	1.6	7.2
	10	0.7	4.9
	15	1.0	0.7
	25	1.1	2.8
	50	1.6	7.2
1900	1	2.3	9.4
2000	1	0.9	5.4
2100	1	1.5	2.3
	10	1.7	4.3
	15	1.4	1.8
	25	1.0	1.1
	50	1.5	5.3
2300	1	1.5	3.9
June 23, 1966			
0000	1	1.7	7.4
0200	1	1.0	0.5
	10	1.1	2.1
	15	0.7	0.4
	25	1.1	2.7
	50	1.1	2.4

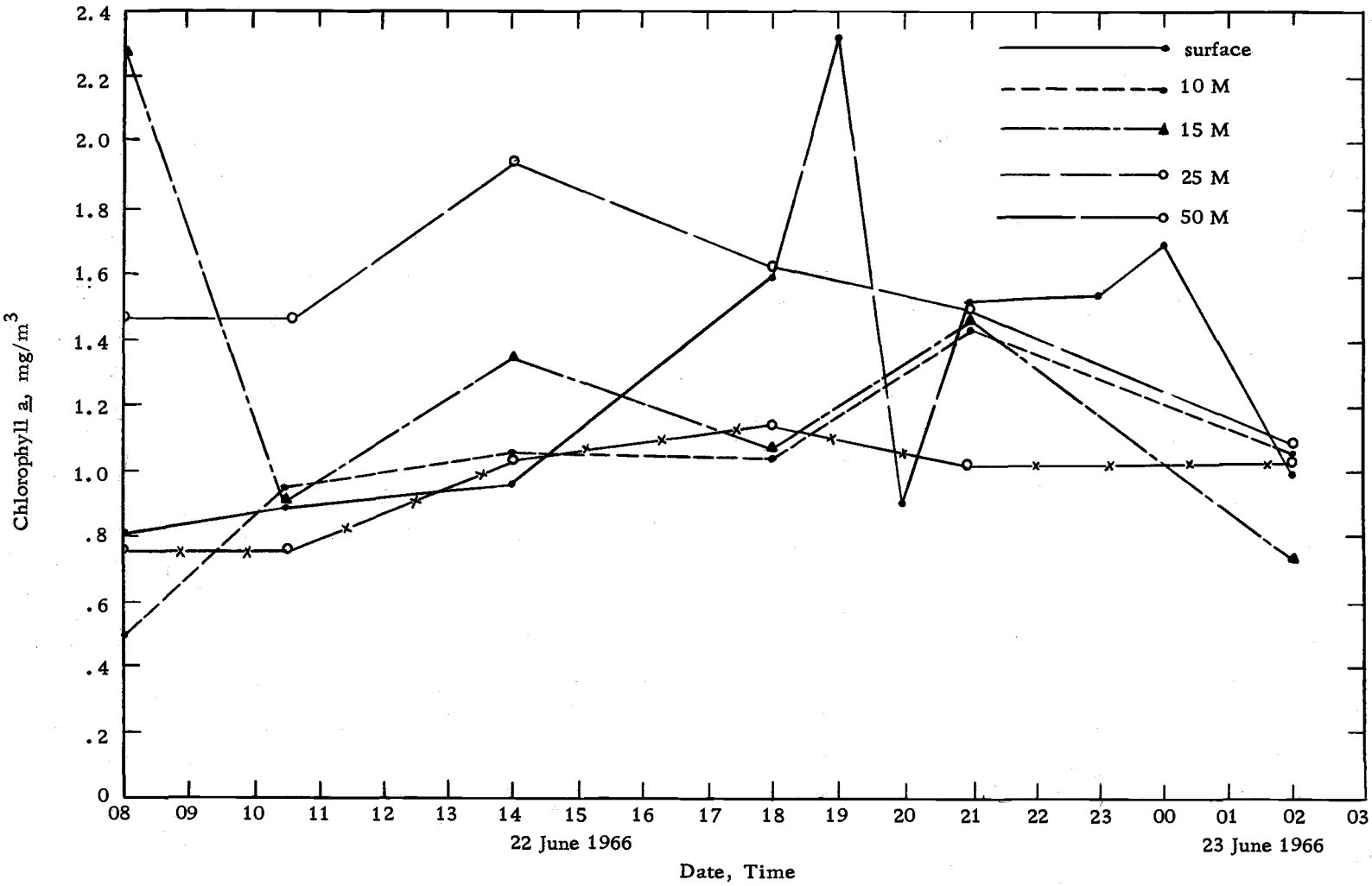


Figure 15. Chlorophyll a, Cruise YALOC-66, Fixed Station "C" (50° 28'N, 176° 14'W), 22-23 June 1966.

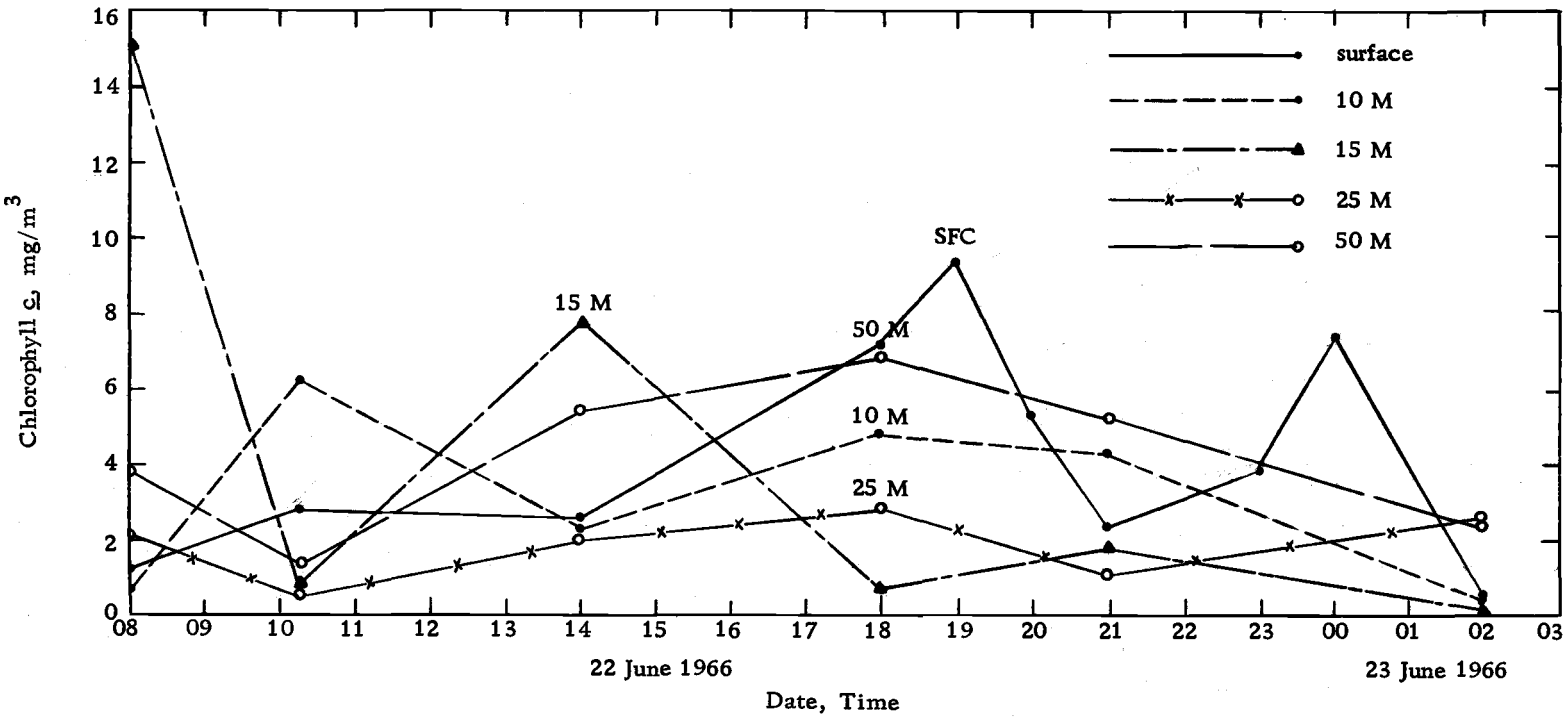


Figure 16. Chlorophyll c, Cruise YALOC-66, Fixed Station "C" (50° 28'N, 176° 14'W), 22-23 June 1966.

Station "A". Chlorophyll c showed a greater variation than chlorophyll a.

Cruise 6701

Cruise 6701 occupied Station NH-25 from 1200 on January 3, to 0700 on January 4, 1967. The weather was overcast, with maximum solar radiation of 0.2-0.3 langleys per minute. Starting at approximately 0600 on the 4th, high winds and rough seas occurred, curtailing the sampling program.

A diel periodicity of chlorophyll a occurred (Fig. 17 and Table 10). The maximum value on the surface occurred at 2230, and the minimum at 0700, the ratio between these two values being 1.7. At 10 meters, the ratio between the maximum at 1920 and the minimum 1200 was 2.7. At 15, 25, and 50 meters, the maximum values occurred at 1700, 1700, and 0300, respectively, and the minimum values at 0100, 2230, and 1200, respectively. Ratios of maximum to minimum pigments at these depths were 2.7, 1.9, and 2.4. The chlorophyll a maximum appears to occur much earlier than during previous cruises when a diel periodicity was noticed.

A diel periodicity of chlorophyll c was seen during the night of the 4th, but this periodicity had two sets of maximum values, one between 1200 and 2230 on the 3rd, and another set of maxima between 2230 and 0700 on the 4th (Fig. 18). This is in contrast to chlorophyll

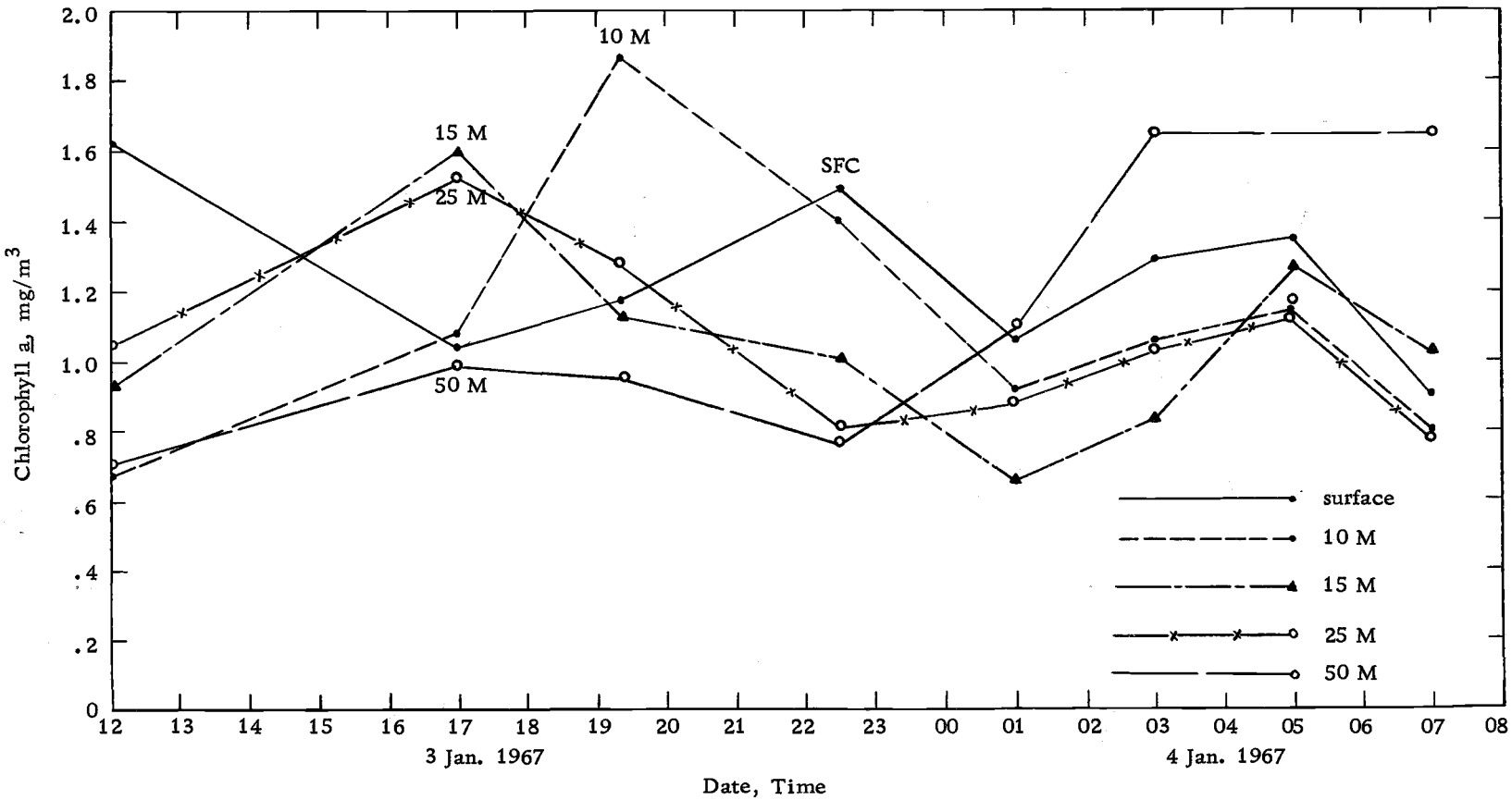


Figure 17. Chlorophyll a, Cruise 6701, Station NH-25, 3-5 Jan. 1967.

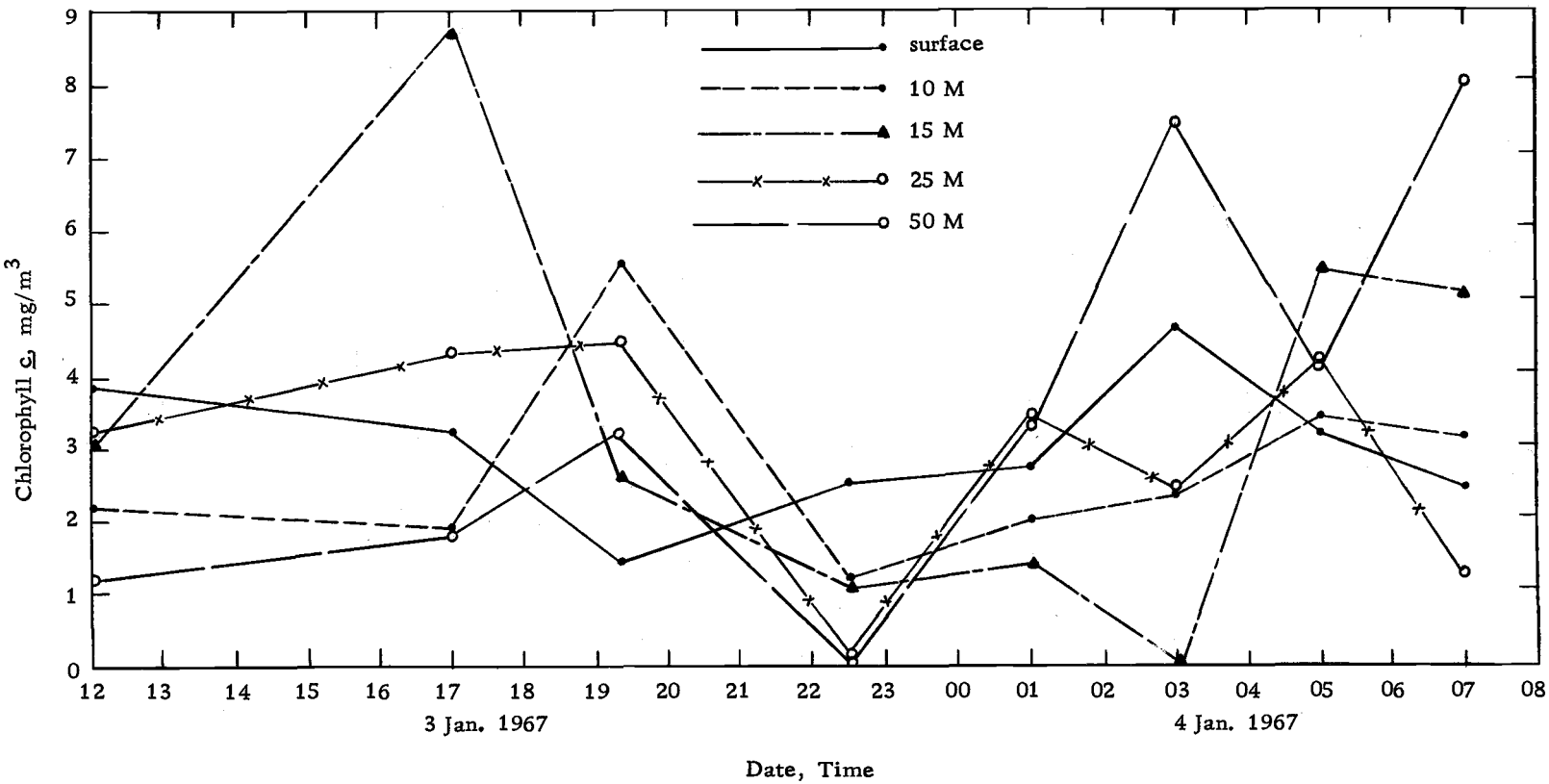


Figure 18. Chlorophyll c, Cruise 6701, Station NH-25, 3-5 Jan. 1967.

Table 10. Chlorophyll Values, Cruise 6701, January 3-4, 1967, Station NH-25.

Date, Time (PST)	Depth, Meters	Pigment Values, mg/m^3	
		Chlorophyll *a*	Chlorophyll *c*
January 3, 1967			
1200	1	1.6	3.8
	10	0.7	2.2
	15	1.0	3.1
	25	1.0	3.2
	50	0.7	1.2
1700	1	1.0	3.2
	10	1.1	1.9
	15	1.6	8.7
	25	1.5	4.3
	50	1.0	1.9
1920	1	1.2	1.4
	10	1.9	5.5
	15	1.1	2.6
	25	1.3	4.4
	50	1.0	3.1
2230	1	1.5	2.5
	10	1.4	1.2
	15	1.1	1.1
	25	0.8	0.0
	50	0.8	0.0
January 4, 1967			
0100	1	1.1	2.7
	10	0.9	2.0
	15	0.6	1.4
	25	0.9	3.3
	50	1.1	3.4
0300	1	1.3	4.6
	10	1.1	2.3
	15	0.8	0.0
	25	1.1	2.3
	50	1.7	7.4
0500	1	1.3	3.2
	10	1.1	3.4
	15	1.3	5.4
	25	1.2	4.2
	50	1.2	4.2

Table 10. Continued.

Date, Time (PST)	Depth, Meters	Pigment Values, mg/m^3	
		Chlorophyll *a*	Chlorophyll *c*
0700	1	0.9	2.4
	10	1.0	3.1
	15	1.1	5.1
	25	0.8	1.2
	50	1.7	8.0

a which shows only a slight increase in concentration between 0100 and 0700 on the 4th. Whether or not these secondary maxima are important is not known.

Contoured Time Series with Depth of Chlorophyll a and c

Chlorophyll profiles plotted against time and depth were made for the following cruises: 6507 (Figs. 36 and 37), 6509 (Figs. 38, 39, and 40), 6511 (Fig. 41), and YALOC-66 (Fig. 42). These time series were integrated and chlorophyll values were presented on a milligrams per square meter basis to a depth of 50 meters (see bottom of figure). Integrated values showed that both chlorophyll a and c were higher at night (Figs. 36-42) which agreed with previous results obtained (Figs. 2-5, 7, 8, 12). These data also showed that chlorophyll concentrations changed quite rapidly with time as evidenced by contours being closely spaced. The times of most rapid chlorophyll changes were generally in the early evening to sunrise. Particularly rapid changes occurred in chlorophyll a and c from 2200 to 0600 on July 20-21, 1965, at Station NH-25 (Figs. 36 and 37); 1900 to 0300 at Station SB on September 25-26, 1965 (Figs. 38 and 39); from 2100 to 0300 at Station NH-35 for chlorophyll c (Fig. 40) and from 1900-2100 at NH-25 on November 29, 1965 (Fig. 41). The most rapid changes were seen for chlorophyll c on the YALOC-66 cruise, Fixed Station "A", from 0500-0900 on June 8, 1966, and 1900-2300 on June 9, 1966 (Fig. 42).

Laboratory Results

Diel Periodicity of Skeletonema costatum Chlorophyll, 12 Hour Light-12 Hour Dark Cycle at Low Light Intensity

A 12-liter culture of Skeletonema costatum was grown in enriched sea water under 400 foot-candles light intensity. After one 12 hour light - 12 hour dark cycle, samples were collected every two hours for 120 hours and analyzed for chlorophylls a and c and cell numbers (Table 11 and Figs. 19 and 20).

Cells were in the log phase of division after 40 hours of growth and reached senescence after about 105 hours; thus measurements of total chlorophyll a in the culture would be meaningless and are not plotted for this experiment. (When cell multiplication is rapid and numbers increase in a geometric progression in a culture, this is known as the logarithmic phase. This phase usually is preceded by a lag phase, when no increase in cell numbers occur. When relative growth declines, and cell numbers become more or less stationary, senescence is reached.) The concentration of chlorophyll a per cell varied in relation to the light-dark cycle (Fig. 19). The concentration of this pigment increased during the light period and reached a maximum value at the end of the light period (on the first day, this maximum occurred four hours into the dark cycle). During the dark cycle the chlorophyll a concentration decreased,

Table 11. Diel Periodicity Experiment Using Skeletonema Costatum, 12 Hour Light - 12 Hour Dark Cycle, 400 f. c. Light Intensity.

Date,	Time	Cells/ml x 10^3	µg Pigment/l		mg Pigment/Cell x 10^{-6}	
			Chl. a	Chl. c	Chl. a	Chl. c
Nov. 8	0915	2.8	1.4	3.6	0.50	1.30
	1100	4.4	1.1	2.2	0.25	0.50
	1300	5.0	1.4	3.6	0.29	0.73
	1500	4.1	2.2	4.4	0.54	1.10
	1700	4.7	3.3	9.6	0.70	2.00
	1900	4.4	2.4	1.2	0.55	2.70
	2100	4.1	4.5	4.4	1.10	1.10
	2300	3.9	4.5	4.4	1.20	1.10
Nov. 9	0100	4.1	2.9	4.0	0.63	0.98
	0300	6.0	2.9	0.0	0.49	0.00
	0500	6.9	2.4	0.0	0.35	0.00
	0700	11.3	2.2	4.4	0.20	0.39
	0900	9.5	7.6	19.2	0.80	2.00
	1100	9.4	8.9	12.4	0.95	1.30
	1300	9.4	8.8	18.4	0.94	2.00
	1500	4.6	9.5	18.8	2.10	4.10
	1700	8.7	12.3	22.0	1.40	2.50
	1900	5.3	14.2	28.0	2.70	5.30
	2100	10.6	16.6	39.2	1.60	3.70
	2300	14.4	16.6	35.6	1.10	2.50
No. 10	0100	20.3	18.8	30.4	0.93	1.50
	0300	29.7	23.9	44.0	0.81	1.50
	0500	37.5	21.3	37.6	0.57	1.00
	0700	48.8	20.1	29.6	0.41	0.61
	0900	40.5	20.9	27.0	0.52	0.67
	1100	84.1	56.4	86.7	0.67	1.00
	1300	74.1	76.4	90.7	1.00	1.20
	1500	111.6	96.8	34.0	0.87	0.31
	1700	105.9	130.1	90.0	1.20	0.85
	1900	174.4	167.0	138.0	0.96	0.79
	2100	166.3	167.4	122.0	1.00	0.73
	2300	245.0	154.2	112.2	0.65	0.46
Nov.11	0100	271.0	168.6	88.6	0.62	0.33
	0300	285.8	174.0	116.8	0.61	0.41
	0500	320.0	150.6	92.0	0.49	0.29
	0700	481.0	195.8	198.1	0.41	0.41
	0900	600.	186.0	6.0	0.31	0.01
	1100	534.	231.2	16.4	0.43	0.03

Table 11. Continued.

Date, Time	Cells/ml x 10^3	µg Pigment /l		mg Pigment/Cell x 10^{-6}	
		Chl. a	Chl. c	Chl. a	Chl. c
Nov. 11 1300	549	338.2	114.0	0.62	0.21
1530	552	526.2	218.2	0.95	0.40
1700	740	625.4	276.4	0.84	0.37
1900	653	686.4	351.8	1.10	0.54
2030	700	722.6	375.8	1.00	0.54
2300	931	726.0	401.8	0.79	0.43
Nov. 12 0100	1172	758.2	609.2	0.65	0.52
0300	1435	734.6	332.6	0.51	0.23
0500	1350	754.2	356.6	0.56	0.26
0700	1500	628.4	795.2	0.42	0.20
0900	2025	795.8	314.6	0.39	0.16
1130	1111	1055.6	434.4	0.95	0.39
1320	1504	1338.2	626.0	0.89	0.42
1510	1476	1534.2	901.6	1.00	0.61
1700	1725	1609.8	800.2	0.93	0.46
1930	1955	1456.6	701.6	0.75	0.36
2115	1780	1499.8	733.2	0.83	0.41
2315	1840	1475.0	582.2	0.80	0.32
Nov. 13 0100	1928	1468.8	783.6	0.76	0.41
0300	2075	1553.8	725.8	0.75	0.35
0500	2300	1558.8	639.0	0.68	0.28
0700	2130	1542.6	779.4	0.73	0.37
1100	1900	1729.2	934.0	0.91	0.49

Light Schedule

Lights On: 0700
Lights Off: 1700

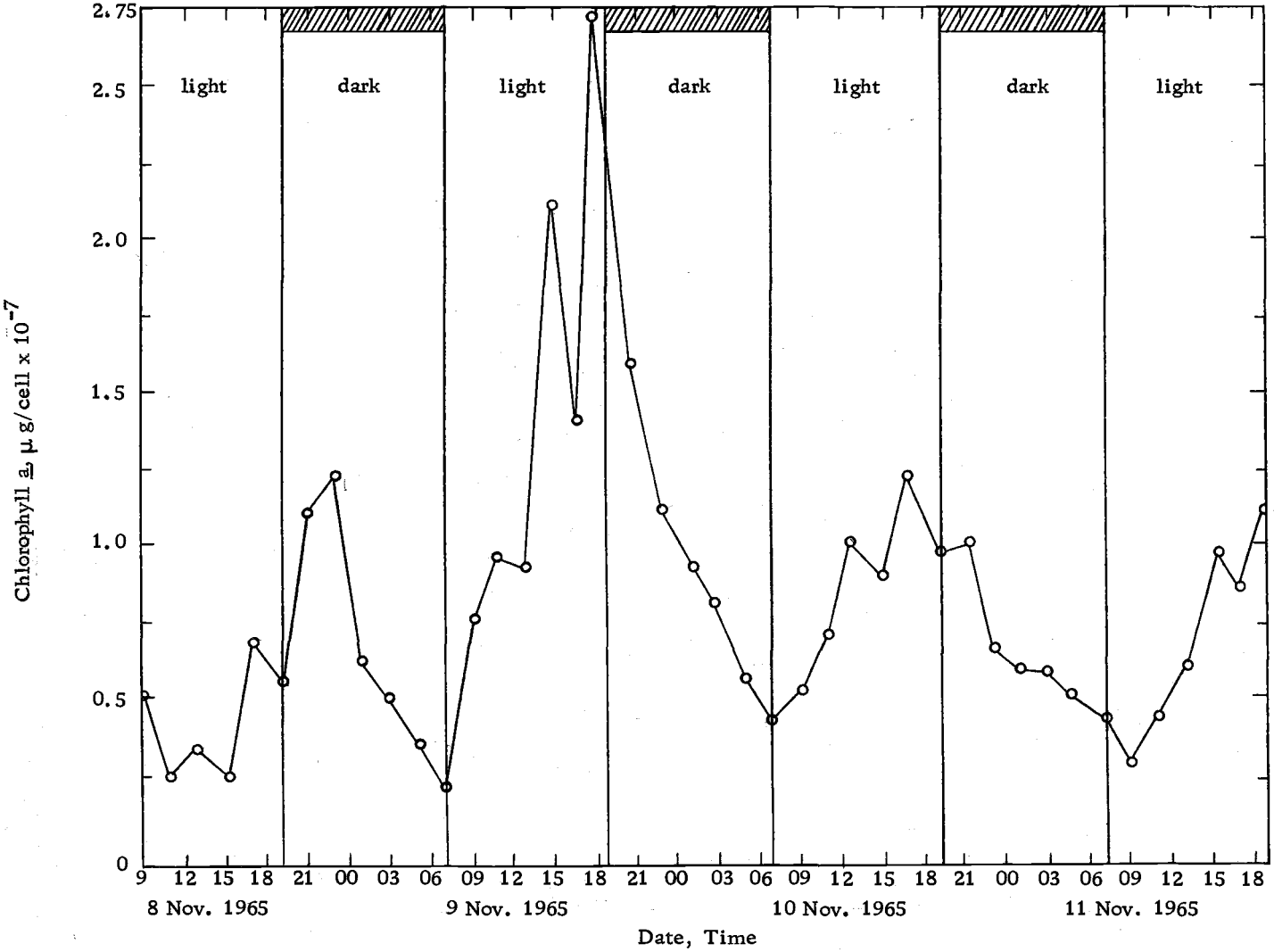


Figure 19. Chlorophyll a per cell of Skeletonema costatum, 12 hour light - 12 hour dark cycle at 400 foot candles light intensity.

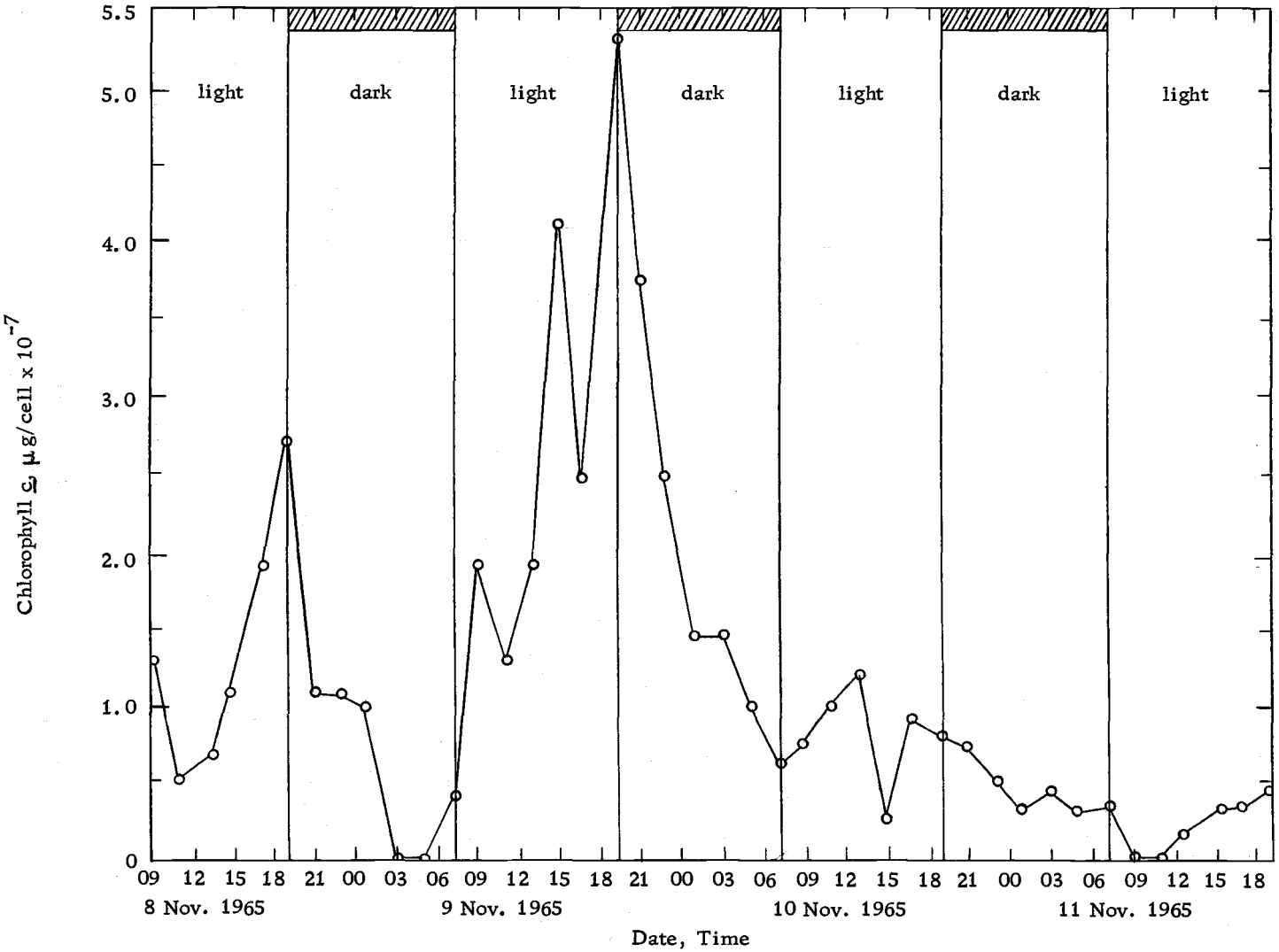


Figure 20. Chlorophyll c per cell of Skeletonema costatum, 12 hour light - 12 hour dark cycle at 400 foot candles light intensity.

reaching its minimum value at the end of the dark cycle. Ratios were calculated of maximum chlorophyll a concentration to minimum concentration at the end of the dark period. These ratios were 6.0, 6.6, 3.9, 2.8, and 1.5 for the first, second, third, fourth, and fifth days, respectively. As the cells become senescent, the magnitude of this diel periodicity apparently decreased.

As with chlorophyll a, chlorophyll c concentrations per cell increased during the light period, and decreased during the dark period (Fig. 20). At the end of the first dark period, the measurable concentration of this pigment dropped to zero. During the second day, the ratio of maximum chlorophyll c concentration (at the end of the light period) to the minimum concentration (at the end of the dark period) was 7.9. On the third day the ratio was 120.0, on the fourth day, 3.4, and on the fifth day, 2.2. Again, the diel periodicity of this pigment decreased in magnitude as senescence began.

No difference was observed in the cell division rate in the dark versus the light period. Therefore, differential rates of cell division in the light and dark periods do not appear to explain the diel periodicity of pigments in this experiment.

Diel Periodicity of Chlorophyll in *Skeletonema costatum* at Simulated Depths in the Ocean, 12 Hour Light - 12 Hour Dark Cycle

In an attempt to simulate the amount and quality of light at selected depths in the ocean, *Skeletonema costatum* was grown in enriched

sea water to apparent logarithmic phase and placed under selected Corning glass filters in the Shever-Gillet Growth Chamber. The cells were cycled for one 12 hour light - 12 hour dark period; then sampling was done approximately every two hours to determine chlorophyll a and c concentrations and cell numbers. These data are presented in Table 12.

Although the cells went into a lag phase, and cell counts showed variability with time, diel periodicity of pigments occurred during the lag phase and senescence. I feel that cessation of log phase growth did not affect the results of this experiment, however.

The total chlorophyll a in the culture at the three simulated depths showed a diel periodicity (Fig. 21). The "surface" sample showed that chlorophyll a increased for the first nine hours of the dark period, then began to decrease. The lowest value was attained towards the end of the light period. The pigment then began to increase in the following dark period, again reaching a maximum value seven hours into the dark period. The chlorophyll a concentration per cell (Fig. 2) reached a maximum three hours into the dark period, then began to decrease. A minimum was obtained during the third hour of the light period, after which an increase in concentration to a maximum again was attained three hours into the dark period. At the simulated 10 meters depth, total chlorophyll a increased during the first dark period and reached a maximum seven

Table 12. Diel Periodicity Experiment Using Skeletonema costatum, 12 Hour Light - 12 Hour Dark Cycle.

Date, Time	Simulated depth, meters	Cells/ml x 10^3	Chlorophyll a µg/L	Chlorophyll a µg/cell x 10^{-6}	Chlorophyll c µg/L	Chlorophyll c µg/cell x 10^{-6}
December 18						
1500	surface	165	133.4	0.81	112.2	0.68
	10	245	140.0	2.47	348.2	1.42
	50	155	196.2	1.26	417.0	2.79
1700	sfc	212	145.0	0.68	218.0	1.03
	10	195	141.0	0.72	258.2	1.38
	50	217	158.2	0.73	269.6	1.26
2000	sfc	150	173.4	1.15	267.6	1.78
	10	127	134.6	1.06	203.6	1.61
	50	118	173.4	1.47	157.6	1.34
2300	sfc	136	162.2	1.19	213.4	1.57
	10	118	172.2	1.46	205.8	1.74
	50	114	170.8	1.50	480.6	2.11
December 19						
0100	sfc	153	158.2	1.03	263.8	1.73
	10	171	153.8	0.90	204.6	1.20
	50	136	157.6	1.16	247.8	1.82
0300	sfc	112	164.4	1.47	181.2	1.62
	10	226	156.6	0.69	299.4	1.32
	50	156	158.2	1.02	154.2	0.99
0500	sfc	138	181.8	1.32	260.0	1.88
	10	187	210.2	1.12	679.6	3.64
	50	157	169.2	1.08	92.6	0.59
0700	sfc	218	194.4	0.89	266.0	2.26
	10	219	272.4	1.24	518.4	1.90
	50	164	189.0	1.15	238.8	1.27
0900	sfc	249	198.4	0.79	247.4	0.99
	10	159	167.0	0.99	208.0	1.31
	50	185	167.2	0.90	178.6	0.97
1200	sfc	150	208.0	1.38	202.0	1.35
	10	162	198.2	1.22	374.6	2.31
	50	185	210.2	1.13	143.6	0.78
1500	sfc	270	212.6	0.76	235.0	0.87
	10	278	200.4	0.72	382.6	1.38
	50	155	220.2	1.42	170.0	1.07

Table 12. Continued.

Date, Time	Simulated depth, meters	Cells/ml x 10^3	Chlorophyll a µg/L	Chlorophyll a µg/cell x 10^{-6}	Chlorophyll c µg/L	Chlorophyll c µg/cell x 10^{-6}
1700	sfc	180	178.2	0.99	144.8	0.81
	10	169	204.4	1.21	248.2	1.47
	50	220	211.0	0.96	105.8	0.48
2100	sfc	150	195.4	1.30	134.4	0.89
	10	222	158.4	0.71	170.2	0.77
	50	253	224.0	0.88	189.6	0.75
2300	sfc	233	212.6	0.91	235.0	1.01
	10	219	205.6	0.94	194.4	0.89
	50	300	227.6	0.76	195.4	0.65
December 20						
0100	sfc	287	206.8	0.72	140.4	0.49
	10	207	262.6	1.28	434.2	2.10
	50	306	240.4	0.78	151.0	0.53
0300	sfc	291	242.0	0.83	196.4	0.67
	10	264	200.0	0.76	177.4	2.10
	50	382	249.2	0.65	247.4	0.53
0500	sfc	241	197.4	0.82	185.8	0.67
	10	190	148.4	0.78	193.6	0.67
	50	175	204.2	1.17	336.4	0.65
0700	sfc	298	202.4	0.68	135.0	0.77
	10	195	152.6	0.78	189.8	1.02
	50	220	163.0	0.74	100.8	1.92
0900	sfc	287	169.4	0.59	413.2	1.44
	10	236	117.2	0.45	202.6	0.86
	50	239	239.8	1.00	519.6	2.18
1200	sfc	203	167.2	0.82	256.4	1.26
	10	295	-----	----	-----	----
	50	252	218.8	0.87	367.6	1.46
1500	sfc	237	173.2	0.73	173.6	0.74
	10	240	135.0	0.56	125.8	0.53
	50	217	249.4	1.15	331.0	1.52
1700	sfc	150	144.0	0.96	90.8	0.61
	10	145	160.2	1.10	193.6	1.34
	50	232	213.4	0.92	251.0	1.08

Table 12. Continued.

Date, Time	Simulated depth, meters	Cells/ml x 10^3	Chlorophyll a µg/L	Chlorophyll a µg/cell x 10^{-6}	Chlorophyll c µg/L	Chlorophyll c µg/cell x 10^{-6}
2000	sfc	170	216.2	1.27	348.2	2.04
	10	162	168.2	1.04	146.6	0.90
	50	210	227.4	1.08	292.6	1.40
2300	sfc	172	208.4	1.20	285.8	1.67
	10	153	176.6	1.16	248.8	1.63
	50	223	236.0	1.06	201.4	0.90
December 21						
0100	sfc	234	235.2	1.10	295.2	1.26
	10	219	188.8	0.86	238.8	1.09
	50	179	265.0	1.48	253.4	1.41
0300	sfc	264	206.0	0.78	178.4	0.68
	10	193	187.2	0.97	308.8	1.60
	50	209	232.4	1.11	329.2	1.57

Light Schedule: See Table 2 for energies involved.

	ON	OFF
Low Light	0600	1800
Medium Light	0800	1600
High Light	1000	1400

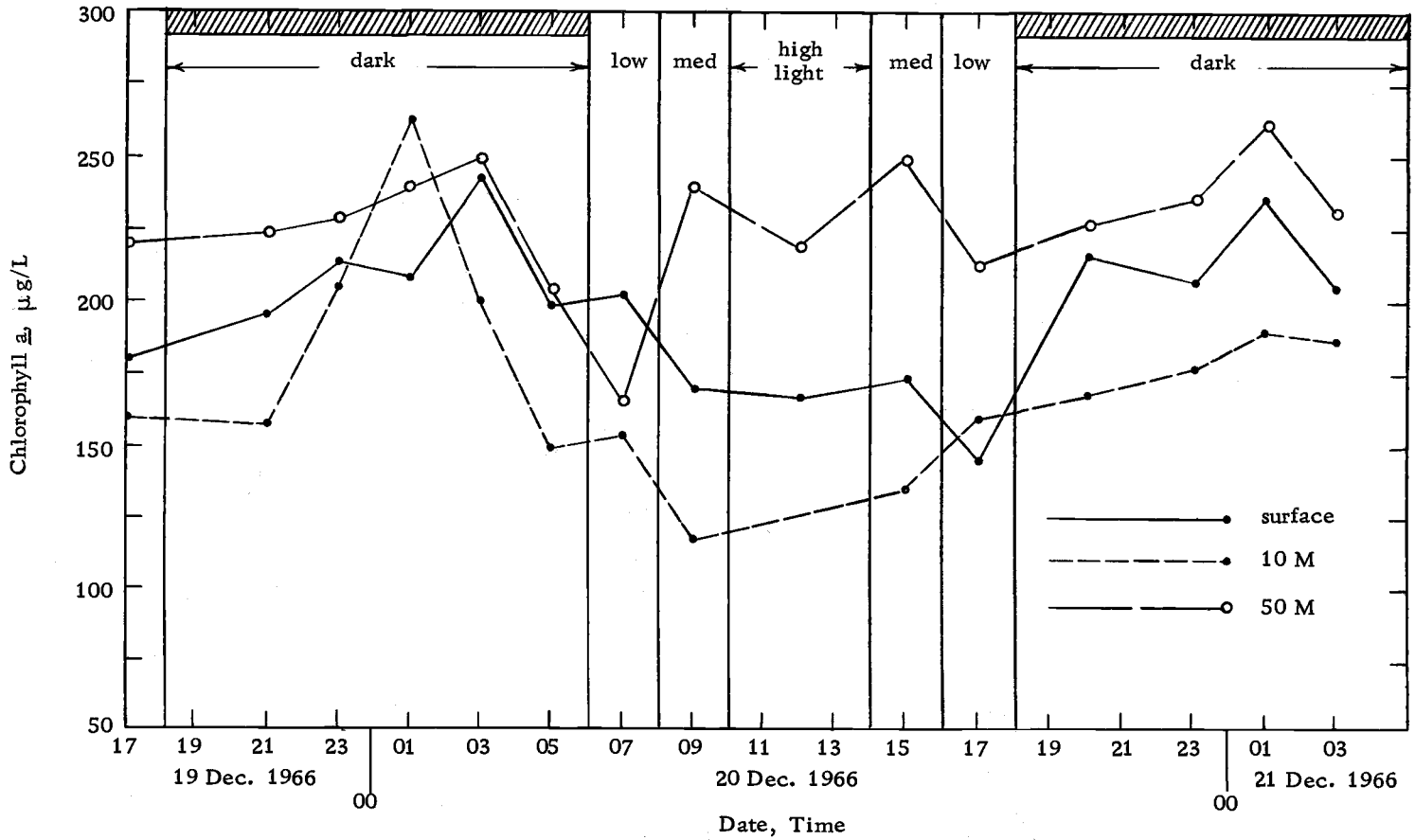


Figure 21. Chlorophyll a, µg/L, in culture of S. costatum, 12 hour light - 12 hour dark cycle.

hours into the dark period. It then decreased in the first three hours of the light period, and increased into the following dark period. The ratio of the peak chlorophyll a in the dark period to the minimum concentration in the light period at "10 meters" was 2.5, as contrasted to a ratio of 2.2 at the "surface." At the "50 meter" depth, the total chlorophyll a in the culture showed maxima during both the light and dark periods, but on a "per cell" basis (Fig. 22). Maximum chlorophyll a occurred toward the end of the dark period. The ratio of maximum to minimum concentration of chlorophyll a per cell was 1.6 at this "depth." No large differences in the concentration of chlorophyll a per cell were apparent at the three light levels used, but at the "10 meter depth" the magnitude of periodicity was slightly higher than at the "surface", and at "50 meters."

Two chlorophyll c maxima at the surface were present, one during the light period, and one during the second dark period (Fig. 23). The same trend was noticed on a "per cell" basis (Fig. 24). At the 10 meter simulated depth, total chlorophyll c reached a maximum value during the middle of the first dark period, decreased to a minimum value nine hours into the light period, then increased in the second dark period. The same trend occurred on a "per cell" basis. The ratio between maximum and minimum concentrations on a "per cell" basis was 4.7. At the 50 meter simulated depth, total chlorophyll c in the culture showed maxima during

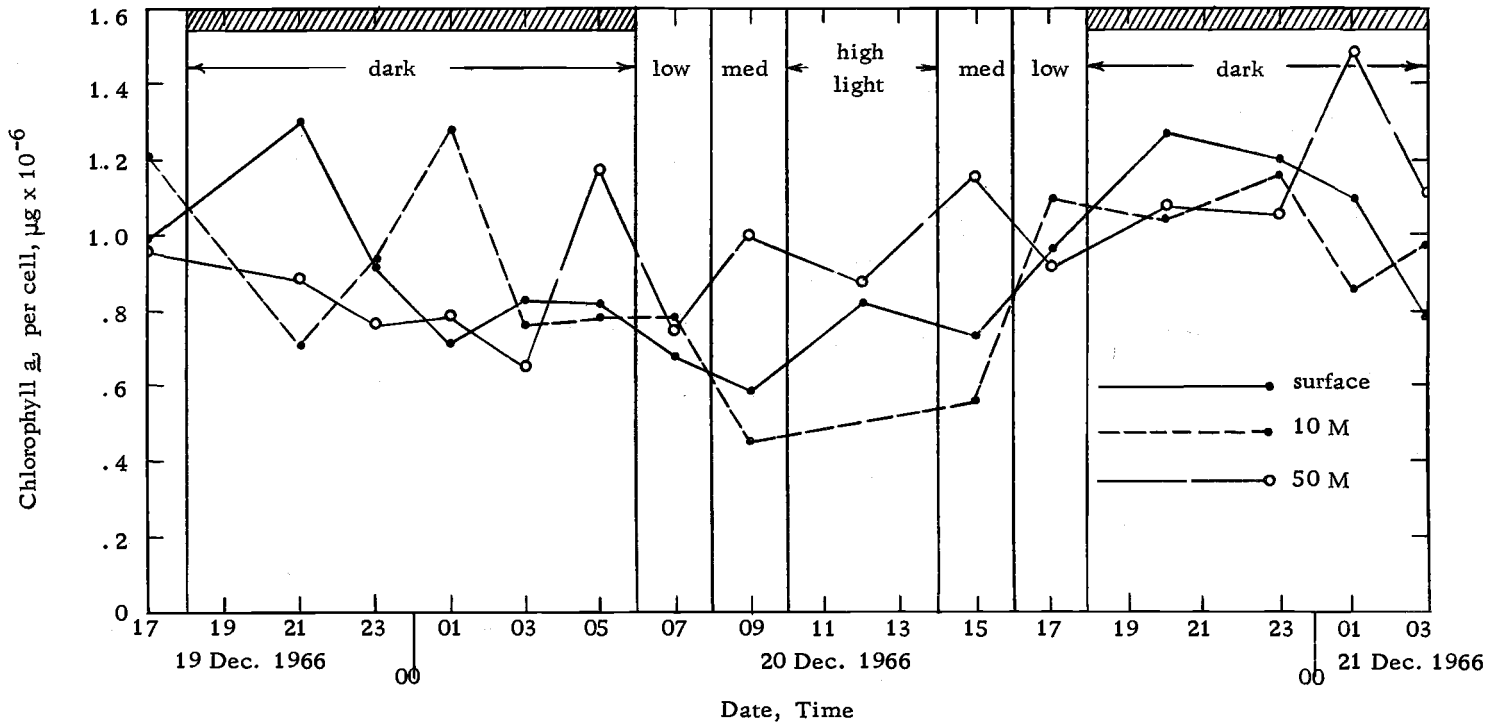


Figure 22. Chlorophyll a, µg/cell, in culture of S. costatum, 12 hour light - 12 hour dark cycle.

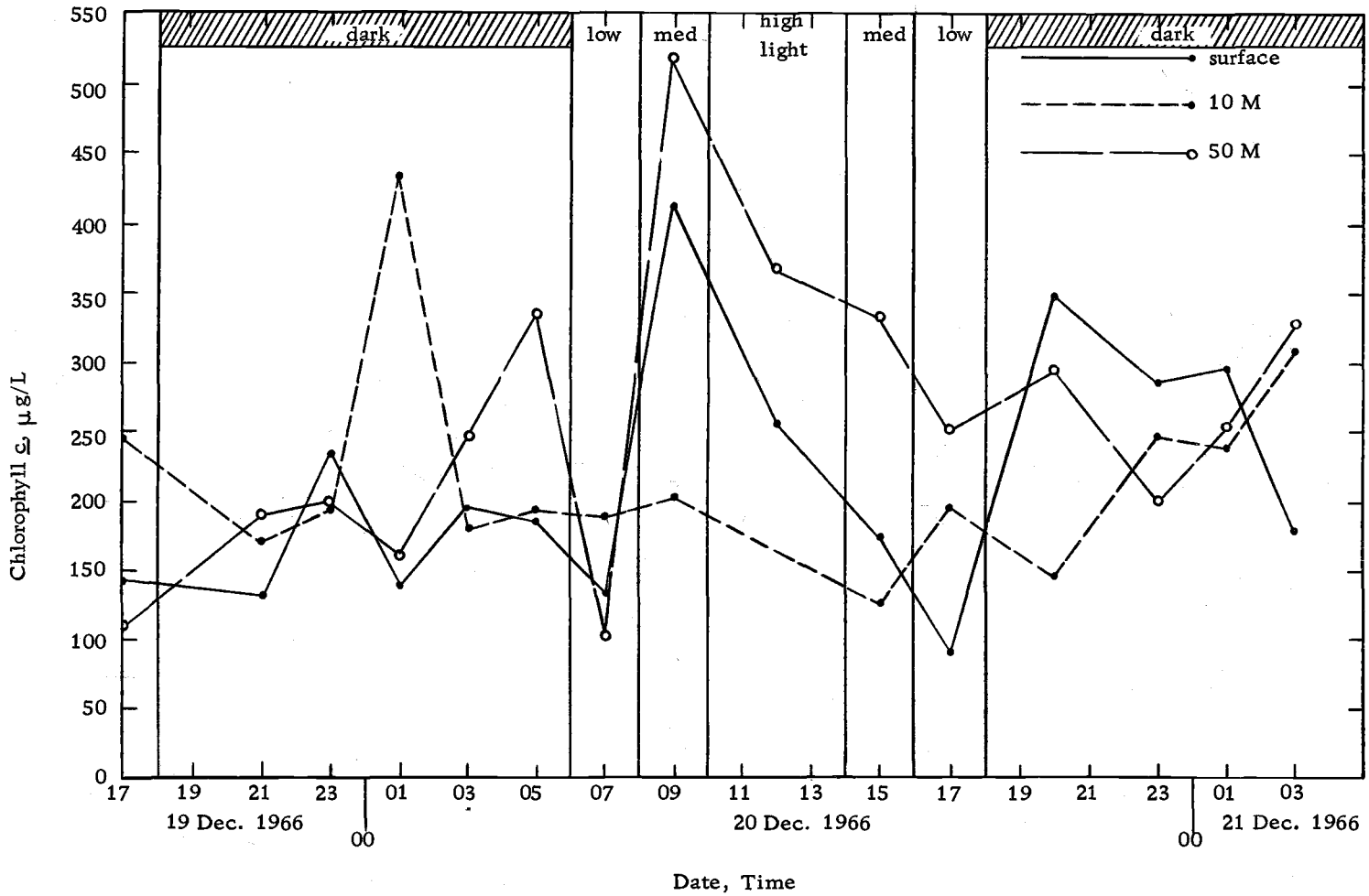


Figure 23. Chlorophyll c, µg/L, in culture of S. costatum, 12 hour light - 12 hour dark cycle.

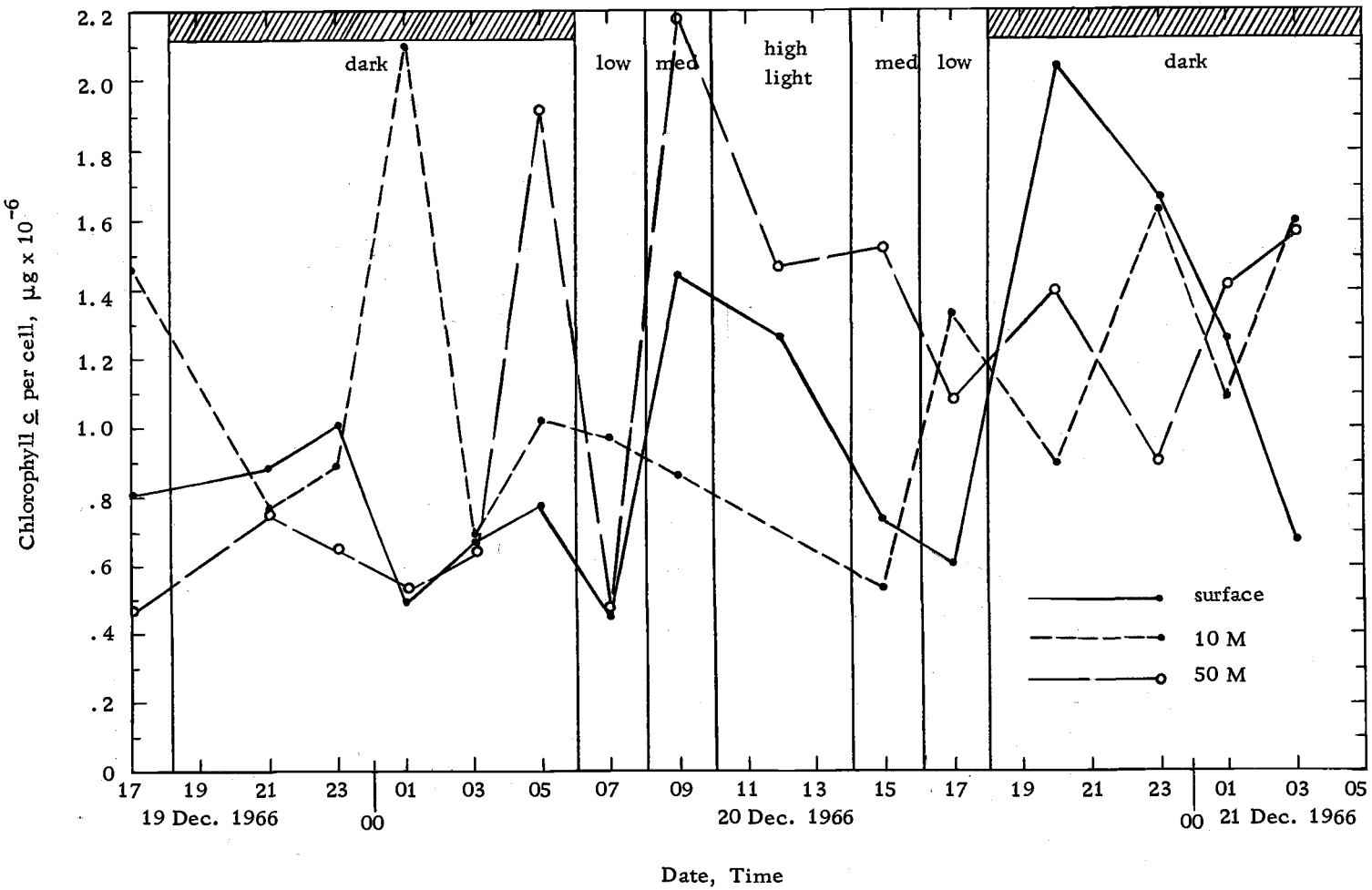


Figure 24. Chlorophyll c, μg/cell, in culture of S. costatum, 12 hour light - 12 hour dark cycle.

both dark periods and the light period, and on a "per cell" basis did the same. The maximum at the "50 meter depth" occurred during the light period.

Thus, a diel periodicity under a 12 hour light - 12 hour dark period was seen with both chlorophyll a and c. At all three simulated depths, greater variability occurred in chlorophyll c than a. The maximum values of both chlorophylls (in terms of concentration per cell) occurred during the dark periods and minimum concentrations in the early portions of the light periods. At the "50 meter depth" chlorophyll a and c showed maxima in both the light and dark periods.

Diel Periodicity of Chlorophyll in Skeletonema costatum at Simulated Depths in the Ocean, 16 Hour Light - 8 Hour Dark Cycle

A 12-liter culture of Skeletonema costatum was grown in enriched sea water at 200 foot-candles and upon reaching log phase, 2.5 liters was transferred into three one-gallon polyethylene screw-capped bags. Each bag was placed under the appropriate filter in the Sherer-Gillet Growth Chamber to simulate 10 and 50 meters depth. Chlorophylls a and c and cell numbers were determined approximately every two hours (Table 12). The cells at the "surface" and "50 meters" appeared to enter a lag phase, while the "10 meter" cells contined to grow in a slow log phase. This experiment was

begun after the culture was allowed to undergo a complete cycle of light and dark, and samples were first collected in the light period after the complete cycle.

On the "surface", chlorophyll a per cell reached a minimum during the middle of the first light period, then increased to a maximum during the first dark period. Then chlorophyll a per cell decreased during the second light period, but again increased somewhat during the second dark period. A decreasing trend into the third dark period was seen. The magnitude of this diel periodicity appeared to be significant. For example, the ratio of the maximum in the second dark period to the minimum during the second light period was 2.2, and to the minimum in the third light period, 2.3. At the simulated 10 meter depth, a diel trend was seen. Here, chlorophyll a per cell reached a maximum three hours before the end of the first light period, then decreased to a minimum at the end of that light period, then slowly increased to another maximum at the beginning of the second dark period, decreased rapidly in this dark period to the beginning of the third light period, then increased to another maximum at the middle of the third light period. The magnitude of these variations also appeared to be significant. For example, the ratio of the maximum at the beginning of the second dark period to the minimum at the beginning of the third light period was 1.9. The ratio of the maximum at the end of the first light

period to the minimum at the start of the first dark period was 2.0. At the "50 meter depth", a diel pattern was also seen. During the middle of the first light period, a maximum was seen, decreasing to a minimum at the middle of the first dark period. The ratio of these two points was 4.8. Chlorophyll a per cell then increased to a maximum at the middle of the second light period, decreased during the second dark period, then increased throughout the third light and dark periods. The sharp increase in chlorophyll a concentration per cell at the end of the second dark period and abrupt decrease may be due to an experimental error as it seems to be too irregular a change in concentration. The ratio of chlorophyll a maximum at middle of the third dark period to the minimum in the third light period preceding it was 1.8, so at "50 meters depth" the magnitude of the diel periodicity decreased with time. The "surface" chlorophyll a generally was higher during the dark periods, while the "50 meter" chlorophyll a generally was lower in the dark periods and greater during the light periods. The "10 meter" samples tended to be intermediate to the "surface" and "50 meter" samples in that the chlorophyll a maximum in the former occurred towards the end of the light period or at the beginning of the dark period (Fig. 25).

At all three "depths", chlorophyll c maxima occurred both in the light and dark periods, so it is impossible to say that a diel

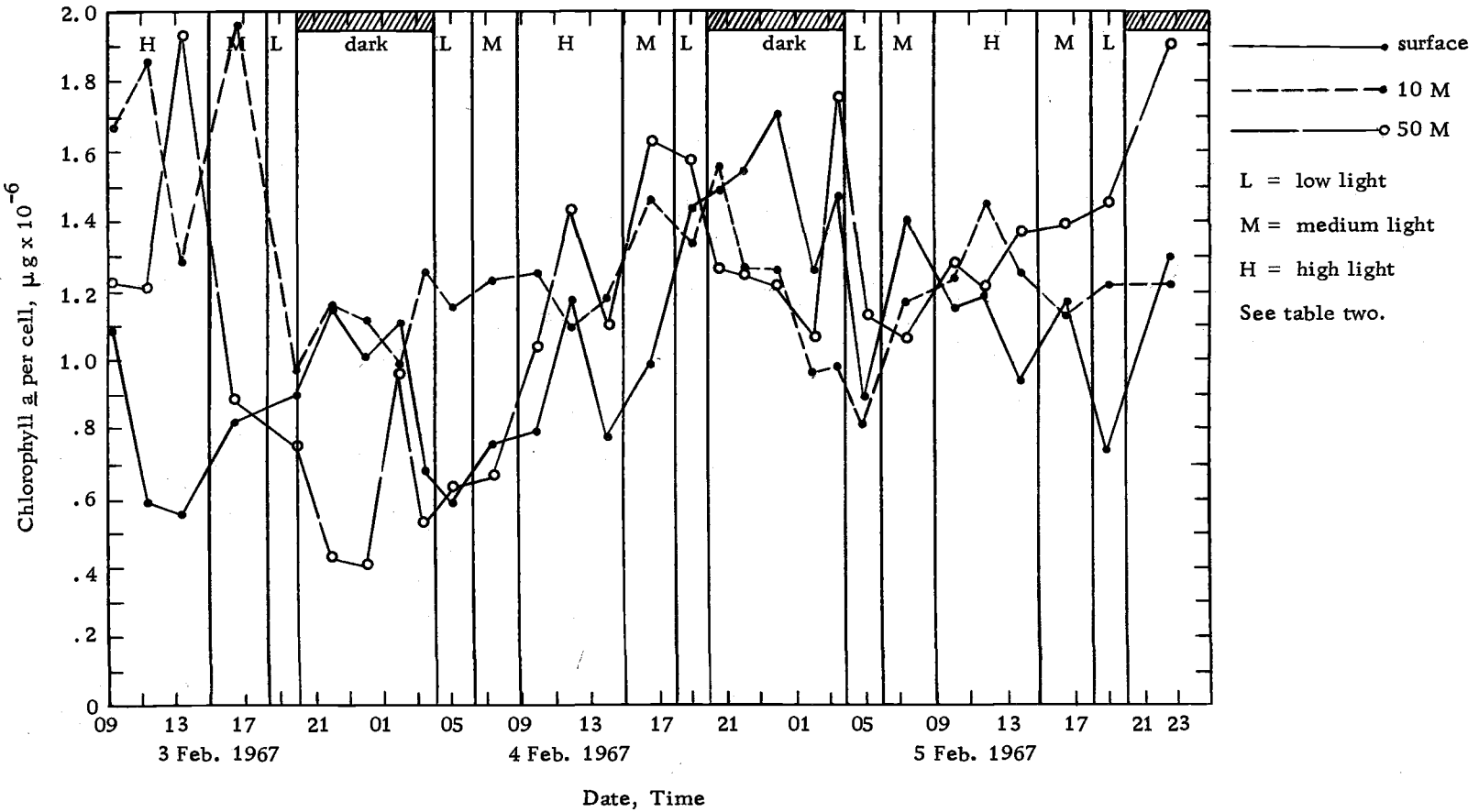


Figure 25. Chlorophyll a per cell of S. costatum, 16 hour light - 8 hour dark cycle.

periodicity occurred in this pigment (Fig. 26).

Diel Periodicity of Chlorophyll in Skeletonema costatum at Simulated Depths in the Ocean, 9 Hour Light - 15 Hour Dark Cycle

A 12-liter culture of Skeletonema costatum was grown to log phase in enriched sea water at a light intensity of approximately 400 foot-candles. Three-liter portions of this culture were then transferred to polyethylene bags and placed under appropriate light filters to simulate the surface (no filter) and depths of 10 and 50 meters. Samples were removed from each of these "depths" periodically to determine chlorophylls a and c and cell concentration (Table 14).

Chlorophyll a showed a diel periodicity (Fig. 27). At the "surface", the concentration of this pigment was highest at the end of the light periods, having increased from a minimum value at the end of the previous dark period. For example, the ratio of the chlorophyll a maximum toward the end of the second light period to the minimum in the dark period preceding it was 2. 4. At "10 meters", no clear maximum of chlorophyll a was seen during the second light period, but a maximum was measured at the end of the third light period. At "50 meters", the same trend in chlorophyll a was observed as at the "surface". A maximum was attained near the end of the dark period. The ratio between the maximum concentration

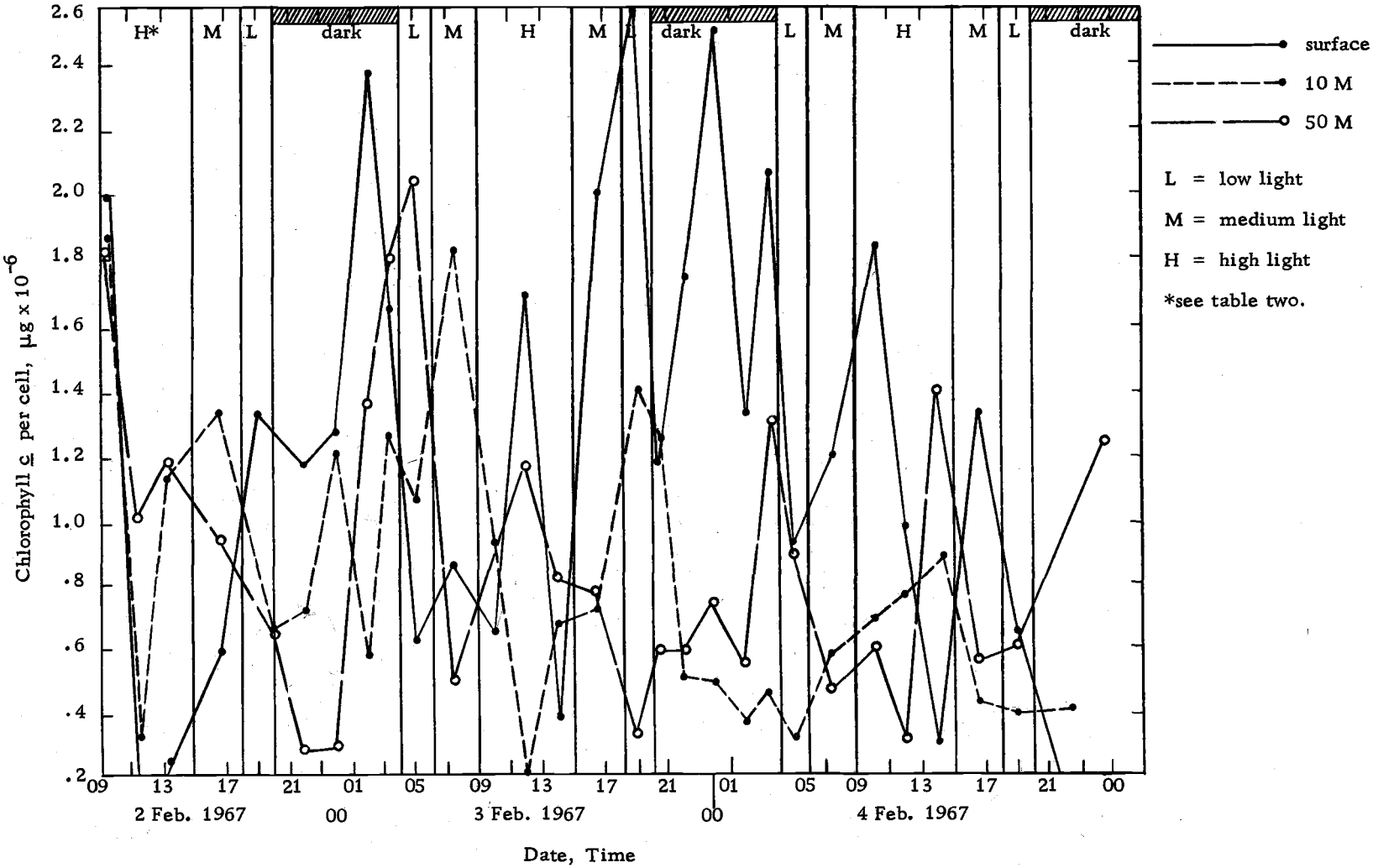


Figure 26. Chlorophyll c per cell of S. costatum, 16 hour light - 8 hour dark cycle.

Table 13. Diel Periodicity Experiment Using Skeletonema costatum, 16 Hour Light - 8 Hour Dark Cycle.

Date, Time	Simulated depth, meters	Cells/ml x 10^3	Chlorophyll a µg/L	Chlorophyll a µg/cell x 10^{-6}	Chlorophyll c µg/L	Chlorophyll c µg/cell x 10^{-6}
February 2						
0930	surface	55	59.4	1.08	110.6	2.02
	10	41	68.5	1.67	77.7	1.90
	50	55	67.7	1.23	101.8	1.85
1130	sfc	54	31.8	0.59	0.0	0.00
	10	86	74.2	1.86	28.1	0.32
	50	67	81.0	1.21	67.7	1.01
1330	sfc	75	41.5	0.55	18.3	0.24
	10	50	64.7	1.28	57.4	1.14
	50	40	77.2	1.93	47.4	1.19
1630	sfc	56	45.9	0.82	33.5	0.60
	10	40	78.5	1.96	54.1	1.35
	50	89	78.9	0.88	85.1	0.95
2000	sfc	61	54.8	0.90	81.0	1.33
	10	102	99.2	0.97	68.8	0.67
	50	126	94.2	0.75	80.6	0.64
2200	sfc	48	55.7	1.16	56.9	1.18
	10	88	102.1	1.16	63.4	0.72
	50	189	89.1	0.43	53.5	0.28
February 3						
0000	sfc	56	56.6	1.01	71.6	1.28
	10	100	111.0	1.11	121.8	1.22
	50	119	81.3	0.41	58.5	0.29
0200	sfc	76	84.6	1.11	182.0	2.40
	10	112	109.2	0.99	65.0	0.58
	50	119	127.6	0.98	163.0	1.37
0330	sfc	105	71.8	0.68	175.8	1.67
	10	96	120.2	1.25	122.2	1.27
	50	134	71.6	0.54	244.4	1.82
0500	sfc	109	64.6	0.59	57.4	0.63
	10	125	143.8	1.15	134.4	1.07
	50	117	73.4	0.63	241.8	2.07
0730	sfc	80	60.8	0.76	69.2	0.86
	10	110	135.4	1.23	203.6	1.85
	50	180	121.2	0.67	90.2	0.50

Table 13. Continued.

Date, Time	Simulated depth, meters	Cells/ml x 10^3	Chlorophyll a µg/L	Chlorophyll a µg/cell x 10^{-6}	Chlorophyll c µg/L	Chlorophyll c µg/cell x 10^{-6}
1000	sfc	97	76.8	0.79	63.4	0.65
	10	154	192.2	1.25	142.6	0.93
	50	132	138.2	1.04	123.2	0.93
1200	sfc	59	69.2	1.17	100.6	1.71
	10	140	152.6	1.10	25.4	0.18
	50	104	148.2	1.43	122.0	1.17
1400	sfc	77	59.6	0.77	30.2	0.39
	10	131	154.0	1.18	89.0	0.68
	50	110	121.2	1.10	90.2	0.82
1630	sfc	72	70.8	0.98	146.2	2.03
	10	149	217.8	1.46	106.6	0.72
	50	104	169.6	1.63	79.0	0.76
1900	sfc	50	71.2	1.43	130.2	2.60
	10	139	184.2	1.33	195.8	1.41
	50	93	146.2	1.57	30.4	0.33
2030	sfc	46	67.8	1.48	54.8	1.19
	10	137	212.2	1.55	173.4	1.26
	50	123	154.6	1.26	73.0	0.59
2200	sfc	52	80.2	1.54	91.8	1.77
	10	136	167.4	1.26	68.8	0.51
	50	126	158.1	1.26	76.3	0.60
February 4						
0000	sfc	47	79.6	1.70	118.0	2.53
	10	135	170.5	1.26	66.3	0.49
	50	128	154.5	1.21	94.8	0.74
0200	sfc	53	67.3	1.25	71.0	1.34
	10	176	168.8	0.96	64.6	0.37
	50	140	147.4	1.05	94.8	0.55
0330	sfc	67	98.2	1.47	139.8	2.09
	10	221	216.0	0.98	101.0	0.46
	50	122	211.0	1.75	159.6	1.32
0550	sfc	86	76.2	0.89	79.6	0.93
	10	226	182.6	0.81	72.2	0.32
	50	131	147.6	1.13	115.8	0.89

Table 13. Continued.

Date, Time	Simulated depth, meters	Cells/ml x 10^3	Chlorophyll a µg/L	Chlorophyll a µg/cell x 10^{-6}	Chlorophyll c µg/L	Chlorophyll c µg/cell x 10^{-6}
0730	sfc	67	94.2	1.41	80.6	1.21
	10	216	253.4	1.14	124.6	0.58
	50	149	157.8	1.06	70.4	0.47
1015	sfc	69	79.2	1.15	128.6	1.87
	10	205	252.8	1.23	143.4	0.70
	50	140	177.8	1.28	79.4	0.60
1200	sfc	82	97.4	1.19	80.0	0.98
	10	215	312.0	1.45	165.2	0.77
	50	137	164.8	1.20	55.2	0.30
1400	sfc	89	84.0	0.94	26.4	0.30
	10	230	287.4	1.25	199.0	0.87
	50	150	205.0	1.37	210.4	1.41
1630	sfc	82	96.2	1.17	110.2	1.34
	10	293	334.8	1.14	137.6	0.43
	50	117	191.2	1.39	66.2	0.56
1900	sfc	150	111.8	0.74	97.8	0.65
	10	275	334.6	1.22	110.0	0.40
	50	132	191.2	1.45	80.8	0.61
2230	sfc	67	87.8	1.31	0.0	0
	10	235	287.4	1.22	97.2	0.41
	50	130	256.2	1.90	166.8	1.25

Light Schedule: See Table 2 for energies involved.

	ON	OFF
Low Light	0400	2000
Medium Light	0600	1800
High Light	0900	1500

Table 14. Diel Periodicity Experiment Using Skeletonema costatum, 9 Hour Light - 15 Hour Dark Cycle.

Date, Time	Simulated depth, meters	cells/ml x 10^3	Chlorophyll a µg/L	Chlorophyll a µg/cell x 10^{-6}	Chlorophyll c µg/L	Chlorophyll c µg/cell x 10^{-6}
March 1, '67						
1330	surface	59.0	32.6	0.55	20.7	0.35
	10	47.0	47.6	1.01	47.7	1.01
	50	43.7	36.6	0.84	37.0	0.85
1530	sfc	50.0	48.6	0.97	62.5	1.25
	10	65.3	64.4	0.99	13.7	2.10
	50	45.3	33.9	0.75	50.9	1.15
1700	sfc	59.5	35.9	0.60	39.1	0.66
	10	64.5	48.7	0.75	39.1	0.61
	50	89.8	55.0	0.62	53.5	0.60
1915	sfc	49.0	62.3	1.27	20.4	0.42
	10	-----	51.5	----	80.4	----
	50	100.0	56.4	0.56	56.3	0.56
2130	sfc	60.0	34.7	0.58	26.8	0.45
	10	86.0	63.7	0.74	42.6	0.50
	50	97.0	80.8	0.83	28.9	0.30
2300	sfc	50.0	38.7	0.77	57.6	1.15
	10	137.0	59.3	0.43	38.3	0.38
	50	141.0	89.7	0.64	217.7	1.53
March 2, '67						
0230	sfc	80.0	42.7	0.53	35.5	0.44
	10	101.5	67.9	0.63	54.9	0.52
	50	146.5	74.6	0.70	33.9	0.32
0500	sfc	81.5	55.4	0.68	64.9	0.80
	10	105.5	67.9	0.63	54.9	0.52
	50	106.5	74.6	0.70	33.9	0.32
0800	sfc	69.0	528.0	0.77	51.4	0.29
	10	142.0	68.7	0.48	30.8	0.25
	50	115.5	70.2	0.61	29.5	0.39
1000	sfc	125.0	62.4	0.50	35.8	0.29
	10	107.5	73.3	0.68	27.0	0.25
	50	83.5	82.0	0.98	32.7	0.39
1200	sfc	78.0	469.0	0.60	37.4	0.48
	10	115.0	863.0	0.75	47.9	0.42
	50	97.5	86.3	0.88	47.9	0.49

Table 14. Continued.

Date, Time	Simulated depth, meters	Cells/ml x 10^3	Chlorophyll a µg/L	Chlorophyll a µg/cell x 10^{-6}	Chlorophyll c µg/L	Chlorophyll c µg/cell x 10^{-6}
1330	sfc	50.0	68.1	0.36	107.6	2.15
	10	168.0	85.5	0.51	72.1	0.43
	50	74.0	99.2	1.34	68.8	0.93
1530	sfc	75.0	65.4	0.87	151.9	2.02
	10	156.0	109.0	0.70	92.3	0.59
	50	76.0	102.4	1.63	116.1	1.53
1700	sfc	65.0	77.7	1.20	78.3	1.21
	10	145.5	126.1	0.87	128.2	0.88
	50	111.0	118.6	1.07	76.7	0.69
1900	sfc	120.5	66.8	0.55	40.1	0.33
	10	154.5	110.4	0.72	52.1	0.34
	50	102.5	108.8	1.06	61.7	0.60
2130	sfc	92.0	84.4	0.92	57.2	0.62
	10	149.0	119.2	0.80	60.6	0.41
	50	105.0	107.3	1.02	54.6	0.52
2400	sfc	99.0	74.0	0.75	60.8	0.61
	10	123.0	120.7	0.98	70.2	0.57
	50	118.0	115.8	0.98	71.1	0.60
March 3, '67						
0230	sfc	137.5	98.0	0.71	15.1	0.11
	10	143.0	127.8	0.40	77.2	0.54
	50	133.0	136.2	1.02	93.8	0.70
0500	sfc	103.0	99.5	0.90	50.0	0.45
	10	165.5	148.1	1.35	99.8	0.63
	50	149.0	143.7	1.30	94.5	0.86
0800	sfc	110.0	99.5	0.90	50.0	0.45
	10	159.0	148.1	1.35	99.8	0.63
	50	110.0	143.7	1.30	94.5	0.86
1000	sfc	120.0	72.8	0.66	43.1	0.36
	10	200.0	127.1	0.64	43.4	0.22
	50	97.0	125.4	1.29	55.6	0.58
1200	sfc	130.0	108.4	0.83	58.4	0.45
	10	190.0	141.8	0.75	40.6	0.22
	50	140.0	152.9	1.09	96.0	0.69

Table 14. Continued.

Date, Time	Simulated depths, meters	Cells/ml x 10^3	Chlorophyll a µg/L	Chlorophyll a µg/cell x 10^{-6}	Chlorophyll c µg/L	Chlorophyll c µg/cell x 10^{-6}
1430	sfc	82.0	81.9	1.00	43.6	0.53
	10	148.0	157.1	1.06	61.4	0.41
	50	139.0	156.8	1.13	69.4	0.47
1700	sfc	95.0	135.4	1.42	117.9	1.14
	10	146.0	198.4	1.36	137.6	0.95
	50	130.0	224.8	1.73	101.2	0.78

Light Schedule: See Table 2 for energies involved.

	ON	OFF
Medium Light	0800	1700
High Light	1100	1400

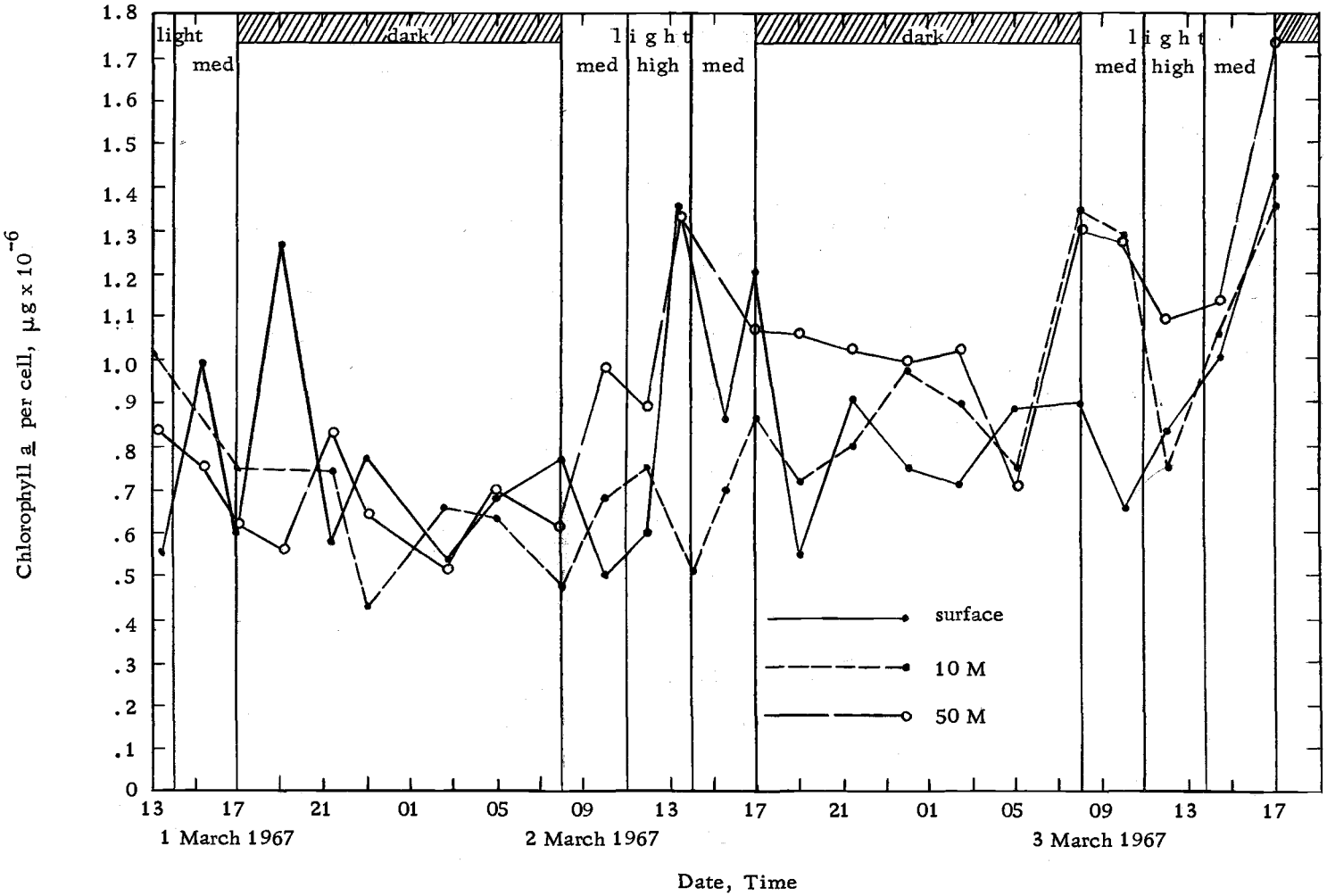


Figure 27. Chlorophyll a per cell of S. costatum, 9 hour light - 15 hour dark cycle.

at the end of the second light period to the minimum at the end of the first dark period was 2. 7. The concentration of chlorophyll a per cell did not appear to be significantly different at the three "depths. "

Chlorophyll c (Fig. 28) gave results similar to chlorophyll a. With the exception of the maxima at the "surface" and "50 meters" during the middle of the first dark period, highest values of this pigment (per cell) occurred at the end of the light period, and lowest values at the end of the dark period. The greatest variation was seen at the surface between the maximum during the second light period and the preceding minimum at the beginning of the light period (a ratio of 7. 4).

Thus, during this experiment, both chlorophyll a and c increased during the light period to a maximum value, and decreased during the dark period to a minimum value near the end of the dark period. This occurred at all three "depths. "

The Effect of High Intensity Light on Chlorophyll Concentration in Skeletonema costatum

A three-liter culture of Skeletonema costatum was grown to log phase in enriched sea water at a light intensity of 200 foot-candles, then placed in the dark at a cell concentration of 210, 000 cells per milliliter. After 24 hours in the dark, the cell concentration had increased slightly to 270, 000 cells per milliliter. Fifty-milliliter

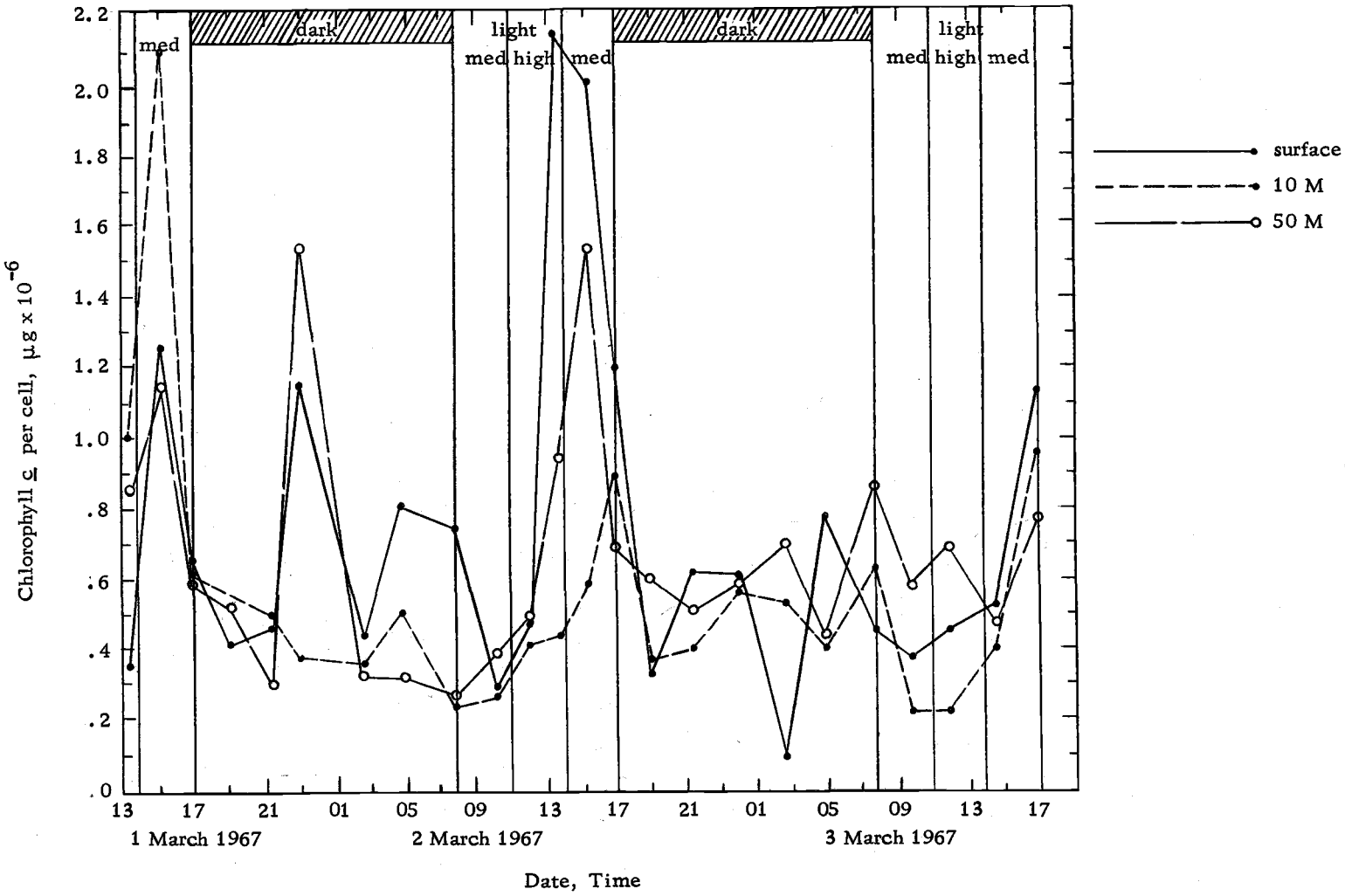


Figure 28. Chlorophyll c per cell of S. costatum, 9 hour light - 15 hour dark cycle.

aliquots of this darkened culture were placed in a filter funnel and exposed to light of 6500 foot-candles from a Sylvania tungsten-iodine lamp for a duration of 5, 30, 60, and 180 seconds. Then the cells were filtered immediately and analyzed for chlorophylls a and c (Table 15, Fig. 29). Dark controls also were run. The experiment was carried out in a darkened room in order to prevent changes in pigment concentration in the ambient light.

Table 15. Effect of High Intensity Light Duration on Chlorophyll Concentration in Skeletonema Costatum, Cells Kept in Dark for 24 Hours Prior to Experiment.

Light Exposure, Seconds	Chlorophyll a		Chlorophyll c	
	µg/L	% Dark Control	µg/L	% Dark Control
0	13.6	100.0	4.0	100.0
5	17.3	122.1	7.2	192.4
30	17.7	130.0	6.1	152.6
60	15.2	111.1	8.6	215.0
180	7.7	56.6	6.7	167.5

The concentration of chlorophyll a increased 22.1 percent in five seconds, but only 30.0 percent in 30 seconds. The concentration declined with longer exposures to the high light intensity. Chlorophyll c showed a rapid increase in five seconds, but then levelled off and did not decline below the concentration of the dark control as chlorophyll a did (Fig. 29).

The same experiment was repeated after the culture was diluted 1:1 by volume with unenriched sea water, and placed under 200

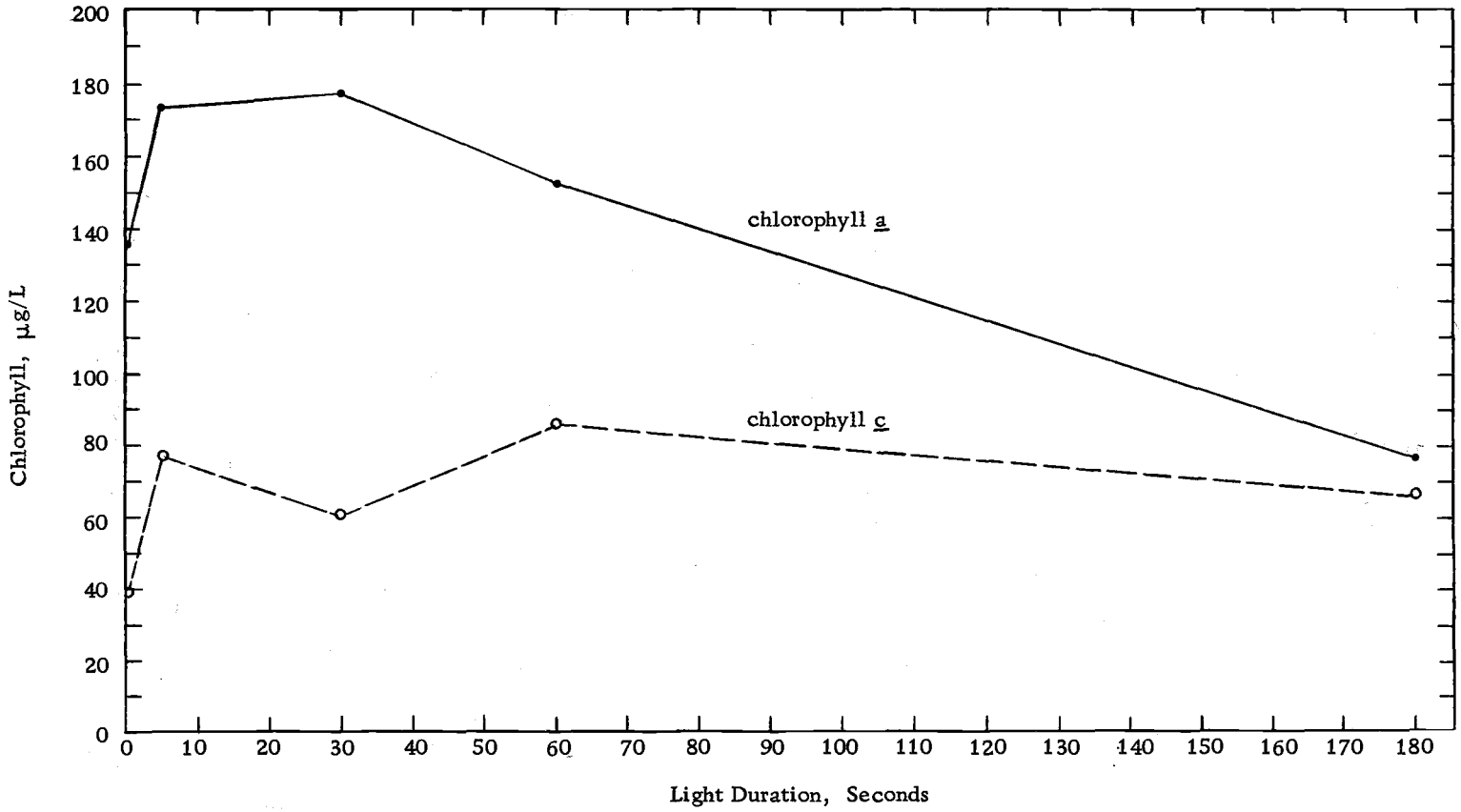


Figure 29. Chlorophyll a and c per cell of S. costatum after exposure to 6500 f. c. light intensity. Cells grown in dark for 24 hours prior to exposure.

foot-candles for 24 hours. The cell concentration was adjusted to 270,000 per milliliter for comparison with the previous experiment After 180 seconds light duration, the tungsten-iodine light source was moved to two different distances from the culture solution in the filter funnel to determine the effect of lower light intensities on the pigments after 180 seconds light duration (Table 16).

Table 16. Effect of High Intensity Light Duration on Chlorophyll Concentration in Skeletonema costatum. Cells were Kept in Continuous Light Prior to Experiment.

Light Exposure, Seconds	Chlorophyll a		Chlorophyll c	
	μg/L	% Control	μg/L	% Control
0	166	100.0	98	100.0
5	166	100.0	109	111.3
30	163	98.3	111	113.4
60	140	84.3	91	94.9
180, 6500 f.c.	103	62.1	89	90.8
180, 3800 f.c.	147	88.6	93	95.0
180, 2100 f.c.	167	100.6	106	115.2

Chlorophyll a (Fig. 30) remained fairly constant up to 30 seconds, but then declined to 62.1 percent of the control after 180 seconds exposure to the light source. Chlorophyll c increased by 11.3 percent after five seconds illumination, but decreased below the control after 60 seconds exposure, with no subsequent decrease at the end of 180 seconds illumination.

Comparing the results obtained with the culture kept in the dark

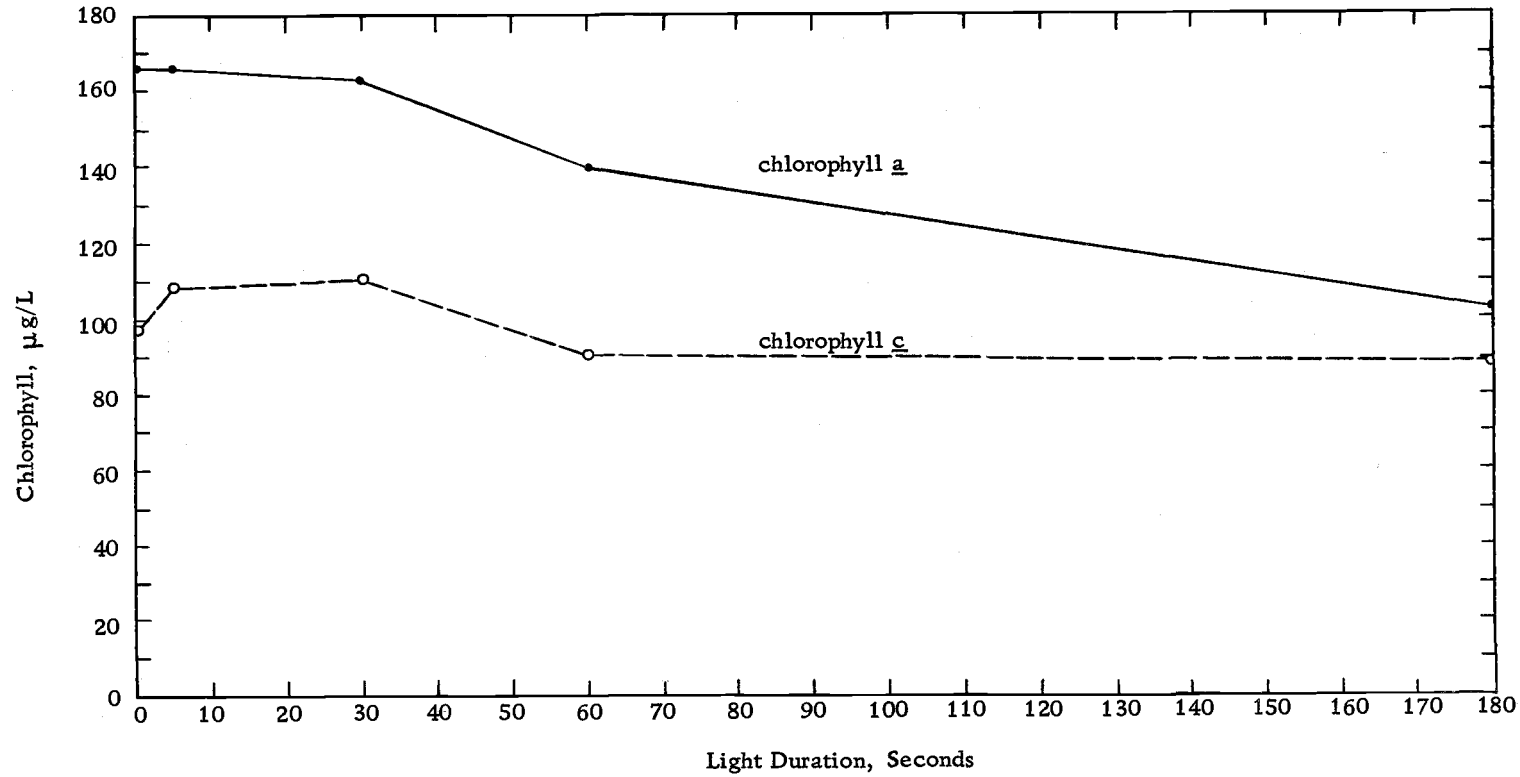


Figure 30. Chlorophyll a and c per cell of S. costatum after exposure to 6500 f. c. light intensity.

for 24 hours, and the culture kept in the light for 24 hours prior to the experiment, it appears that after five seconds illumination in the dark culture, some precursor of chlorophyll a and c was converted into these two pigments. But in the culture kept in the light, the precursor had already been converted to the respective chlorophyll, so no increase was seen. Also, in the light-grown culture, decrease of the chlorophyll a began after 30 seconds exposure to the light source, but in the dark-grown culture, a decrease did not occur until after 60 seconds exposure to the source. In the case of chlorophyll c, no decrease of this pigment took place in the dark-grown culture at the different time intervals of exposure to the light, but decreases were noted in the culture kept in the light. It appeared that previous light history of the cultures might influence the reaction of pigments to high light intensities. In this experiment, the light-grown cells appeared to be more sensitive to higher light intensities than the dark-grown cells. However, if one compares the chlorophyll at end of 180 seconds to the maximum value formed after short exposure to the light, the dark cultures showed a 56.5% decrease compared to 38.9% in the culture kept continuously in the light.

When the light-grown cells were exposed to 180 seconds of lower light intensity, both chlorophyll a and c approached the controls as light intensity decreased. Thus, there appears to be an intensity-time relationship involved in pigment decline that is induced

by higher light intensities.

The Effect of Heterotrophic Nutrition on the Decline of Chlorophyll in the Dark

Two liters of Skeletonema costatum culture were grown in nutrient-enriched sea water. Upon the attainment of the log phase of growth, five milligrams of carbon as glucose and five milligrams of carbon as pyruvic acid were added per liter. The culture was then placed in the light bath at an intensity of 400 foot-candles and cycled for 12 hours light and 12 hours dark. The culture flask was covered with a black hood in the dark to prevent any light from entering. After one light-dark cycle, samples were withdrawn every hour for four hours in the light and five hours in the dark and analyzed for chlorophylls a and c. In addition, cell counts were made to put pigment results on a "per cell" basis. The data are presented in Table 17. Compared to the control, the concentration of chlorophyll a per cell in the culture to which the external carbon source was added stayed fairly constant in the dark for a period of four hours (Fig. 31). Then, a decrease in concentration was noticed. In the control, the concentration of this pigment decreased throughout the dark period. The concentration of chlorophyll c decreased throughout the dark period in the control. In the culture where the external carbon source was added, the chlorophyll c

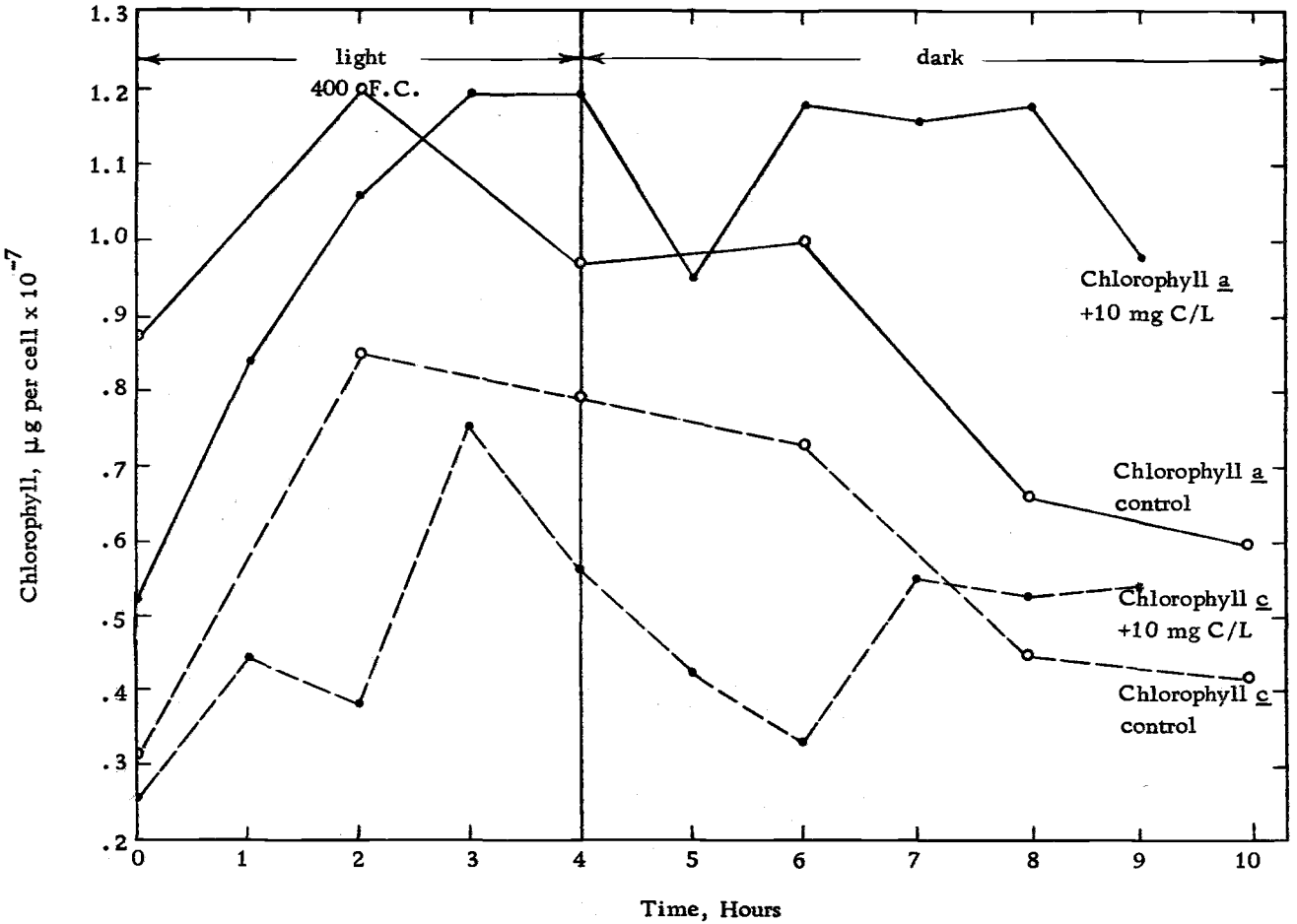


Figure 31. Chlorophyll a and c per cell of S. costatum grown with external carbon source.

Table 17. Chlorophyll Concentration in Skeletonema costatum Cells Supplied with an External Carbon Source.

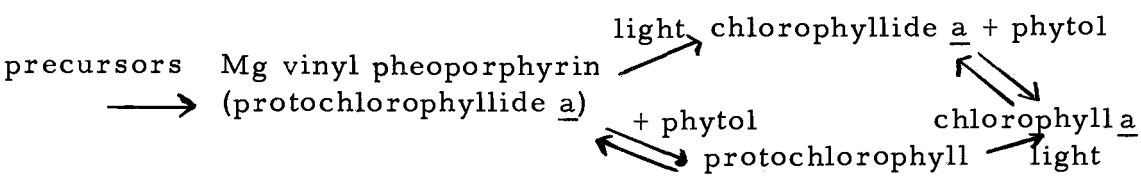
Time of Sample, Hours	Cells/ml	Chlorophyll a		Chlorophyll c	
		μg/L	μg/Cell x 10^{-7}	μg/L	μg/Cell x 10^{-7}
0	450,000	23.3	0.52	11.0	0.25
1	397,000	34.6	0.84	17.6	0.44
2	362,000	38.6	1.06	13.8	0.38
3	396,000	47.4	1.20	29.5	0.75
4	391,000	46.6	1.19	22.0	0.56
5	456,000	43.5	0.95	19.2	0.42
6	455,000	53.3	1.18	15.3	0.33
7	420,000	49.3	1.16	23.2	0.55
8	395,000	46.5	1.18	20.9	0.53
9	432,000	422	0.98	23.4	0.54
		Control (no external carbon)			
0	112,000	96.8	0.87	34.0	0.31
1	-------	----	----	----	----
2	106,000	130.1	1.20	90.0	0.85
3	-------	----	----	----	----
4	174,000	167.0	0.96	138.0	0.79
5	-------	----	----	----	----
6	166,000	167.4	1.00	122.0	0.73
7	-------	----	----	----	----
8	245,000	159.2	0.65	112.2	0.46
9	-------	----	----	----	----
10	271,000	168.6	0.62	88.6	0.33

decreased in the dark for the first two hours, but then increased to a fairly constant value for the next three hours. Apparently, the added carbon source maintained the chlorophyll in the cell in the dark.

DISCUSSION

The Biosynthesis of Chlorophyll

Several biochemical studies have been made upon the biosynthesis and degredation of chlorophyll which are pertinent to the interpretation of the field and laboratory experiments on chlorophyll periodicity. The biosynthesis of chlorophyll a has been summarized by Aronoff (1959) as follows:



Light is necessary for chlorophyll a synthesis, but not for protochlorophyll synthesis, or the conversion of chlorophyllide a to chlorophyll a. Wolff and Price (1957) had previously shown that light was necessary to convert the protochlorophyllide to the chlorophyllide a which can be converted in the dark to chlorophyll a with the addition of the alcohol, phytol. They also showed that chlorophyll a can revert back to the chlorophyllide by action of the enzyme chlorophyllase.

Virgin (1958) believed that protochlorophyll was the most important precursor of chlorophyll a and could be formed in the dark. Light is necessary to convert protochlorophyll to chlorophyll a.

This conversion takes place fairly rapidly. Madsen (1963) found that chlorophyll a was formed from protochlorophyll within four milliseconds after a one millisecond illumination period. Virgin (1964) found that this conversion in a leaf grown in the dark consisted of a rapidly completed stage after a two minute illumination in ordinary day light, followed by a slower stage which was followed by another rapid stage of chlorophyll a synthesis. He felt that all chlorophyll a was formed from protochlorophyll.

The lack of experimental evidence implicating chlorophyllide a in chlorophyll a synthesis was explained by Holden (1963), who found that chlorophyllide a was rapidly converted to chlorophyll a as soon as it was formed from protochlorophyllide a. She believed that the enzyme chlorophyllase played an important role in the synthesis of chlorophyll a, because when etiolated leaves were placed in the light she noticed an increase in activity of this enzyme.

As we have seen, some studies on the diel periodicity of pigments in algae have shown increases of chlorophyll in the dark. There is evidence that this pigment can be synthesized in the dark. Seybold and Egle (1938) saw chlorophyll increases in the dark in certain plants which were previously grown in the dark, exposed to light, then returned to the dark. Kutiurin (1956) felt that decomposition and restitution of the chlorophyll molecule takes place both in the light and the dark. Godnev, Shlyk, and Rotfarb (1959) showed

chlorophyll synthesis in the dark in several higher plants, mainly gymnosperms, although the chlorophyll levels in dark-grown plants were never as high as those in light-grown plants. Sudyina (1963) showed that chlorophyllase activity greatly increased during the first few minutes of illumination, and concluded that this enzyme does not take part in chlorophyll breakdown in vivo. This worker showed chlorophyll synthesis in the dark in several gymnosperms with the oldest plants on an evolutionary scale showing the greatest synthesis in the dark.

Studying Pinus jeffreyi embryos, Engvild (1964) found chlorophyll synthesis in the dark with up to two-thirds the level of chlorophyll in light controls being formed. Certain mineral elements, urea, and sucrose induced considerable chlorophyll synthesis in the dark, and B-vitamin and amino acid additions enhanced the synthesis. Bogorad (1965) also reported that several algae can synthesize chlorophyll in the dark, and therefore must have enzyme systems capable of reducing protochlorophyll or protochlorophyllide a.

A possible reason for the decrease of chlorophyll level in the dark was given by Franks and Kenney (1955). Using corn seedlings, they noticed that chlorophyll decreased in the dark and decided that light was necessary for the maintenance of this compound in vivo. They grew seedlings for one week in the light and then placed them in the dark and measured chlorophyll with time. After 120 hours in

the dark, the pigment had disappeared. Additions of three percent sucrose solution protected chlorophyll depletion for a time, and prevented the total destruction of chlorophyll.

I mentioned previously that certain researchers such as Yentsch and Scagel (1958) attributed the decline in chlorophyll concentration during the day to photo-oxidation of the pigment (Yentsch and Lee, 1966). Several studies have been made on the effect of high light intensity on chlorophyll. Aronoff (1959) found that at high light intensities chlorophyll a was converted to a leucochlorophyll which did not absorb light at the same wavelength as chlorophyll a. Coleman and Rabinowicz (1959) showed evidence of the formation of a stable, pink-colored chlorophyll at high light intensities which they named eosinophyll. This compound was formed at the high light intensities at which light saturation of photosynthesis occurs. It appeared to be similar to the leucochlorophyll that Aronoff had previously mentioned.

Photo-oxidation processes were studied in Chlorella by Sironval and Kandler (1958). They demonstrated that bleaching of the chlorophyll molecule occurred only at light intensities greater than 4650 foot-candles, with a seven-fold increase in bleaching occurring between 6500 and 9300 foot-candles. Above 6500 foot-candles rapid destruction of the chlorophyll molecule took place. The effect of temperature on this process was negligible. The induction of

bleaching was reversed in the dark. Kandler and Sironval (1959) found that during the initial period of bleaching, photosynthesis was inhibited, endogenous respiration increased, and oxidative assimilation and the rate of phosphorylation decreased. They believed that radicals were formed along with peroxides which led to bleaching. They felt that this bleaching was a secondary effect that took place when the stable chloroplast structure was disrupted or partially destroyed as a result of the inhibition of metabolism. Holden (1963) also believed that chlorophyll could be bleached by non-enzymatic processes. It appears that pigment bleaching can account for the decrease of pigments during the higher light portions of the day, giving support to the reasons for diel pigment periodicity as advanced by Yentsch and Scagel (1958) and Yentsch and Lee (1966).

The possibility that an endogenous rhythm caused the diel variations in photosynthetic ability was advanced by Hastings, Astrachan, and Sweeney (1961). They indicated that, in Gonyaulax grown under a 12 hour light - 12 hour dark cycle, maximum photosynthesis occurred at the eighth hour of the light period. When the cells were transferred to continuous dim light, the rhythm continued, but the rhythm was not noticed under a continuous bright light. Lowest photosynthetic ability was found during the dark period. These authors found no periodicity of chlorophyll. Several studies have shown that pigment synthesis may be a phenomenon of red and

far red light. Price and Klein (1961) found that chlorophyll synthesis was stimulated by red light, but reversed by far red light. Mitrakos (1963) reported that both the synthesis of protochlorophyll and chlorophyll were involved with the red-far red phenomenon. Henshall and Goodwin (1964) studied chlorophyll synthesis in pea seedlings and discovered that short exposures to red light prior to constant illumination reduced the lag period of chlorophyll synthesis which was normally seen after the exposure of etiolated seedlings to light. Far red light reversed this stimulation.

Thus many factors appear to influence the biosynthesis of chlorophyll a. Little is known about chlorophyll c biosynthesis and degradation in plants. Granick (1949) proposed that chlorophyll c might arise from protochlorophyllide a, for no phytol is present in either compound, nor is part of the ring D structure reduced.

The biosynthetic pathways for chlorophyll synthesis are summarized in Fig. 32.

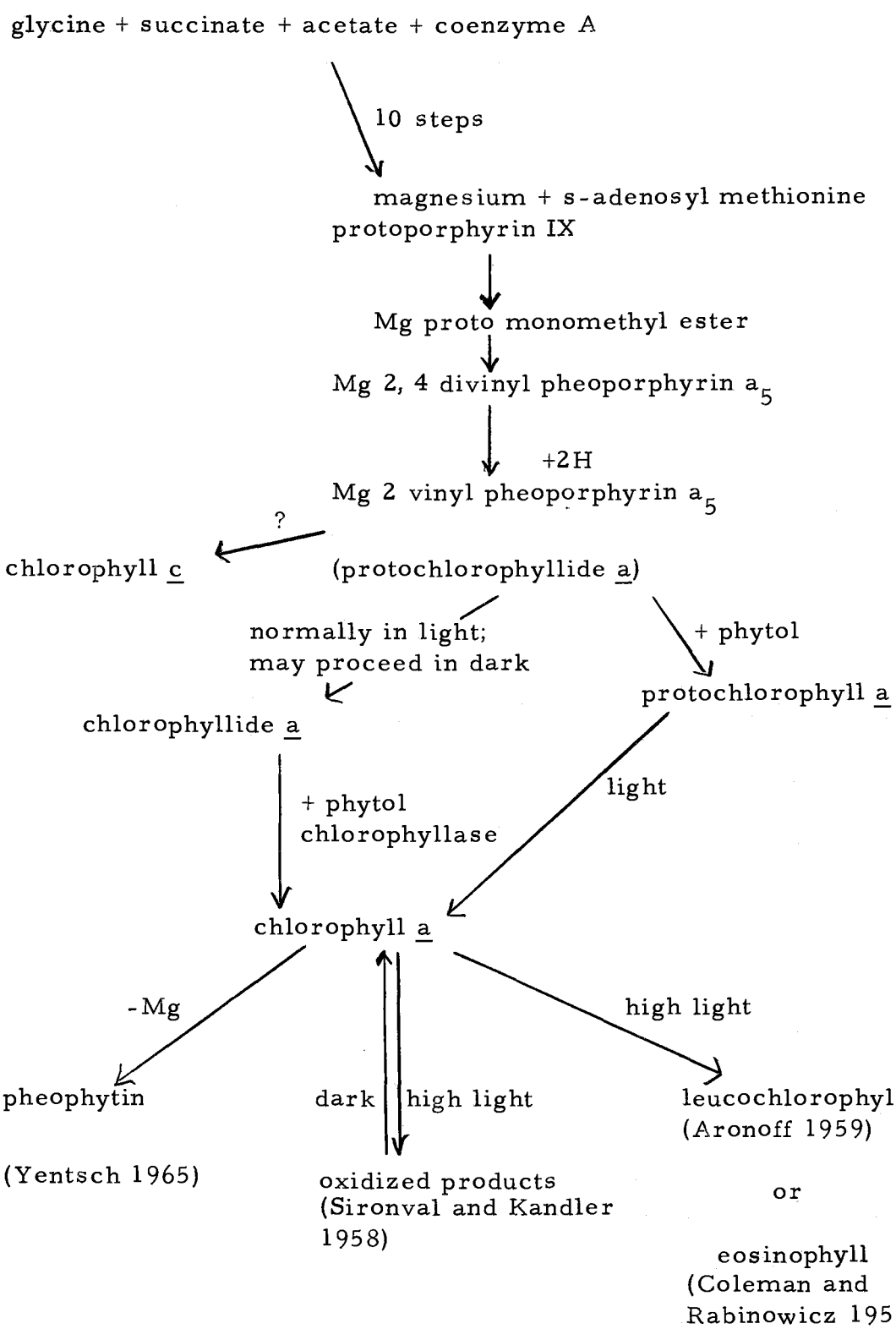


Figure 32. The Pathways of Chlorophyll Biosynthesis (Bogorad 1965).

Diel Periodicity of Chlorophyll *a* and *c* in Laboratory Cultures of *Skeletonema costatum*

The laboratory experiments were chosen to examine diel pigment periodicity under a range of photoperiods and light intensities. The 12 hour light-12 hour dark cycle was chosen as it is typical of early spring and fall photoperiods off the Oregon coast, and of tropical areas during most of the year. The 16 hour light-8 hour dark cycle would be found at higher latitudes during the summer, and the 9 hour light-15 hour dark cycle would be typical of winter light conditions off the Oregon coast. The light intensities used in these experiments were not as high as found in nature due to the type of lights that were available for use in the laboratory. The light intensity of 0.05 langleys per minute (equivalent to 1200 foot-candles) used to simulate surface conditions, was lower than the 1.9 langleys per minute that can occur during bright summer days at the surface off of the Oregon coast at noon. This may not be a serious problem because on most cruises bright, clear days were not encountered, especially during seasons other than summer.

The first laboratory experiment (Figs. 19 and 20) consisted of examining the diel periodicity of chlorophyll *a* and *c* in *Skeletonema costatum* exposed to 12-hour periods of light and dark. Both chlorophylls increased in the light period to maximum value at the end of the light period, then decreased (to the value found at the beginning

of the light period) during the following dark period. The ratio of maximum concentration of chlorophyll a at the end of the light period to the minimum concentration at the end of the following dark period was as high as 6. 6. The same ratio for chlorophyll c was as high as 120. 0, a ratio that appears to be much higher than found in nature so it may be in error. In general, the magnitude of these diel fluctuations decreased with time during the five-day period of the experiment; hence cell age may be a factor influencing the magnitude of diel periodicity that takes place. The results of this experiment agreed for the most part with previous work done on other algal species by Gibor and Meehan (1961), Yentsch and Reichert (1963), Edmonds (1965), Eppley and Coatsworth (1960 and Jorgensen (1966). However, the latter author explained synchronous cell division during the light period as the cause of this diel periodism. Synchronous cell division during either the dark or light period was not observed in any experiment done in this study. Also, synchronous cell division does not explain the reason for the decrease in pigment concentration observed during the dark period. Unfortunately, the authors expressed their results as the amount of chlorophyll found per given volume of culture solution. However, in a dividing population of cells, results expressed on a chlorophyll per cell basis are more meaningful. The results of this particular experiment did show that light appears to be necessary either directly or indirectly for

chlorophyll synthesis. Both chlorophyll a and c increased during the light period. Light may be necessary for production of a chlorophyll precursor. Or, photosynthesis may provide energy for chlorophyll synthesis, hence the requirement for light.

In an attempt to observe the nature of diel periodicity of chlorophyll at higher light intensities, the same experiment was repeated in the Sherer-Gillet Growth Chamber using Corning glass filters to simulate light intensity and quality at the depths of 10 and 50 meters in the ocean. In this experiment, cell numbers were fairly constant with time so results were plotted as micrograms chlorophyll a and c per liter (Figs. 21 and 23), and as micrograms per cell (Figs. 22 and 24). Chlorophyll a at the "surface" increased in the dark, reaching a peak value approximately three hours into the dark period. After this maximum, the concentration of chlorophyll a decreased to the middle of the following light period followed by an increase with chlorophyll a at the simulated "10 meter depth", but at the "50 meter depth", maxima were seen both in the light and dark periods with the maximum values occurring towards the end of the dark periods. The greatest amount of diel variation occurred at the "10 meter depth." The concentration of chlorophyll a per cell was not significantly different at the three "depths" used in this experiment. As for chlorophyll c, no definite diel periodicity was seen at the "surface" and "50 meters", but at "10 meters", this pigment was highest during the

dark decreasing to a minimum value about three hours before the end of the following light period. Chlorophyll a at the "surface" and "10 meters", and chlorophyll c at "10 meters" showed maximum concentrations during the dark period, with a subsequent decrease in concentration to the middle of the light period, and increases again into the following dark period. This is in contrast to the increase of pigment in the light and decrease in the dark as seen in the previous experiment. Both Castenholz (1964) and Yentsch (1965) found chlorophyll could increase in the dark in marine diatom species, but neither of these authors looked at these changes in chlorophyll over several light-dark cycles to see if a periodicity was present.

The same experiment was repeated using a 16 hour light-8 hour dark cycle, (Figs. 25 and 26). Both at the "surface" and "10 meters depth", chlorophyll a reached maximum values early in the dark period, decreased in the light period, then again increased to a maximum in the following dark period. At "50 meters", the opposite was seen with maximum concentrations of this pigment occurring in the light and minimum concentrations during the dark period. The chlorophyll c results (Fig. 26) did not show a diel periodicity.

In the 9 hour light-15 hour dark experiment, chlorophyll a per cell showed a diel periodicity at all three "depths" with maximum values occurring during the light periods, and minimum values in the dark periods in Fig. 27. Chlorophyll c (Fig. 28), showed

similar results. Again, no significant differences occurred among the chlorophyll contents of the cells at the three light levels that they were exposed to. Also, chlorophyll c again showed more variability than did chlorophyll a.

The results of the three experiments performed in the growth chamber are summarized in Fig. 33 ("surface"), Fig. 34 ("10 meters"), and Fig. 35 ("50 meters"). These results indicate that photoperiod length influences the diel periodicity of pigments as predicted by Terborgh and Thimann (1964). At the lower light intensities, used in the first experiment (400 foot-candles), at the simulated 50 meter depth, and at shorter light durations (9 hours), it appears that chlorophyll a and c are synthesized in the light. Previous work on chlorophyll biosynthesis by Virgin (1958) and Aronoff (1959), and the review article by Bogorad (1965), all show that this light-induced synthesis is to be expected. However, at the higher light intensities used, i. e., at the intensities used at the "surface" and "10 meters depth", both chlorophyll a and c were at their lowest concentrations during part of the light period, and increased to a maximum concentration in the cell during the dark period. These observations are in conflict with the results obtained at lower light intensities, but are explainable.

Chlorophyll a per cell reached a minimum value towards the middle to end of the light period at the higher light intensities used.

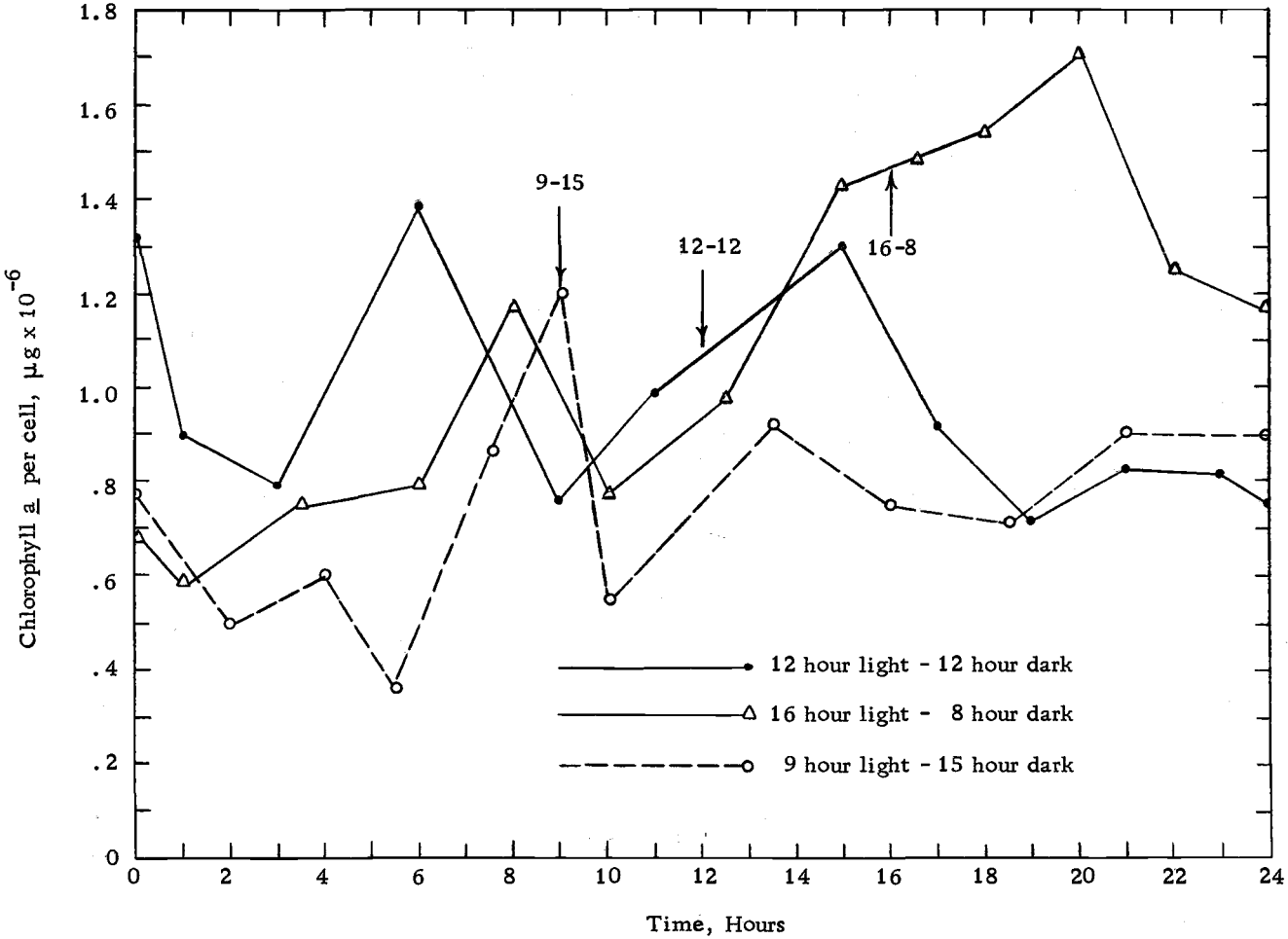


Figure 33. Chlorophyll per cell of S. costatum grown at three photoperiods under simulated surface conditions.

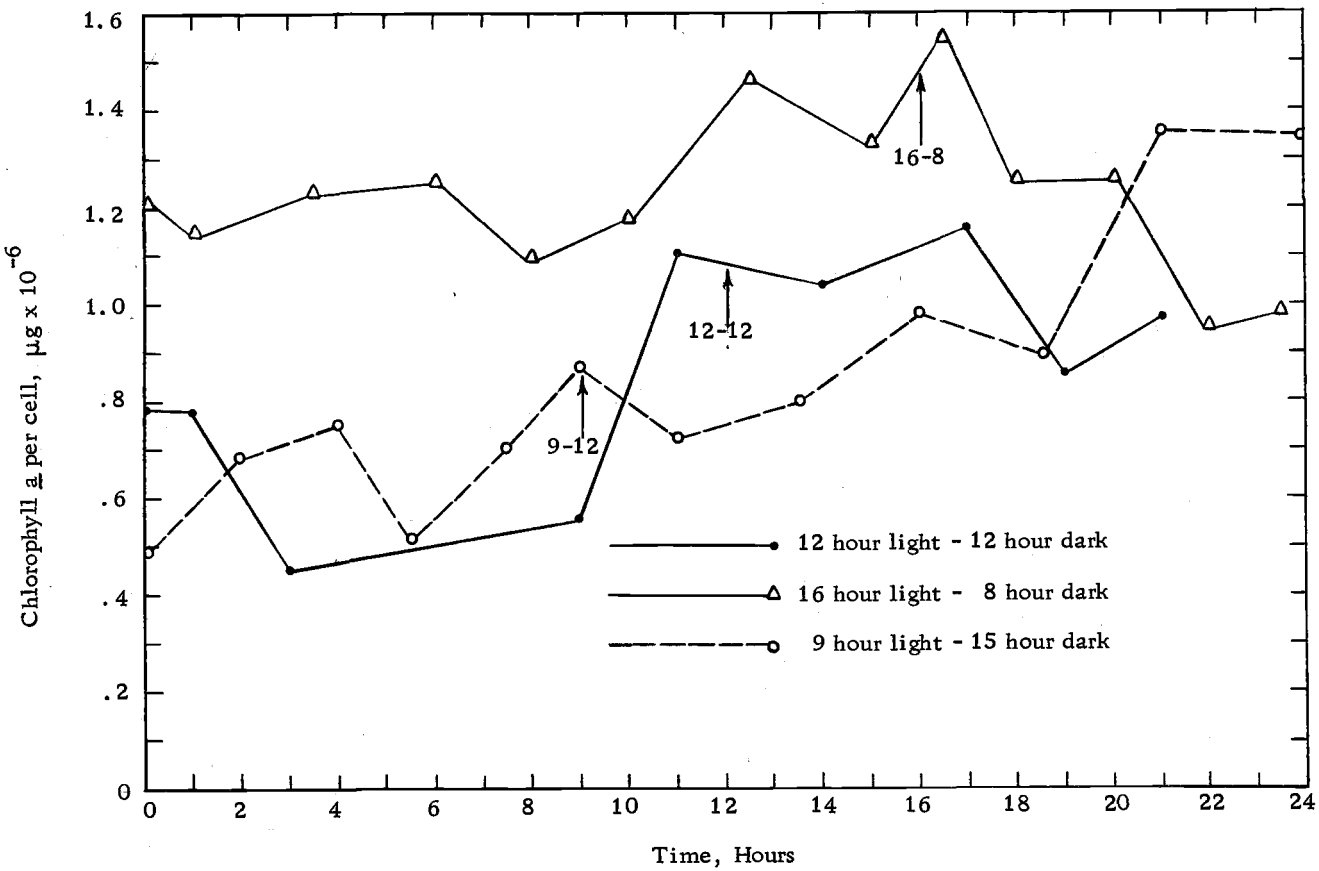


Figure 34. Chlorophyll per cell of S. costatum grown at three photoperiods under simulated 10 meter surface conditions.

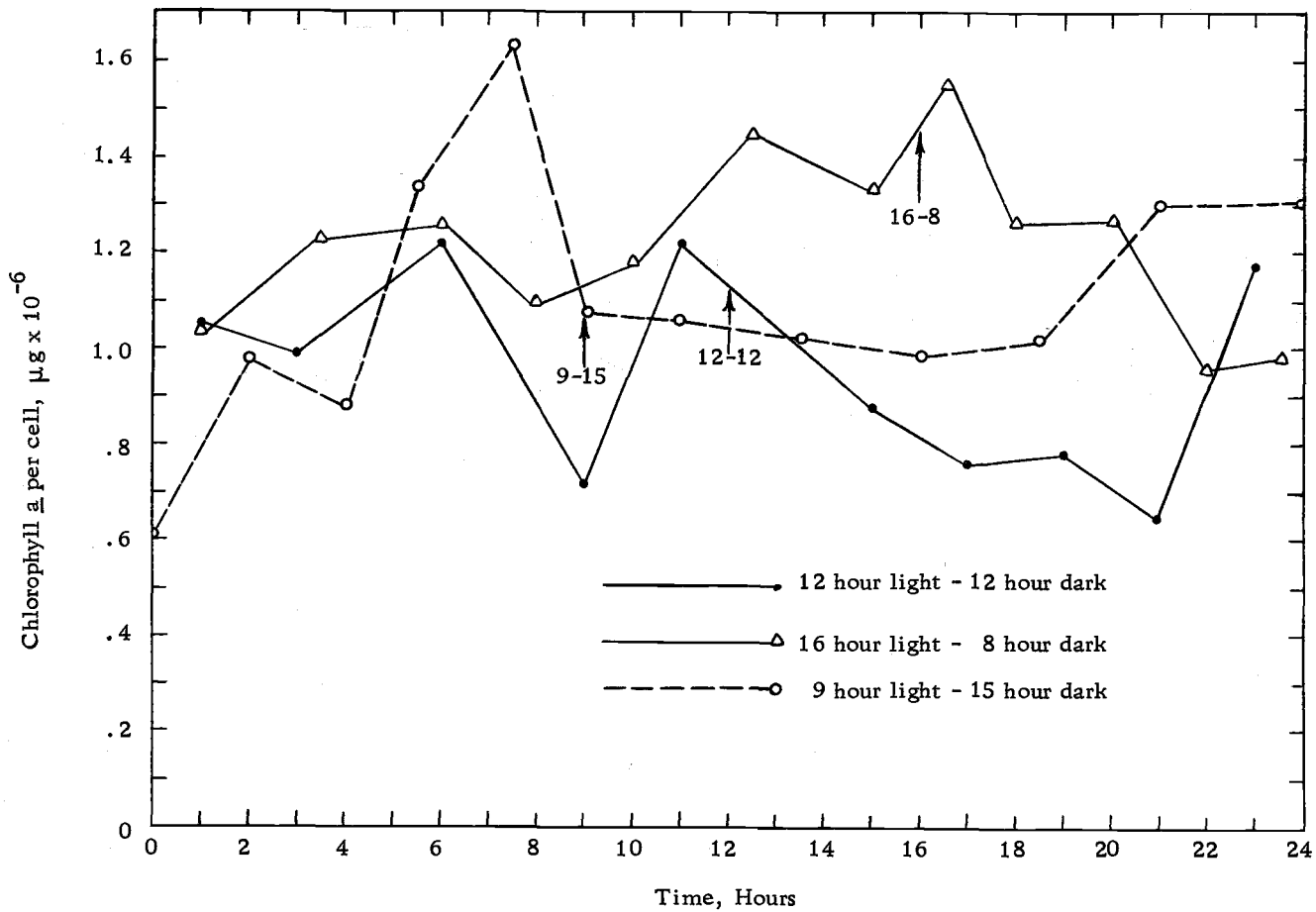


Figure 35. Chlorophyll per cell of S. costatum grown at three photoperiods under simulated 50 meter surface conditions.

Then, the concentration of this pigment started to increase during the latter part of the light period. The initial decrease at the beginning of the light period is most likely caused by a bleaching of chlorophyll by light at these higher intensities.

After a certain time interval in the light period at the higher intensities, the decrease of chlorophyll a and c ceased, and the level of this pigment began to increase. The reason for this beginning of synthesis in the light period after bleaching may be an internal adjustment to the high light intensity. Yentsch and Lee (1966) concluded that short-term adjustments rather than long-term adaptations in internal chlorophyll concentrations were made by marine phytoplankton in response to varying conditions of light. The results of these experiments offer proof of their hypothesis that the chlorophyll content of a cell is a balance between photo-oxidation at higher light intensities and pigment synthesis at lower light intensities. At light intensities approaching natural conditions, chlorophyll bleaching appears to be the reason for minimum concentrations of this pigment occurring during the light period. At lower light intensities, pigment synthesis occurs continually throughout the light period. This may explain the discrepancies in the literature in which investigators found chlorophyll increases during the light period. For example, Gibor and Meehan (1960) used 250 foot-candles, Yentsch and Reichert (1963) - 800 foot-candles, Edmonds (1965) - 330 foot-candles,

and Jorgensen (1966) - 280 foot-candles. All of these investigators, found chlorophyll synthesis throughout the light period probably because of the relatively low light intensities that they used. When low light intensities were used in this study (such as at the light level simulating 50 meters depth, and under the 400 foot-candle illumination used in the first laboratory experiment) chlorophyll increased only during the light period. At the higher light intensities, bleaching occurred during initial parts of the light period giving the diel pigment patterns that were seen.

The exact mechanism of the bleaching process is not felt to be important for the purposes of this study. The bleaching may be due to the conversion of chlorophyll to a leucochlorophyll as proposed by Aronoff (1959) or to the apparently similar eosinophyll (Coleman and Rabinowicz 1959). Also, the photo-oxidation of the chlorophyll molecule could explain this observed decrease in concentration (Sironval and Kandler, 1958, and Kandler and Sironval, 1959). Bleaching can occur quite rapidly after the exposure of cells to higher light intensities. In order to determine the speed of this process, an experiment was done by exposing Skeletonema costatum cells to the high light intensity of a tungsten-iodine lamp at 6500 foot-candles. The cells kept in the dark for 24 hours prior to exposure to light synthesized chlorophylls a and c for the first 30 seconds of the light exposure (Fig. 29). The rapid chlorophyll a and c synthesis during the

initial five seconds light exposure was probably due to conversion of some dark-formed chlorophyll precursor such as protochlorophyll to chlorophyll a (Madsen, 1963). Then, chlorophyll a decreased due to bleaching, until at 180 seconds exposure to light, 56.5 percent of the chlorophyll a formed at the end of 30 seconds exposure had been bleached. In the cells which were kept continuously in light (Fig. 30), 38.9 percent of the chlorophyll a was bleached at the end of 180 seconds exposure. Thus, the light-grown cells were more resistant to pigment bleaching than those kept in the dark for 24 hours, indicating that light history of the cells is an important factor in controlling the amount of diel chlorophyll variation in cell populations. Chlorophyll c appeared to be more resistant to bleaching even though it also showed diel variations in the previous experiments (Figs. 29 and 30). A relationship between intensity and exposure time shown was in this experiment. When the light intensity was reduced to 3800 foot-candles at a duration of 180 seconds, (Table 16), the levels of both chlorophyll a and c returned to that of the controls, while at 2100 foot-candles the levels of chlorophylls a and c were 88.6 and 95.0 percent of their controls, respectively.

During the first three or four hours of the dark period (depending on the experiment performed), both chlorophyll a and c per cell continued to increase in Skeletonema costatum when the cells were grown at higher light intensities and a photoperiod of at least 12 hours.

Dark synthesis of chlorophyll in diatoms was reported by Castenholz (1964) and Yentsch (1965). In higher plants, especially gymnosperms, dark chlorophyll synthesis was reported by Seybold and Egle (1938), Kutiurin (1956), Godnev, Shylk, and Rotfarb (1959), Sudyina (1963), Engvild (1964), and Bogorad (1965). The terminal steps of chlorophyll biosynthesis require light according to Virgin (1958), Aronoff (1959), and Madsen (1963). Therefore, either the terminal steps of chlorophyll biosynthesis in the dark in diatoms and certain gymnosperms must be different than in other plants, or the photoreduction of the chlorophyll a precursors, protochlorophyll and/or protochlorophyllide a, must be replaced by a dark enzyme system as postulated by Bogorad (1965).

The experiments performed in this study indicates that dark synthesis of chlorophylls a and c occurred only when cells were grown at higher light intensities for a duration of at least 12 hours. These results suggested that energy or a certain precursor is required for the dark synthesis of chlorophyll, and that sufficient quantities of the substrate that supplies this energy or acts as a precursor can only be formed when cells are grown at higher light intensities for a minimum amount of time. Engvild (1964) found evidence that certain metabolic substrates such as sugars, organic acids, and amino acids greatly stimulated dark synthesis of chlorophyll a in embroys of Pinus jeffreyi. The substrate required to supply this

energy may be one of the normal components of the glycolytic or tricarboxylic cycles of the plants, or a compound such as an amino acid which can be transformed into a component of one of these pathways.

In my study both chlorophyll a and c declined at the end of the light period when cells were grown at lower light intensities, and after several hours in the dark when grown at higher light intensities. The possible reason for this observation was given by Franks and Kenney (1955). They suggested that light was necessary for the maintenance of chlorophyll levels in corn seedlings, as the level of chlorophyll in these seedlings decreased in the dark. This decrease of chlorophyll in the dark was delayed in time by several hours (and decreased in magnitude) by additions of three percent glucose to the seedlings. This work suggests that a substrate is needed to provide energy or act as a precursor to prevent the chlorophyll a molecule from reverting back to one of its precursors in the dark, or possibly forming a degredation product such as phaeophytin. Franks and Kenney's work suggests that the energy-yielding substrate (or precursor) that maintains chlorophyll a levels in the dark can be formed heterotrophically in the dark when a substrate such as glucose is fed to the plant. Not enough is known about the biosynthesis of chlorophyll c to speculate about why it decreases in the dark.

Supporting evidence that energy-yielding substrate is necessary

in the dark to maintain the level of chlorophyll was obtained in the experiment in which cells of Skeletonema costatum were provided with an external carbon source consisting of five milligrams carbon per liter each of glucose and pyruvic acid. The concentration of chlorophyll a in the cell (Fig. 31) remained fairly constant in the dark after increasing in the light for four hours. The control, to which no external carbon was added, showed the typical decrease of chlorophyll in the dark as soon as the light was turned off. Chlorophyll c showed the same trend. It appears that the addition of an energy-yielding substrate or precursor can keep chlorophyll from decreasing in the dark. If cells in nature grew under conditions in which they had stored great quantities of the necessary substrate, they might retain chlorophyll in the dark for relatively long periods of time. Cells grown under conditions in which the substrate was not accumulated would not retain chlorophyll in the dark.

Diel Periodicity of Chlorophyll a and c at Sea

In the previous section, it was shown that when laboratory cultures of Skeletonema costatum were exposed to higher light intensities, peak chlorophyll a and c values occurred after several hours in the dark period, and lowest concentrations of these pigments were found around the middle of the light period. At lower intensities of light, such as those occurring in the ocean at 50 meters depth, chlorophyll

concentrations reached peak values in the light period, and minimum values in the dark period. Therefore, if light is the main factor controlling the diel periodicity of chlorophyll a and c in marine phytoplankton, and S. costatum is a representative species, the relations mentioned above should be seen in situ in the oceans.

A summary of chlorophyll a and c values measured during various cruises with the time of maximum and minimum pigment values at each depth and the ratio between these two extremes is shown in Table 18. Unfortunately, cell counts were not available during earlier cruises. Starting with Cruise 6602, samples for cell counts were collected, but difficulties were encountered in trying to concentrate the samples for reproducible cell counts. This lack of cell number data was not felt to be a serious problem as Yentsch and Ryther (1957), and Yentsch and Scagel (1958) reported that the magnitude of diel periodicity of pigments was greater than could be explained by changes in cell numbers.

In the samples in which a definite diel pigment periodicity was observed, concentrations of chlorophyll a and c showed trends similar to those observed in the laboratory experiments (Table 18). At the surface and at 10 and 15 meters depths where cells would be subjected to higher light intensities, maximum chlorophyll a and c concentrations were found at night, and minimum concentrations in the day, usually in the late afternoon. For example, during Cruises

Table 18. Summary of Times of Maximum and Minimum Chlorophyll at Sea; Ratios of Maximum to Minimum Chlorophyll.

Cruise, Date, Station	Depth, meters	Chlorophyll a Time of max.	Chlorophyll a Time of min.	Ratio max./min.	Chlorophyll c Time of max.	Chlorophyll c Time of min.	Ratio max./min.
6507							
July 20-21, '65	surface	0300	1700	3.3	0300	2100	9.8
NH-25	10	0100	1700	2.8	2300	2100	4.8
	15	2300	1700	3.4	2300	2100	6.7
	25	2100	1700	2.1	1025	2100	6.9
	50	2100-0800	1500	3.0	1025	2300	14.0
6509							
Sept. 25-26, '65	surface	0100	1400	3.5	0100	1115	5.2
SB	10	not clear	1200	---	2300	0600	3.0
	15	2300	1115	2.9	2300	0100	*
	25	maxima not apparent			1115	1400	7.5
	50	fairly constant			maxima not apparent		
6509							
Sept. 28-30, '65	surface	2335	1218	12.1	2335	1905	
NH-55	10	0240	1403	2.0	2335	1403	
	15	0240	2050	2.2	2335	0445	
	25	2335	1625	2.5	2125	0315	
	50	1403	2335	10.0	2125	2335	
6511							
Nov. 28-30, '65	surface	no definite maximum			data too variable,		
NH-25	10	0300-2250			maxima not apparent		
	15	maxima irregular			"	"	"
	25	maxima irregular			"	"	"
	50	maxima irregular			"	"	"
6602							
Feb. 19-21, '66	all depths	no definite maxima			no definite patterns		
NH-25		"	"	"	"	"	"
		"	"	"	"	"	"
		"	"	"	"	"	"
		"	"	"	"	"	"
YALOC-66							
June 8-10, '66	surface	0800			pattern irregular due to		
29°12'N, 161°	10	0420			cloud cover during day.		
30W	15	1015			See discussion.		
	25	0800					
	50	0400					

Table 18. Continued.

Cruise, Date, Station	Depth, meters	Chlorophyll a Time of max.	Chlorophyll a Time of min.	Ratio max. / min.	Chlorophyll c Time of max.	Chlorophyll c Time of min.	Ratio max. / min.
YALOC-66							
June 22-23, '66	surface	1900	0800	2.9	1900	0200	18.8
50°28'N,	10	2100	0800	3.4	1800	0200	2.3
176°14'W	15	2100	0200	2.0	1400	1030	19.5
	25	1800	0800	1.4	1800	1030	4.7
	50	1400	0200	1.7	1800	0200	5.2
6701							
Jan. 3-4, '67	surface	2230	0700	1.7	pattern too variable		
NH-25	10	1920	1200	2.7	"	"	"
	15	1700	0100	2.7	"	"	"
	25	1700	2230	1.9	"	"	"
	50	0300	1200	2.4	"	"	"

* At times, pigment not measurable.

6507 and 6509 at Stations SB and NH-55 down to 25 meters, maximum chlorophyll a values occurred at night and minimum values during the day. The opposite occurred at 50 meters on Cruise 6507 for chlorophyll a, and at 25 and 50 meters for chlorophyll c. Maximum pigment values occurred during the day, as at the 50 meter depth at station NH-55 for chlorophyll a. During the YALOC-66 cruise, at 29° 12'N, 161° 30'W, this pattern was not as apparent (discussed later), but at 50° 28'N, 176° 14'W maximum chlorophyll a values occurred in the early evening at depths down to 25 meters. In general, chlorophyll c data was much more variable than chlorophyll a, which probably reflected the nature of the method and equations used in measuring chlorophyll c (Richards and Thompson, 1952). Also, during cruises in the late fall and early winter, (Cruises 6511, 6602, and 6701), the presence of a diel periodism was hard to ascertain.

High pigment values at night, and lowest pigment values during the daylight hours at depths shallower than 25 meters, generally were in accord with the previous results of Yentsch and Scagel (1958) and El-Sayed and Mandelli (1965). Yentsch and Ryther (1957), Menzel and Vaccaro (1961), Lorenzen (1963), McAllister (1963), and Wood and Corcoran (1966) found that maximum chlorophyll concentrations occurred during the daylight hours. The data of Wood and Corcoran show increased chlorophyll at night when chlorophyll values were

plotted on a "per cell" basis. It is possible that a chlorophyll maximum could occur during the day if light was low due to cloud cover or if particularly heavy grazing by zooplanktonic herbivores had occurred during the previous night (McAllister, 1963). However, in Oregon coastal waters and at the two stations occupied during the YALOC-66 cruise in the North Pacific, chlorophyll values were highest at night above the 25 meter depth.

If grazing of phytoplankton by such zooplanktonic herbivores as copepods and euphausiids was the cause of diel pigment variations, then one would expect the number of phytoplankton cells to be lowest at night. Zooplankton have been shown to undergo diel vertical migrations by such investigators as Russell (1927), Clarke (1934), Cushing (1951), and Bainbridge (1961). The latter author summarized this vertical migration of animals as consisting of a rise to the surface layers of the ocean in the late afternoon around sunset due to a positive swimming towards a source of decreasing light intensity. This continues into the night, and at dawn, the animals migrate downward away from the increasing light. Evidence for this vertical migration is the deep scattering layer, an echo sounding trace which ascends toward the surface in the evening around sunset, remains there during the night and descends in the early morning around sunrise. Moore (1950), Tucker (1951), and Bary, Barraclough, and Herlinveaux (1962) have correlated this sound-reflecting layer with

the presence of euphausiids. These may graze on phytoplankton, or feed upon copepods which also graze on phytoplankton (Ponomareva, 1957). Thus the phytoplankton predators appear to be feeding at the surface layers at night and therefore should decrease the standing stock of phytoplankton (as evidenced by lower chlorophyll) at night. In the cruises since February 1966, this deep scattering layer was measured by using the echo sounder on the ship. The layer was generally below about 150 meters during the daylight hours, began to ascend at sunset to within 15-20 meters of the surface by 1900 or 2000 hours, and stayed there until sunrise when it began to descend. If grazing was a cause of pigment decrease at night as proposed by McAllister (1963) and Wood and Corcoran (1966) then highest values of pigment should be found during the day, not around midnight (Table 18). Even when no definite periodicity of chlorophyll was seen (Cruises 6511, 6602, and part of 6701), chlorophylls a and c were not lower at night than during the day. When total chlorophyll a and c down to 50 meters was integrated (Figs. 36-42), chlorophyll was generally higher at night, again conflicting with the idea of grazing causing a decrease of the standing stock at night. Also, in these figures, chlorophyll values were contoured in order to depict the spatial distribution of this pigments with time. Areas where contours are close together are indicative of rapidly changing chlorophyll concentrations. Where contours are widely spaced, chlorophyll values

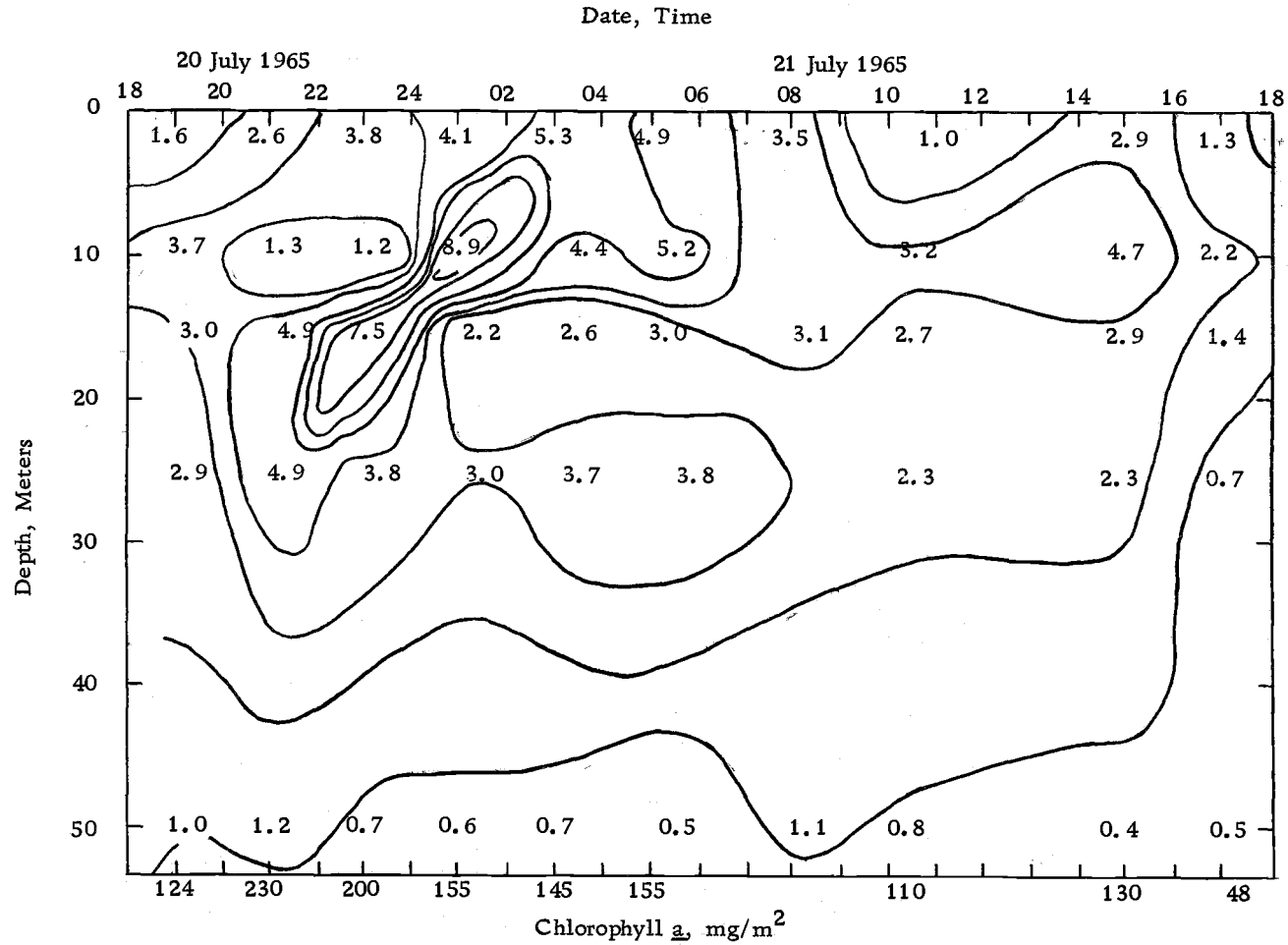


Figure 36. Chlorophyll a at depth vs. time, Cruise 6507, Station NH-25, 20-21 July, 1965, Contour interval = 1.0 mg/m^3.

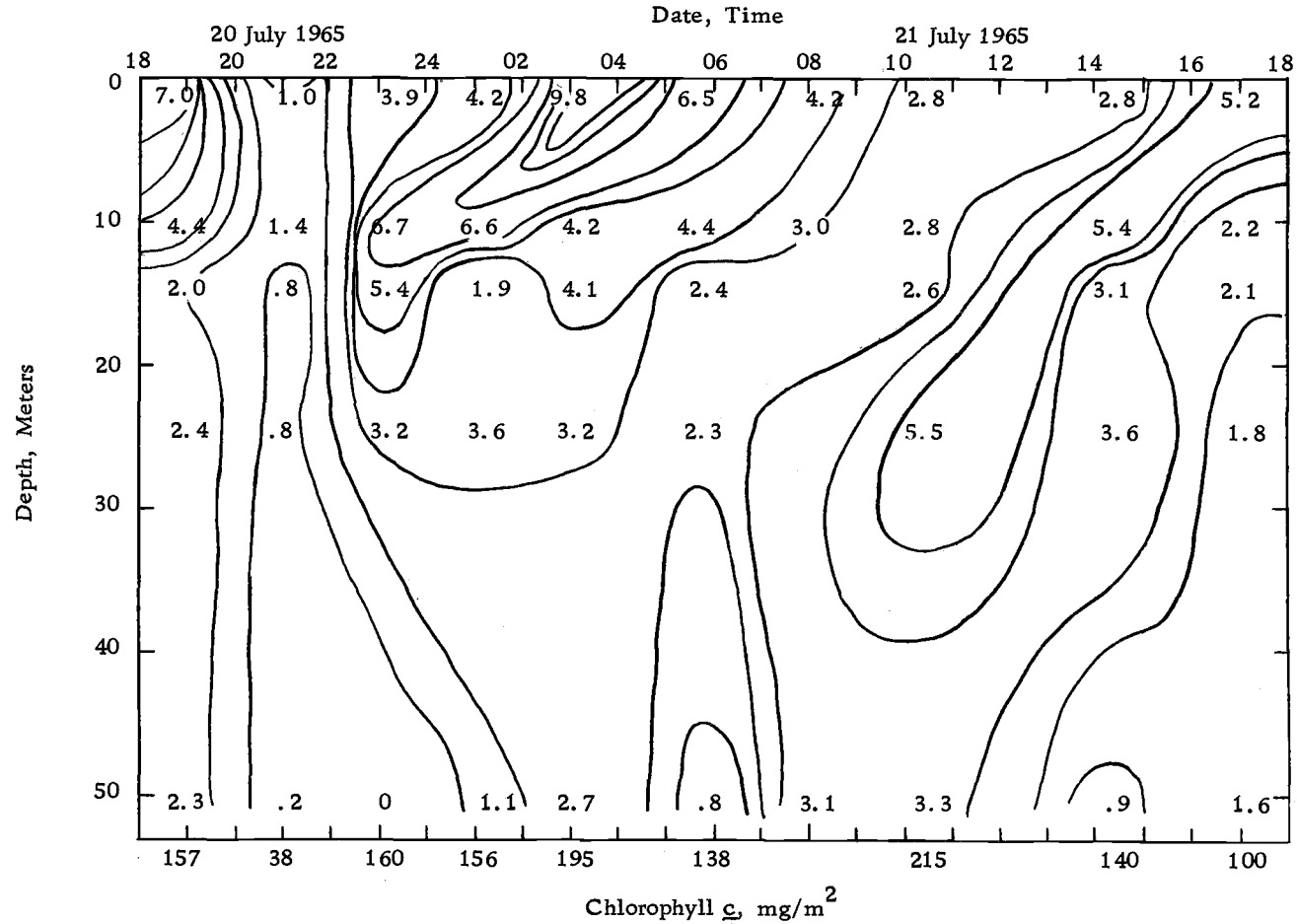


Figure 37. Chlorophyll c at depth vs. time, Cruise 6507, Station NH-25, 20-21 July 1965, Contour interval = 1.0 mg/m^3.

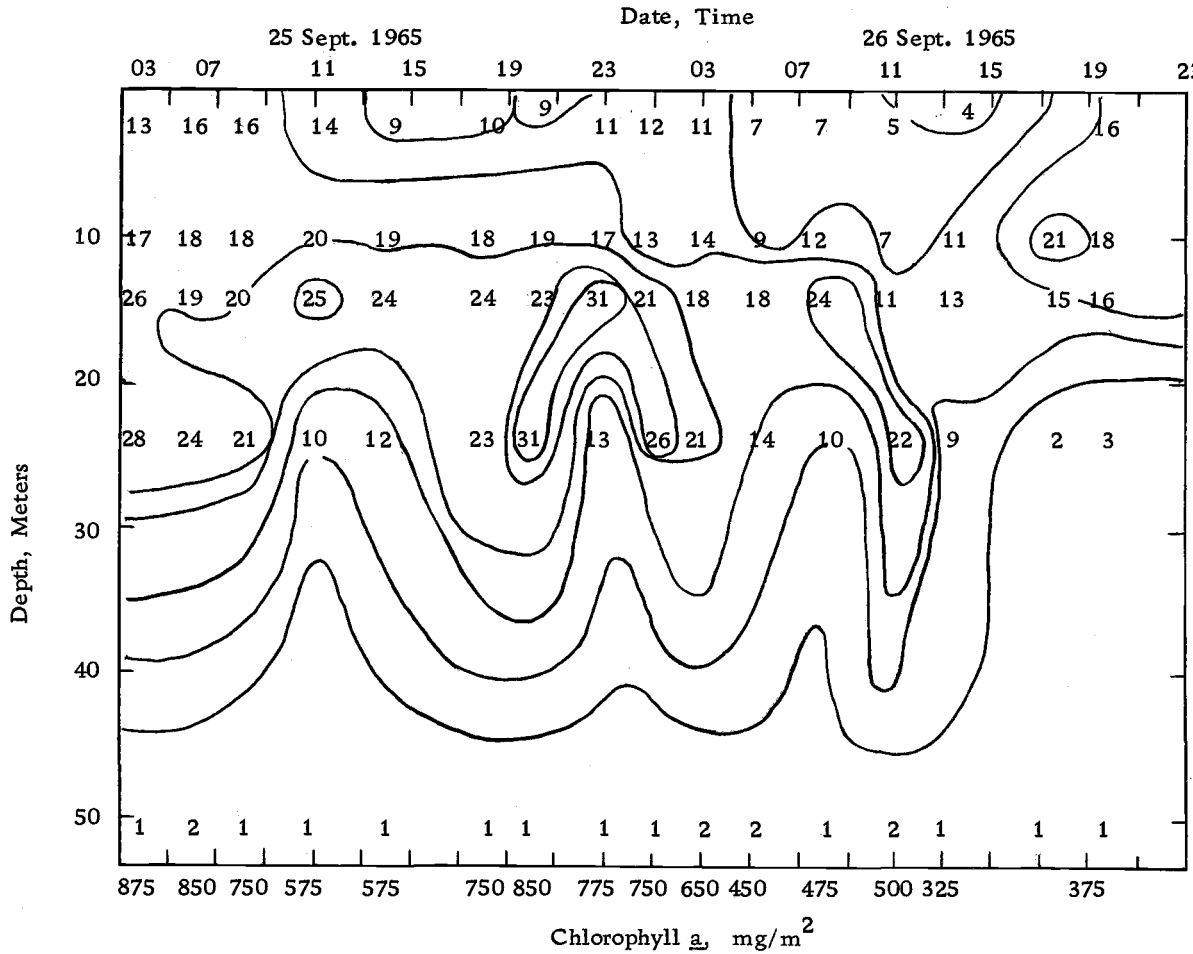


Figure 38. Chlorophyll a at depth vs. time, Cruise 6509, Station SB, 25-26 Sept. 1965, Contour interval = 5.0 mg/m^3.

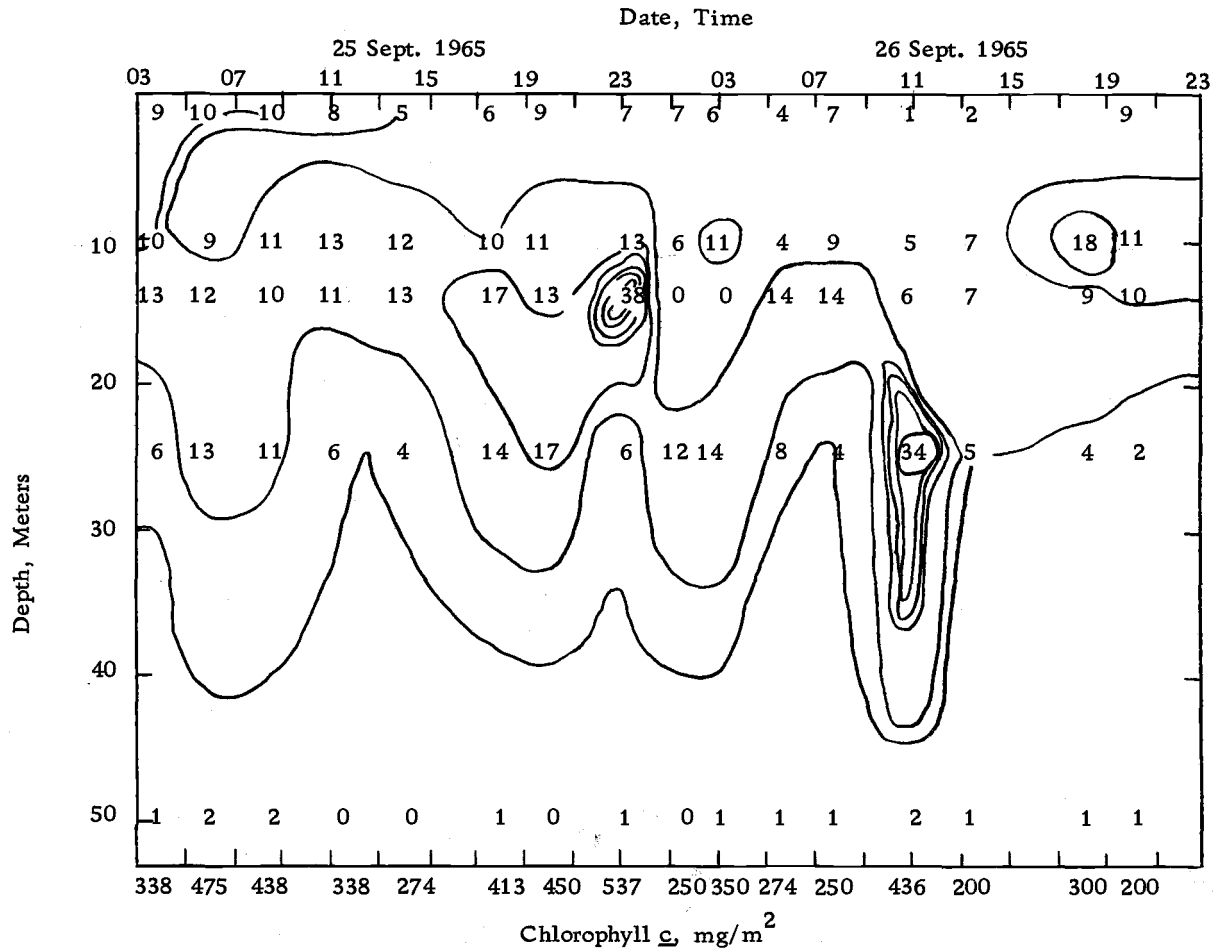


Figure 39. Chlorophyll c at depth vs. time, Cruise 6509, Station SB, 25-26 Sept. 1965, Contour interval = 5.0 mg/m^3.

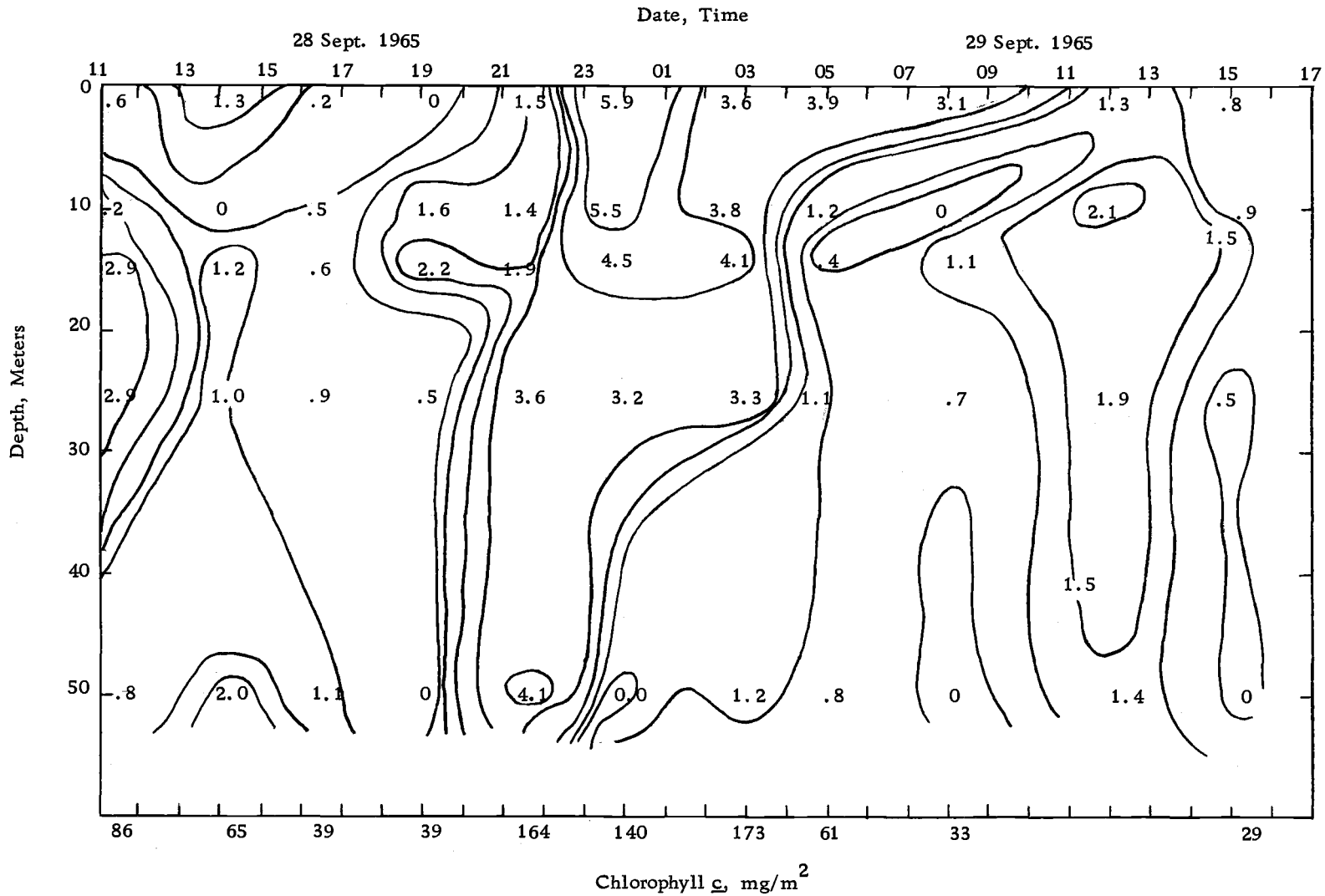


Figure 40. Chlorophyll c at depth vs. time, Cruise 6509, Station NH-55, 28-30 Sept. 1965, Contour interval = 0.5 mg/m^3.

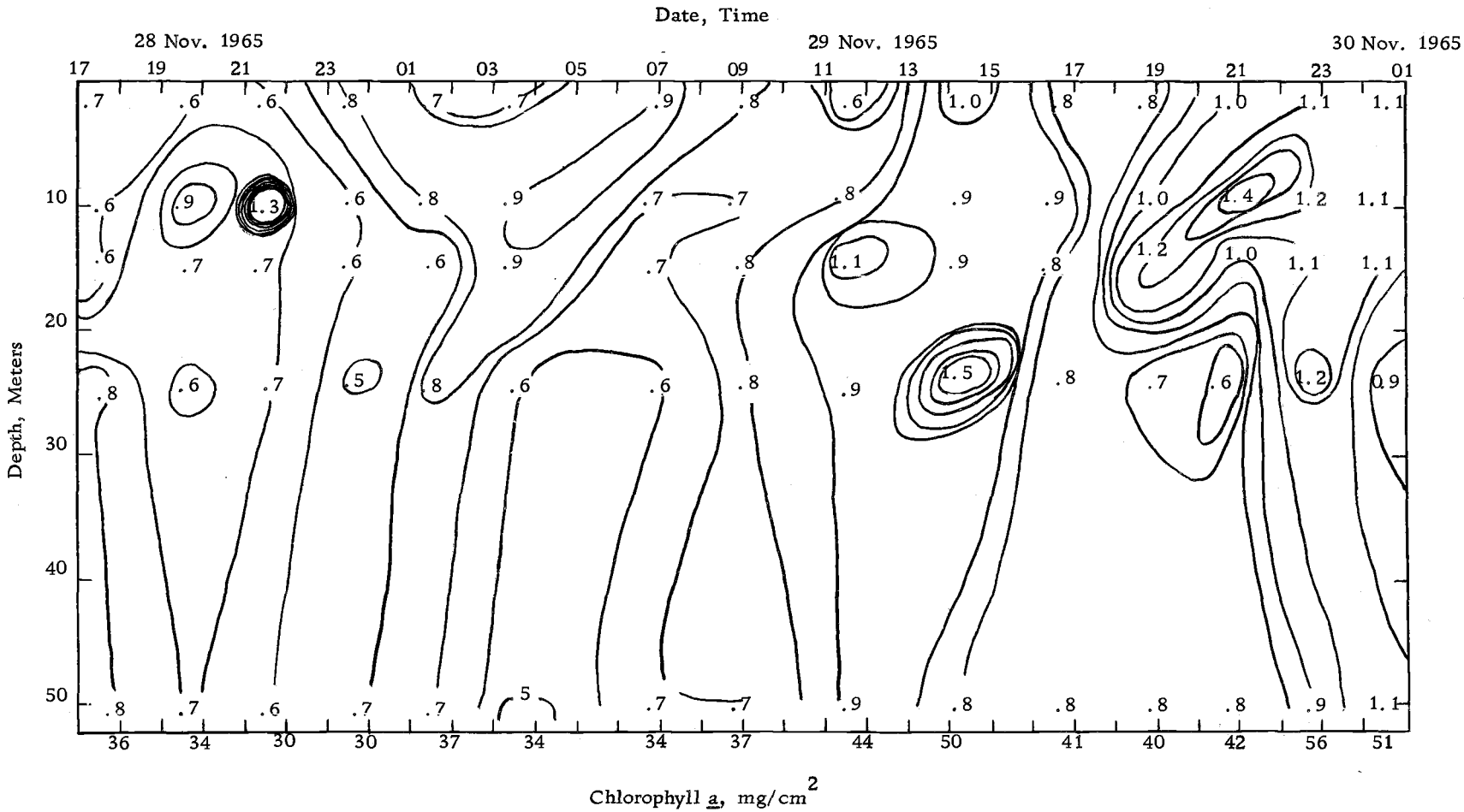


Figure 41. Chlorophyll a at depth vs. time, Cruise 6511, Station NH-25, 28-30 Nov. 1965, Contour interval = 0.1 mg/m^3.

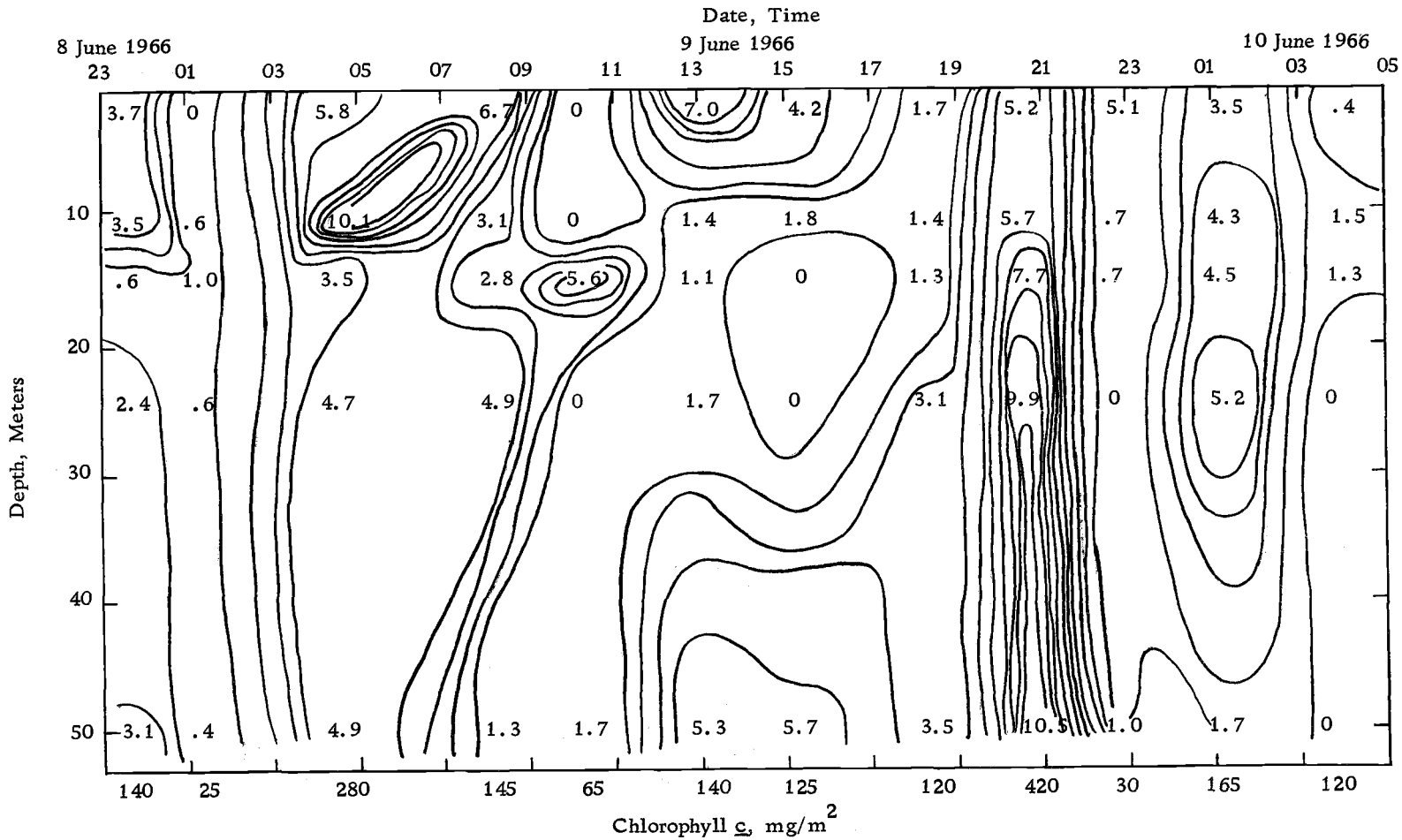


Figure 42. Chlorophyll c at depth vs. time, Cruise YALOC-66, Fixed Station "A", 29° 12'N, 161° 30'W, 8-10 June 1966, Contour interval = 1.0 mg/m^3.

would tend to be constant. These plots show that close time-interval sampling for pigments is necessary at those times when contours are fairly close in order that samples might be statistically valid. In the late fall (Fig. 41), chlorophyll is higher at night as evidenced from the intergrated values; however, when plotted (Fig. 8), this increased concentration at night was not apparent. No clear diel periodicity at given depths was seen for chlorophyll c at Fixed Station "A" on the YALOC-66 cruise (Fig. 14). However, the chlorophyll c concentration was higher in the water column at night on a milligrams per square meter basis (Fig. 42). The advantage of such a plotting method is that integrated values show chlorophyll changes that may be unseen if waters are well-mixed due to turbulence. The chlorophyll undulations seen below 25 meters in Figs. 38 and 39 may have been caused by an internal wave.

Light duration and intensity appears to be the controlling factor for the diel periodism of chlorophyll a and c in situ in the oceans. Except at the 50 meter depth, both chlorophylls a and c were in minimum concentrations in the middle to late afternoon, increased to a maximum value during the early to middle portion of the night, then began to decrease during the night into the daylight hours to the minimum value. Also, maximum-to-minimum chlorophyll ratios appear to be similar in laboratory experiments and in situ in the oceans, especially for chlorophyll a. Cruise 6507 (Fig. 2) showed

evidence for the light control of diel periodism of chlorophyll a. The maximum concentration of this pigment came earlier in time with depth. At the surface, the maximum concentration of this pigment occurred at 0300 while at 10 meters, the maximum came two hours earlier at 0100. At 15 meters, the maximum came two hours earlier at 2300, and at 25 meters, two hours earlier at 2100. This showed that at depth, where light intensity of a given intensity occurred for shorter durations, cells were not able to store enough energy-producing substrate or chlorophyll precursor to continue the dark synthesis of chlorophyll.

Evidence of the relationship between light intensity and pigment changes on a short-time interval were seen (Fig. 13). The amount of incoming solar radiation increased until approximately 1200, when suddenly a cloud cover came in and stayed until about 1500. Chlorophyll a concentration had started its normal decrease due to bleaching, but as the cloud cover increased, the concentration of this pigment, even at depth, began to increase. Then, at 1500, when the cloud cover disappeared and light intensity increased, the concentration of chlorophyll again decreased until around sunset, when it started to increase again. Bleaching caused by higher light intensities appears to be the reason for the minimum chlorophyll concentration occurring in the afternoon hours. The data at this particular station also showed that bleaching is a fairly rapid process.

The lack of diel pigment periodicity in the late fall and winter could be due to hydrographic causes. During Cruises 6511, 6602, and the latter part of 6701, rough seas were encountered. This turbulence might have mixed the water column quite thoroughly so that cells were rapidly exposed to varying light intensities. This may have masked pigment changes that are seen when cells stay at a particular depth. Yentsch and Scagel (1958) also felt that hydrography could influence diel pigment periodicity.

The Ecological Significance of Diel Chlorophyll Periodicity

The Use of the Chlorophyll Method of Estimating Primary Production

Basically, the chlorophyll and light method of Ryther and Yentsch (1957) is an attempt to estimate the amount of primary production taking place in a given volume of water from measurements of chlorophyll concentration in the water and the incoming solar radiation. This method has the apparent advantage of eliminating primary production measurements by oxygen evolution or the uptake of carbon-14. These latter methods are time-consuming, especially if done *in situ*.

These workers estimated the amount of photosynthesis taking place in a given volume of water by the following relationship:

$$P_d = R_d \times C_d \times A$$

where P_d = daily photosynthesis in grams carbon per cubic meter at depth d.

R_d = relative photosynthesis at depth d from a graph correlating surface radiation with percent light transmission at depth d.

C_d = grams chlorophyll per cubic meter at depth d.

A = the assimilation number, i.e., grams carbon assimilating per hour per gram chlorophyll. These workers reported A as 3.7.

In the definition of assimilation number, Ketchum et al. (1958) suggested chlorophyll a as the pigment used. However, McAllister, Shaw, and Strickland (1964) found that at lower light intensities, the sum of chlorophyll a and c gave better results. In this study, however, chlorophyll c variations were much greater, suggesting that chlorophyll a would be better. Curl and Small (1965) proposed that the assimilation number was influenced by nutrient levels in the water column and that numbers less than three were indicative of nutrient depletion, three to five, borderline depletion, and five to ten, nutrient sufficiency. Thus, to estimate the maximum possible production that would take place in a given volume of water, a correction factor to take into account the concentration of the major and minor nutrients in the water seems necessary.

One of the advantages of the chlorophyll and light method for measuring production is that a cruising research vessel can collect samples for pigment analysis at any time of the day or night. These samples are collected at all hours. However, the values obtained

from nocturnal samplings will give chlorophyll values that are higher (or at least different) than those which would occur during the day when photosynthesis was actually going on. It would then be advantagious to refer the chlorophyll a and c values obtained to a standard time in order to correct for diel variations. This time should be chosen so that it represents a time when photosynthesis would take place, yet chlorophyll bleaching has not advanced too far. This limits the standard time to the morning, and the time of 1000 is proposed for this purpose. At this time, bleaching appears not to have advanced too far, yet grazing herbivores should be below 50 meters depth. Figure 43 gives correction factors to convert chlorophyll a to the standard 1000 time, while Fig. 44 gives the conversion factors for chlorophyll c. These correction factors, when multiplied by the chlorophyll a or c concentration at any given time of sampling will convert these values to the chlorophyll concentration that should be present at the standard 1000 time. These corrections factors are averages from Cruises 6507 at Station NH-25 and 6509 at Stations SB and NH-55, so are probably only applicable to spring, summer, and early fall conditions off the Oregon coast.

The formula of Ryther and Yentsch can be modified:

$$P_d = R_d \times (C_d \times D_d) \times A$$

where D_d = the chlorophyll correction factor used to convert the concentration at time t to the standard 1000 time at depth d.

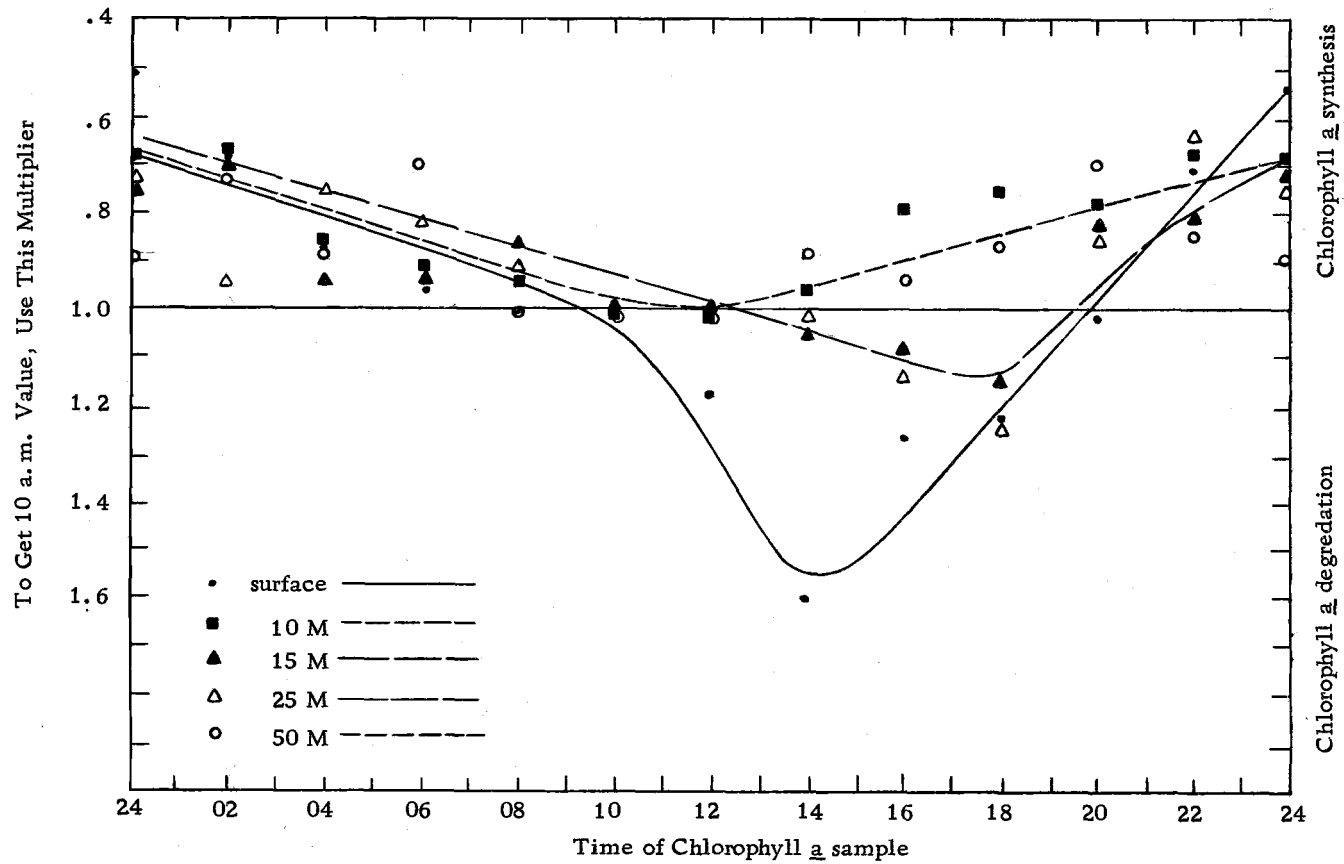


Figure 43. Correction curve for Chlorophyll a to 10 a.m. standard time.

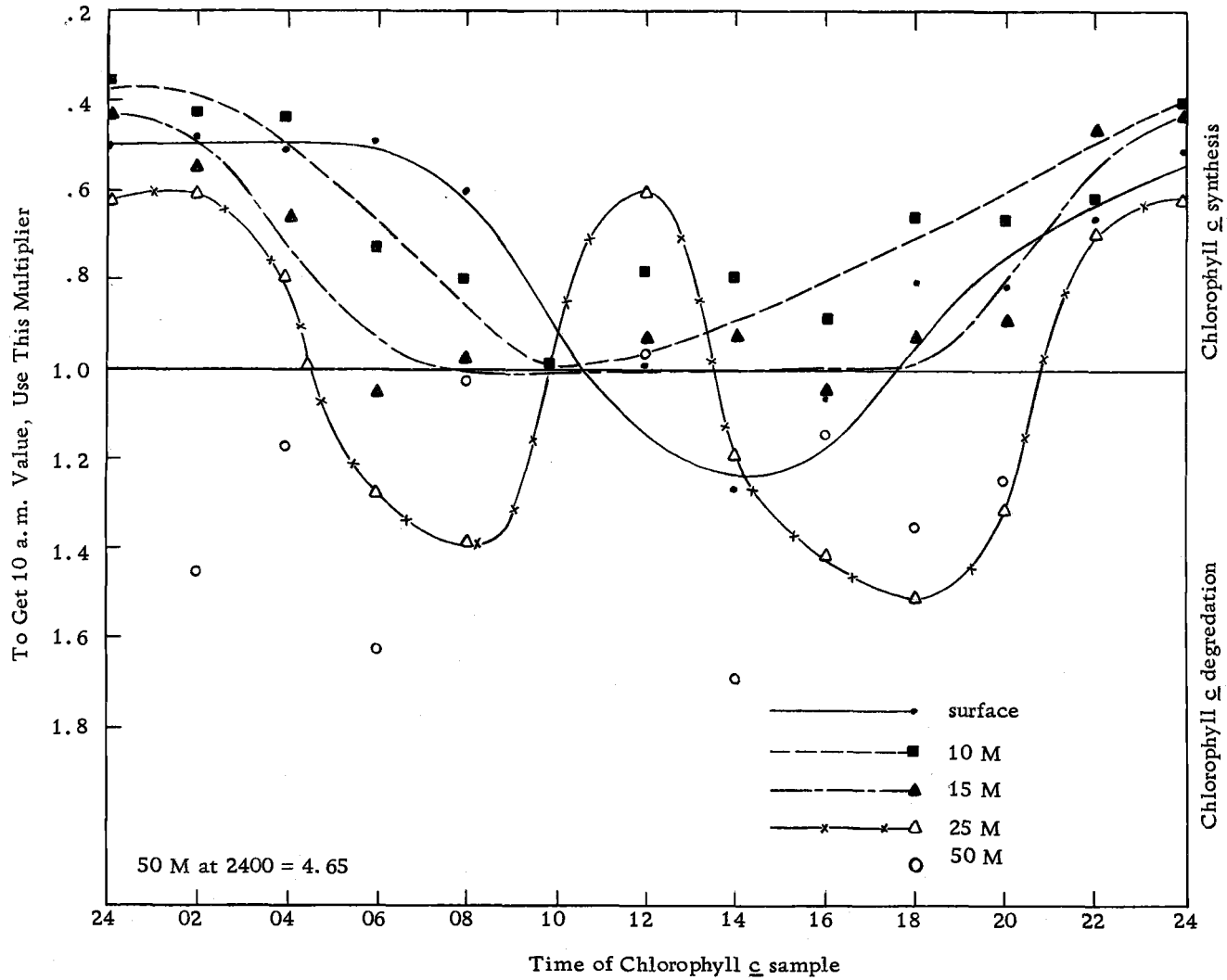


Figure 44. Correction curve for Chlorophyll c to 10 a. m. standard time. 50 meter curve omitted.

However, if repeated measurements are made throughout the daylight day on one station, the diel correction is unnecessary providing that chlorophyll samples are taken at least every two hours, or sooner if at a time of rapid concentration change such as the early evening, or after the night concentration maximum when chlorophyll decreases quite rapidly. Also, the ship's position must be kept so that the same water mass and phytoplankton patch is sampled. A reference parachute drogue would seem adequate for this purpose with the parachute at the depth of maximum chlorophyll (usually 15 meters).

Light Adaptation - Do "Sun" and "Shade" Species Exist?

Ryther and Menzel (1959) advanced the idea that marine phytoplankton existed as "sun" or "shade" species in reference to their position in the water column. This terminology referred to the adaptation of the photosynthetic process in these organisms to different light intensities. Steeman-Nielsen and Hansen (1959) stated that shade species had chlorophyll twice the concentration of temperate surface species, which in turn had twice the concentration of chlorophyll of tropical surface species. In other words, their "shade" species contained a greater concentration of chlorophyll than "sun" species, although they offered no experimental evidence to back up their beliefs about chlorophyll concentration. Humphrey (1963) examined three typical marine phytoplankton species grown at 420

and 680 foot-candles to determine if chlorophyll concentrations per cell were different at the two light intensities. Only one of the species, Nitzschia closterium, had higher chlorophyll at the lower intensity, while the other two species, Skeletonema costatum and a Gymnodinium, had no significant differences in chlorophyll at the two light levels.

In this study (Figs. 22, 24, 25, 26, 27 and 28) when Skeletonema costatum was grown at three different light intensities (Table 2), no significant differences in cellular chlorophyll content were apparent. There always is the possibility that if these cells were grown over long periods of time, the cellular concentration of chlorophyll might have been higher at the lower light intensities. But the experimental evidence seems to indicate that short-term adjustments, as proposed by Yentsch and Lee (1966) takes place in regards to intra-cellular chlorophyll concentration. This would mean that "sun" and "shade" species do not exist in terms of chlorophyll content as proposed by Steeman-Nielsen and Hansen (1959), and Ryther and Menzel (1959).

The ecological significance of diel periodicity of chlorophyll in marine phytoplankton appears to be that of keeping a constant photosynthesis rate. At lower light intensities, chlorophyll is synthesized and assimilation increased due to the higher concentration of this "catalyst" of photosynthesis. However, at higher light intensities, bleaching decreases the concentration of chlorophyll. This then may

prevent, at higher light intensities, the photosynthetic manufacture of substances harmful to the cell such as proposed by Sironval and Kandler (1958) and Kandler and Sironval (1959).

Suggestions for Further Research

The following are a list of suggested topics for further investigation:

1. The nature of the biosynthetic pathways in the planktonic forms of algae of chlorophyll a, and especially chlorophyll c.
2. The role of chlorophyll c in photosynthesis.
3. The role of the red, far-red system in diel pigment periodicity.
4. The vertical distribution of zooplanktonic herbivores off the Oregon coast such as copepods and euphausiids, the nature of their feeding, and time of their grazing.
5. The effect of nutrition on diel pigment periodicity, especially elements that are directly involved in chlorophyll synthesis such as iron.
6. The reason for maximum pigment concentrations occurring during the day, even when light intensity appears to be quite high (Yentsch and Ryther, 1957; Ryther, Menzel, and Vaccaro, 1961; Lorenzen, 1963).
7. The exact cause of the chlorophyll decrease after several hours in the dark.

CONCLUSIONS

At higher light intensities, laboratory cultures of the marine diatom, Skeletonema costatum, showed a diel periodicity of chlorophyll a and c. Highest concentrations of these pigments occurred several hours into the dark period when cells were grown under photoperiods of 9, 12, and 16 hours. The lowest concentrations of these pigments occurred in the light period. It is felt that bleaching of chlorophyll is the reason for the minimum concentration in the light period. Chlorophyll synthesis does occur in the light period after the cell adjusts in some manner to the higher light intensity, and continues in the dark period to a maximum value. The decline of chlorophyll in the dark period begins after the cells become deficient in some chlorophyll precursor or energy-yielding substrate, as the addition of an external carbon source prolonged the period in the dark before the decline of chlorophyll in the cell began. At lower light intensities, chlorophyll synthesis occurs only in the light, and minimum concentration of the pigment is reached during the dark period due to an insufficient amount of energy-yielding substrate or precursor synthesized during the light period.

The same findings appear to explain the diel periodicity of chlorophyll a and c in situ in the ocean. Here, at depths down to 15 meters, and occasionally to 25 meters, maximum concentrations

of these pigments also occur during the night except on days with very small quantities of incoming radiation. As grazing takes place at night, which should decrease the amount of chlorophyll in the water column, it is felt that light, not grazing, is the cause of the diel periodicity of photosynthetic pigments in the ocean.

These conclusions are summarized in Figure 45.

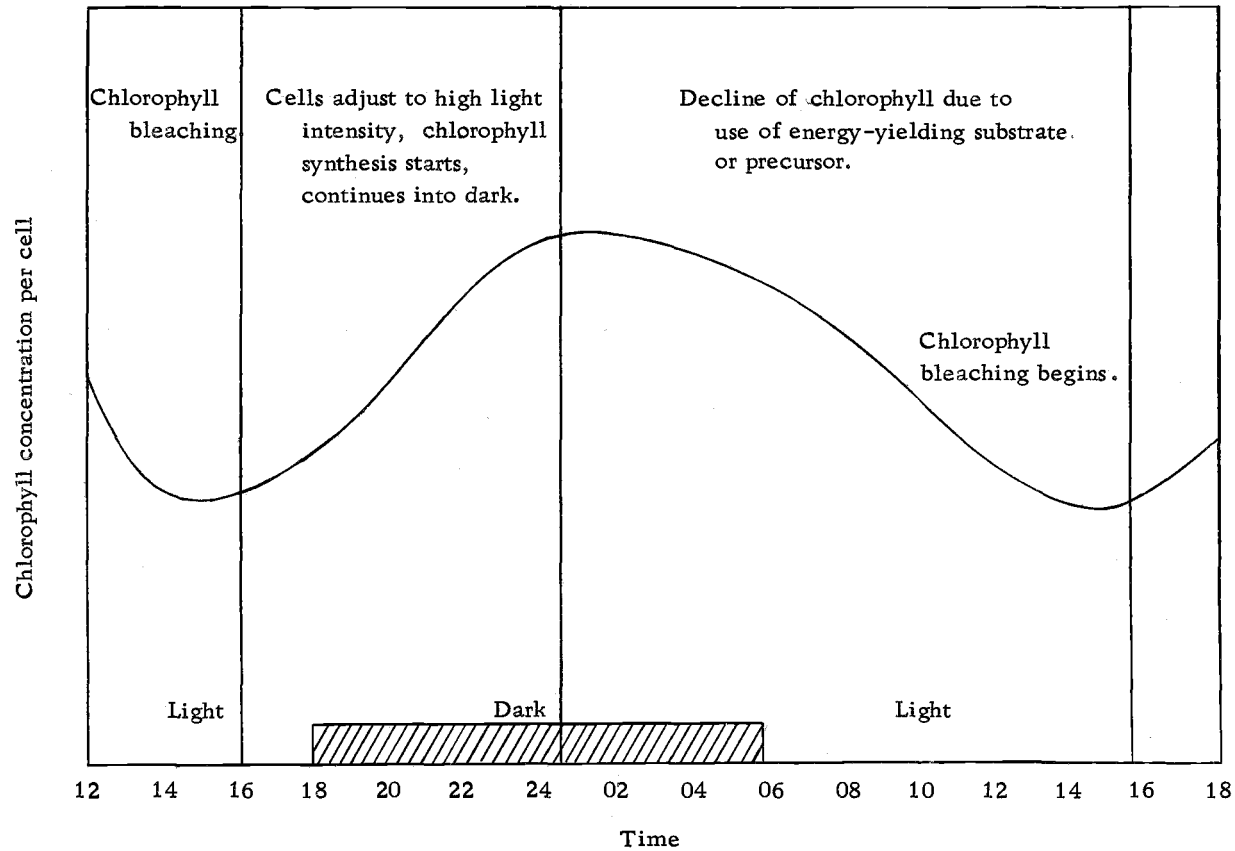


Figure 45. Summary of diel chlorophyll changes in marine phytoplankton under surface conditions of light.

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