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DIURNAL STUDY OF PHYTOPLANKTON PIGMENTS
AN IN SITU STUDY IN EAST SOUND, WASHINGTON

By

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ABSTRACT

The effect of light intensity on the quantities of chlorophyll and carotenoid pigments in a natural marine diatom community has been observed. Marked daily fluctuations in the quantities of these pigments, which showed highest concentrations around mid-day and at night, resulted from changes in quantities within the cells. Rates of synthesis and decomposition throughout the day have been computed. Highest cellular concentrations of chlorophyll and carotenoid pigments corresponded to optimal light intensities for photosynthesis. The differential effect of light on chlorophyll a and on carotenoid pigments, where chlorophyll changes are more extreme, caused the ratios of these pigments to change during the day. The significance of this change is discussed.

INTRODUCTION

Plant pigment destruction and photosynthetic inhibition by strong light may be observed most easily in land plants and algae which are adapted to living at low light intensities. The typical “shade” characteristics of planktonic algae suggest that, in the surface of lakes and oceans, high and low daily light intensities cause marked

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Approximately thirty years ago Dr. T. G. Thompson, working with Gran (Gran and Thompson, 1930), recognized the high phytoplankton productivity of East Sound. Since then, whenever possible, he has encouraged students interested in phytoplankton problems to work in the area.
fluctuations in the pigment content of planktonic populations. In a recent paper, Yentsch and Ryther (1957) observed a diurnal fluctuation in the chlorophyll content of a natural population of phytoplankton enclosed in a floating carboy; highest chlorophyll \( a \) concentrations were observed during morning and afternoon and lowest concentrations around mid-day and early evening. Although other workers have described diurnal variations in chlorophyll concen-

Figure 1. Map of East Sound; circled cross indicates station location.
trations (see Rabinowitch, 1945: chapt. 15), little information is available concerning the rate of pigment synthesis and decomposition under natural conditions of illumination. Where laboratory studies have been made, few have approached intensities comparable to mid-day values. Virgin (1955) observed optimal chlorophyll $a$ synthesis in barley leaves at 500 foot-candles, while Halldal (1958) observed highest concentrations of chlorophyll and carotenoid pigments in blue-green algae at 200–400 and at 400–800 foot-candles, respectively.

During the summer of 1954, while in residence at the University of Washington’s Friday Harbor Marine Laboratory, we conducted a diurnal study of phytoplankton pigments throughout a water column in East Sound, Orcas Island (Fig. 1). The purpose of this study was to observe the relationship of light intensity to chlorophyll and carotenoid pigment concentrations in a natural crop of phytoplankton.

The writers are indebted to Dr. R. H. Fleming who made arrangements for use of the R. v. BROWN BEAR, to Captain Princehouse and crew for their cooperation, and to Carey McAllister, Francis Harris, Ruth Palmer, Alan Duxbury, and Tamio Yamagiwa who aided in shipboard operations and analyses. This work was partially supported by the U.S. Office of Naval Research (Contract N00014-520/II Project NR083012) and by the National Research Council of Canada.

**METHODS AND PROCEDURES**

Water samples for phytoplankton pigments were taken with the Nansen-Knudsen three-liter closing bottle. After each sample had been divided into approximate halves, each half was filtered on board through a millipore type AA filter following the procedure outlined by Creitz and Richards (1955). At the shore laboratory the concentrate from half of each sample was scraped from the filter and allowed to settle in a measured volume of 4% formalin. Cell-counting was done in a modified Sedgwick-Rafter counting chamber of known volume under 100 and 400× magnification. Pigment extraction and determination were also done at the shore laboratory, following the method outlined by Richards with Thompson (1952). Chlorophyll $a$ values are expressed in milligrams, while chlorophyll $c$ and carotenoid pigments are given in specific plant pigment units;
one specific pigment unit of plant carotenoid pigment is closely equivalent to one milligram.

Temperature and salinity were measured with Nansen bottles fitted with reversing thermometers. Chlorinity was determined by the Mohr method as modified by Thompson (1928). Densities were calculated from the hydrographic tables of Knudsen (1901). Light intensities in the water column were measured with a standard Clarke submarine photometer which was fitted with a green filter and which had previously been calibrated against a Weston foot-candle meter. Intensities for given depths were corrected by means of the foot-candle extinction coefficients for green light given by Utterback and Wilson (1940).

Because of the prevailing clear weather and a small range in tide in mid-July 1954, it was decided to occupy a station in East Sound on July 13. At approximately 0900 the R. v. BROWN BEAR dropped anchor at the head of the Sound in 27 m of water (Fig. 1). Sampling periods were 1015, 1615, 2100, and 2400 on July 13, and 0520 and 0845 on July 14. During each sampling period a six-bottle cast for phytoplankton collections was made at depths of 0, 3, 5, 10, 15, and 25 m. A second six-bottle cast at the same depths followed, using Nansen bottles. While the thermometers were coming to temperature, a bathythermograph and the light meter were lowered.

Water Mass Conditions, July 13–14. Stratification in the water column persisted day and night. Surface temperatures varied from 11.67 to 12.78° C, following a trend indicative of diurnal heating and cooling. The maximum thermal gradient was between 3 and 5 m, below which the temperature was constant at 10.0° C. During daylight hours of July 13 the surface waters were practically flat calm; between 1000 and midnight a 10 knot breeze ruffled the surface, but no detectable deepening of the mixed layer was evident. Salinities throughout the water column showed almost no variation throughout the day, the salinities at the surface being 28.91 \%oo, those at the bottom 29.81. The average difference between $\sigma_t$ at the surface and at 25 m was 1.81.

Poor flushing in East Sound can be expected because of relatively little freshwater inflow, the general morphology of the basin, and a small sill which partially obstructs the entrance and thus restricts circulation.

Measurements of oxygen and inorganic phosphate in the surface
water during the study revealed a marked inverse correlation between these properties. Supersaturated oxygen values were consistently observed during the day, and the ratio between the apparent oxygen utilization and phosphate were comparable to ratios associated with oxidation or reduction of organic material (Redfield 1934, 1942; Fleming 1940).

These observations, of a widely varying nature, lead us to conclude that advective influences were minimal during this study. The discussion which follows assumes that advection and eddy diffusion were of negligible magnitude and that the same populations were sampled repeatedly.

**Phytoplankton Community, July 13–14.** The phytoplankton population in East Sound comprised almost exclusively diatoms belonging to the genera, *Chaetoceros, Skeletonema, Nitzschia, Asterionella, Thalassiosira, Thalassionema, Biddulphia,* and *Navicula.* Of these the most abundant species were, *Skeletonema costatum, Chaetoceros decipiens, Nitzschia closterium, N. delicatissma, Thalassiosira nordenskioldii,* and *Asterionella japonica.* Diatom counts alone for surface waters exceeded $3 \times 10^6$ cells per liter. Dinoflagellates belonging to the genera *Peridinium, Dinophysis,* and *Ceratium* did not exceed $2 \times 10^4$ cells per liter.

**TABLE I. Cell Numbers $\times 10^6$ in One Liter, East Sound, July 13–14, 1956**

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Sampling Periods</th>
<th>July 13</th>
<th>July 14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0515</td>
<td>0845</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>3.0</td>
<td>9.0</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>9.0</td>
<td>9.0</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>18.0</td>
<td>13.0</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>13.0</td>
<td>10.5</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>5.0</td>
<td>11.5</td>
</tr>
<tr>
<td>25</td>
<td></td>
<td>9.0</td>
<td>6.4</td>
</tr>
</tbody>
</table>

The cell counts (Table 1) showed that there was an active flowering in the upper 3 m, with cell numbers at the surface increasing 30-fold during the July 13–14 period. During hours of most intense illumination, cell numbers increased at all depths except at 25 m. Maximum numbers occurred between 5 and 10 m during the brightest portion of the day, whereas during darkness the largest numbers occurred between the surface and 3 m.
Figure 2. Concentrations of chlorophyll a and c and carotenoid pigments in East Sound, July 13 and 14.

Diurnal Variations in Phytoplankton Pigments. Fig. 2 illustrates pigment measurements at each depth plotted as a function of time. Largest concentrations of all plant pigments were found between 3 and 10 m in the region of the thermocline while lowest concentrations were observed at the surface and at 15 and 25 m. Highest concentrations of pigments were observed at 5 m during the morning (0845, 1015) and at 10 m in the afternoon (at 1600). At 2400 and 0520 the depth of greatest pigment concentration was at 3 m. Pigment values at the 15 and 25 m level remained more nearly constant throughout the day. Chlorophylls at 0, 3, 5, and 10 m underwent marked fluctuations from day to night. For example, chlorophyll a values at 0 and 3 m were five times higher at midnight
than at mid-day. Although all pigments at the 5 m level showed a steady decline from 1015 to 1615, the decrease there was not as great as that observed at the 0 and 3 m levels. Chlorophyll \( a \) values at 10 m showed only slight variations while chlorophyll \( c \) values at this depth fluctuated considerably. Both chlorophylls were relatively constant at the 15 and 25 m levels.

The extreme changes in chlorophyll content in surface waters throughout the day were not accompanied by similar extreme changes in carotenoid pigments. Thus the pigment ratios were greatly altered throughout the day. This is best illustrated by comparing proportions of chlorophyll \( a \) to carotenoid pigments (Table II). The Chl \( a \):Carot ratio ranged from 7.0 to 3.1. In general it tended to be higher in the early morning and at night in surface waters, but at depths greater than 3 m it was higher during mid-day.

### TABLE II. OBSERVED Chl\( a \): CAROT RATIOS IN EAST SOUND

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Average Sampling Times</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0520 0845 1015 1230 1615 2100 2400</td>
</tr>
<tr>
<td>0</td>
<td>5.34</td>
</tr>
<tr>
<td>3</td>
<td>5.81</td>
</tr>
<tr>
<td>5</td>
<td>5.55</td>
</tr>
<tr>
<td>10</td>
<td>5.36</td>
</tr>
<tr>
<td>15</td>
<td>5.71</td>
</tr>
<tr>
<td>25</td>
<td>4.48</td>
</tr>
</tbody>
</table>

**Changes in the Pigment Concentration of Cells.** At times, changes in the concentration of pigment could be associated with comparable fluctuations in cell numbers (Table I); however, at other times, pigment fluctuations were too great to be accounted for by an increase or decrease in the number of cells, hence these must have resulted from changes in cellular contents. The diurnal trend of cellular content was observed at all levels except at 25 m (Fig. 3), and it was found that the amounts of chlorophyll \( a \) per cell were low at 0, 3, 5, 10, and 15 m during mid-day but were high at these same depths at 0845, at 1015, at 2100, and again at 2400. During daylight hours, above 3 m, chlorophyll \( a \) diminished 2- to 3-fold, whereas below 3 m only small decreases occurred after 2100. The
Figure 3. Chlorophyll and carotenoid pigment concentrations per cell in East Sound, July 13 and 14.

Large decreases in chlorophyll $a$ per $10^8$ cells at the surface and at $3$ m during darkness was probably due to the consummation of cell divisions started in light. The general trend of chlorophyll $c$ followed chlorophyll $a$; however, deviations did occur which indicate that these two chlorophylls were not in phase (Table III). The ratios
of Chl a:Chl c range from a high of 13.10 to a low of 0.86. Although no consistent trend in the ratio of these chlorophylls is indicated, the trends differed most during hours of darkness and early morning. Compared to the magnitude of the chlorophyll changes throughout the day, the carotenoid pigment per cell at that time was almost constant. Differences between the rates of change of chlorophyll and carotenoid pigments is further shown when the rates of synthesis and decomposition are computed for each pigment.

Comparison of the rate of change in cellular chlorophyll a and c and carotenoid pigments is shown in Fig. 4. These rates have been computed for sampling intervals, using concentrations at the beginning and end of each interval. The rates are expressed at each depth in milligrams or in specific plant pigment units per cell per hour. At depths other than 25 m, the highest rate of pigment increase occurred during the morning between 0845 and 1015, the maximum rate of synthesis during this period being observed at 15 m. Between 1015 and 1230, chlorophyll a and carotenoid decreased at all depths except at 25 m, with the maximum again occurring at 15 m. Between 1230 and 1615, little synthesis or decomposition was observed, and, excepting surface observations, small rates of synthesis were observed between 1615 and 2100. Thereafter, during darkness, only small rates of synthesis, if any, were observed although marked rates of decomposition were apparent at times. The active processes of chlorophyll synthesis and decomposition are strikingly apparent. Chlorophyll a rates of synthesis and decomposition are approximately five times greater than the carotenoid rates.

### TABLE III. RATIO OF Chl\(^a\): Chl\(^c\) IN EAST SOUND

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Depth Aver.</th>
<th>Sampling Times</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0520 0845 1015 1320 1615 2100 2400</td>
</tr>
<tr>
<td>0</td>
<td>1.31</td>
<td>1.26 0.86 0.94 1.26 1.34 2.40</td>
</tr>
<tr>
<td>3</td>
<td>1.46</td>
<td>1.90 1.19 1.24 1.18 1.36 1.54 1.62</td>
</tr>
<tr>
<td>5</td>
<td>1.42</td>
<td>1.60 1.49 1.38 1.31 1.05 1.54 1.60</td>
</tr>
<tr>
<td>10</td>
<td>3.28</td>
<td>13.10 1.46 1.44 3.43 1.22 1.38 0.91</td>
</tr>
<tr>
<td>15</td>
<td>2.12</td>
<td>8.02 1.10 0.97 1.14 1.32 1.23 1.09</td>
</tr>
<tr>
<td>25</td>
<td>1.42</td>
<td>3.73 0.99 1.13 0.64 0.63 1.02 1.41</td>
</tr>
</tbody>
</table>

Daily Averages

5.65 1.25 1.17 1.43 1.14 1.34 1.51
Figure 4. The rate of chlorophyll and carotenoid pigment synthesis (+) and decomposition (−). Units are $10^{-8}$ milligrams or specific plant pigment units per cell per hour. Solid line is chlorophyll a, dashed line chlorophyll c, dotted line carotenoid.
Figure 5. Relationship between in situ light and pigment concentrations at six depths.
Relationship between Light Intensities, Chlorophyll a, Chlorophyll c, and Total Plant Carotenoid Values. The largest concentrations of all pigments corresponded to \textit{in situ} light intensities of approximately 1500 foot-candles (Fig. 5), the lowest corresponding to the highest \textit{in situ} intensities of 10,000 f-c. Although the range in pigment concentrations is large for a given intensity, curves drawn through the extremes describe a curve which is comparable to Ryther’s (1956) photosynthesis-light curve for natural populations of marine phytoplankton. Thus the shape of the pigment-intensity curves is interpreted to mean that phytoplankton near the surface during a brighter portion of the day were photosynthetically inhibited and that their pigment was bleached by the intense light. In contrast to this, phytoplankton in deeper water, exposed to light intensities below 1000 f-c, were light-limited.

In Fig. 6 the observed pigment content of cells are plotted against the \textit{in situ} light intensities. Since development and decomposition of pigments within a cell is dependent upon both light intensity and its duration, a wide scatter is expected when these two measurements are taken simultaneously and are plotted against one another. A solid line encloses points of similar depth. Highest concentrations of chlorophyll \textit{a} per cell occurred at light intensities between 100 and 2000 f-c while highest concentrations of carotenoid pigments per cell corresponded to intensities between 100 and 2500 f-c. Values of chlorophyll \textit{a} per cell associated with high light intensities of 2000 were 60 \text{\%} lower than those associated with intensities of about 1500 f-c. In comparison, values of carotenoid per cell at high intensities (2500) were only 50 \text{\%} lower than those at optimal intensities (1500). Chlorophyll \textit{c} quantities differ from those of the other two pigments in that high concentrations were found at both high and low intensities. The trend of points suggests that relatively low chlorophyll \textit{c} values occur at intensities where the chlorophyll \textit{a} per cell is highest.

Note the differential effect of light on pigments at different depths. Surface and near-surface phytoplankton underwent a wide change in light intensity with only a small change in pigment. In contrast, the pigment content of deeper phytoplankton was altered considerably by small changes in illumination. This distinction appears to be comparable to observations made by Harder (1930, 1933) on “light acclimatization” in aquatic plants. Thus the rates of photosynthesis and of pigment synthesis at different light intensities appear to be functions of the average intensity to which the cell is
Figure 6. Relationship between in situ light and pigment concentrations per cell at six depths.
acclimated; hence phytoplankton living at deeper levels, in contrast to surface forms, synthesize pigment optimally at a lower intensity and show signs of bleaching earlier as the intensity increases.

Significance of Changes in Pigment Ratios. Halldal (1957) has pointed out that two ideas have been presented to explain the increasing proportion of carotenoid pigments relative to chlorophylls at higher light intensities. One hypothesis states that higher carotenoid pigments develop in intense light as a protective screen for the chlorophyll a pigment. The second idea, which suggests a differential rate of synthesis and bleaching, is more applicable in accounting for changes in the pigment ratio observed in the surface waters of East Sound. However, these pigment changes do not appear to follow a pattern indicative of chromatic adaption. Indeed, at times lower Chl a:Carot ratios were found in deeper waters where green light would be the only portion of the spectrum available for photosynthesis. Nevertheless, the inconsistency of the Chl a:Carot ratio makes the wavelength utilization theory seem doubtful. It seems more logical to interpret variations in the Chl a:Carot ratio as an accompaniment to changes in the cellular composition of phytoplankton. It has been shown that changes in cellular nitrogen in cultures of marine phytoplankton are associated with a change in the proportion of chlorophyll and carotenoid pigments (Yentsch and Vaccaro, 1958). This familiar process of nutritional chlorosis in plants involves a general loss of plant pigment where the more rapid loss of chlorophyll and its protein complex, as compared to the slower decomposition of carotenoid pigments, changes the proportion of pigments. Hence it appears that a light-induced chlorosis is not different from a nutritional one, since the level of cellular nitrogen is indirectly independent on light through photosynthesis.

The difference between light intensity curves for chlorophyll a and for chlorophyll c suggests that chlorophyll c may serve as a precursor pigment of chlorophyll a since low values of chlorophyll c were at times found at light intensities optimal for chlorophyll a. Further support of the precursor function of chlorophyll c comes from Granick (1949) who has observed that the structure of chlorophyll c is more closely identical to protochlorophyll in higher plants than to chlorophyll a.

Diurnal Chlorophyll-Photosynthesis Curves. Some workers have felt that diurnal changes in the chlorophyll content of natural pop-
ulations are due to internal rhythms (see Rabinowitch, 1945: chap. 15) rather than to changing light intensities. The diurnal pigment variations observed in this study lead us to conclude that light is the causative factor. Since photosynthesis is a function of chlorophyll concentration at light intensities up to saturation, it would appear that Yentsch and Ryther's (1957) conclusion is correct, i.e., that diurnal fluctuations of the photosynthetic capacity of natural phytoplankton populations result from changing chlorophyll content within the population and not from internal rhythms, as suggested by Doty and Oguri (1957). However, the magnitude of the diurnal curve and the vertical distribution of phytoplankton pigments can be expected to be modified by both ecological and physiological factors. For example, the amount of pigment synthesized or decomposed depends on the duration as well as the intensity of the light reaching the cells. Therefore, cells held in surface waters by marked stability in the water column will be bleached more rapidly in intense light than a cell moving about in a mixed layer. Also, varying intensities of light arising from cloud interference will certainly lower the average intensity reaching each cell. The degree to which the cellular content of pigment is altered will be affected also by the previous light history as well as by the age of a cell. Cells growing at low light intensities will be more sensitive to light changes than those growing in high intensities. Furthermore the saturating light intensity for both chlorophyll synthesis and photosynthesis is temperature dependent, and both would be lower in colder than in warmer waters. Lastly, the inconsistency in periodicity curves could result from nutritional causes, since the amount of photosynthesis as well as chlorophyll synthesis depends on the availability of nutrients (see Ketchum et al., 1958; Yentsch and Vaccaro, 1958); at optimal light intensities pigment synthesis is markedly reduced by depletion of available nutrients from a culture medium. In summary, different conditions of either light or temperature or nutrients can be expected to modify the daily periodicity curves for chlorophyll and photosynthesis.

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