

# Growth and Dissipation of Phytoplankton in Chesapeake Bay. I. Response to a Large Pulse of Rainfall<sup>1</sup>

M. E. LOFTUS, D. V. SUBBA RAO and H. H. SELIGER

*McCollum-Pratt Institute and Department of Biology  
The Johns Hopkins University  
Baltimore, Maryland 21218*

**ABSTRACT:** Approximately 90 Km<sup>2</sup> of Chesapeake Bay contiguous with the Severn, South, Rhode and West Rivers were surveyed by *in vivo* chlorophyll fluorescence and captured samples following a large pulse of rainfall in summer, 1971. The growth and subsequent dissipation of blooms containing chlorophyll *a* concentrations up to 40 × pre-bloom values were completed within 21 days. A distinction is made between the blooms produced by nutrient pulses and dinoflagellate blooms normally observed in the fall. In the former, there is a complete change of phytoplankton relative species composition. The latter is a phototactic segregation of species already existing within the water column. The methodology is presented and a mathematical description is attempted.

## Introduction

The major value of a descriptive model of a complex system lies in its predictive capacity and in its ability to identify causal relationships. The difficulties with a strict mathematical model are primarily due to our present incomplete knowledge of the nutritional physiology (including light) of the organisms, the interrelationships among the various species, the control of populations by predation and nutrient turnover due to turbulent mixing and predation. On the other hand, the large variances observed in populations of the higher trophic levels from one season to another and for the same month or season from one year to the next make baseline comparisons "before and after" a man-made perturbation a very imprecise technique except for drastic effects such as fish or crab kills which are usually visible to the non-scientist as well.

We have approached the problem of predictive capability by concentrating our studies on

plankton populations. Owing to the short generation times (hours to days) of phytoplankton and their low motility, one would expect that their growth and physiological state might be strongly coupled to the physical and chemical environmental parameters characteristic of their particular geographic locations. As the consequence of this strong coupling it should be possible to describe the kinetics of growth and dissipation of phytoplankton standing crops in terms of a strongly damped, quasi-stable system driven by a forcing function corresponding to a defined natural perturbation of the ambient physical and chemical environment. This perturbation could be a delta function of nutrients brought in by heavy rainfall such as described in the present paper. It may be temperature changes in early spring or late fall or it may be a specific delivery of toxic materials. The diffusional loss of excess nutrients and organisms as the result of water exchange and the loss of phytoplankton as the result of predation by zooplankton are included as damping factors.

Assuming a knowledge of the more important physiological rate constants and their functional dependence, including feedback, it should be possible to define the limits of

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stability of the system, i.e., to predict the maximum steady-state levels of the physical and chemical environmental parameters (nutrients, temperature, light, etc.) such that the natural plankton system will recover from large natural perturbations. The difference between those maximum levels and the observed levels (at any degree of significance) would then be a measure of the stability of the plankton system. It is not immediately obvious how to describe the coupling to the higher trophic levels of the benthic and pelagic species in such a model. The life cycles of most of the economically important species are long and involve complex developmental and migration patterns. However, a study of plankton growth kinetics would appear to be the logical place to begin this modeling approach.

A goal of our plankton studies is to measure quantitatively the changes in natural plankton communities in response to well-defined natural changes of the physical and chemical environment. In principle the study of the kinetic responses of any system to defined stimuli may be able to establish causal relationships with man-made perturbations.

The present paper represents our first attempt to follow the course of a large natural "delta function" perturbation. On July 31, 1971, following a month of drought, a total of 120 mm of rain fell in the Baltimore-Annapolis area within a few hours. We measured the subsequent growth and dissipation of extensive phytoplankton blooms in a 90 Km<sup>2</sup> portion of Chesapeake Bay between Sandy Point (north of the Bay Bridge) and the West River, including the mouths of the Severn, South, Rhode and West rivers, and we have attempted to derive some of the kinetic rate constants for the coupled system model.

## Methodology

### *In Vivo* FLUORESCENCE

Because of the patchy nature of phytoplankton blooms in the bay, an intensive synoptic study of the phytoplankton distributions was undertaken. The method of choice was the *in vivo* continuous chlorophyll fluorescence technique (Lorenzen, 1966; Flemer, 1969). (From the sizes and shapes of the phytoplankton patches observed previously it would have been a physical impossibility to

obtain good spatial resolution over the large area to be covered by a uniform grid of captured samples each having to be individually assayed for chlorophyll *a*.) A vertical tube mounted off the side of a 21-foot cabin cruiser was fixed to sample at a depth of 0.7 m. Water was drawn through the flow cell of a Turner fluorometer and then past dissolved oxygen (D.O.) and temperature probes set in line with the fluorometer flow cell. To produce minimal cavitation effects a Manostat® peristaltic pump was used at a pumping speed of approximately 2.5 ml/sec. The outputs of the fluorometer and alternately, of the D.O. and temperature probes were recorded on a 2-channel Sanborn Model 320 recorder at constant chart speed. Transects were always run at constant motor RPM between fixed markers in the bay. Therefore, if the beginning and end of a transect were marked on the recorder paper, recorder readings could be correlated to positions on a chart of the bay by dividing the recorder paper into as many equal parts as were considered necessary for resolution. In this way, positions on the bay chart could be determined independent of true boat speed, provided the wind and tide could be considered constant over the time period of a transect. The contours as shown are certainly the result of subjectivity on the part of the plotter. However, in drawing the contours we have made use of the continuity properties of the medium and our previous experience in other areas. In many cases where visible blooms were observed the boat course was modified to include the general extent of the bloom area. Therefore some bloom areas shown may not be directly coincident with the transect lines shown in Fig. 2.

Samples were taken from the output hose just past the D.O. and temperature probes for plankton identification, for microscopic counting, for nutrient analysis and for chlorophyll extraction in order to calibrate the fluorometer. On occasion the boat was stopped and depth distributions of chlorophyll fluorescence, temperature and salinity as well as captured samples for chlorophyll extraction and calibration were obtained.

We were able to make slight changes in the baffling and the collimation of the fluorometer so that for any given natural population the *in vivo* fluorescent intensity was directly proportional to extractable chlorophyll *a* concentra-

tions. By the use of a Corning 3-73 combined with a Corning 5-58 filter for the exciting light and Corning 2-64 filters for the emitted fluorescence we have been able to obtain a significant reduction in the contribution of pheo-pigments to the observed fluorescence.

The use of the *in vivo* fluorometer depends on the instrument calibration ratio, R, and the constancy of this ratio.

$$R = \frac{\text{in vivo fluorescent intensity (relative units)}}{\text{extractable chlorophyll } a \text{ } (\mu\text{g/liter})} \quad (1)$$

Strickland (1968a), Strickland and Parsons (1968), and Flemer (1969) have separately reported variations in R for different natural mixed populations measured by the *in vivo* fluorometric technique. We have measured R for our own instrument, using unialgal laboratory cultures of phytoplankton ranging in size

from a 2  $\mu$  unidentified ultraflagellate to *Gymnodinium nelsonii*, a dinoflagellate of ca. 70  $\mu$  major axis and 40  $\mu$  minor axes. These data, shown in Fig. 1, indicate one major source of error of the *in vivo* chlorophyll fluorescence technique applied to natural populations.

The major phytoplankton standing crop in the Chesapeake Bay consists of nanoplankton considerably smaller than 20  $\mu$ . Since larger dinoflagellates are often dominant in phytoplankton blooms, it is possible to seriously underestimate the chlorophyll concentrations in blooms unless the R value of the fluorometer is determined both inside and outside of the blooms as delineated by the continuous recorder readings.

### CHLOROPHYLL PIGMENTS

Known volumes of freshly captured samples were immediately filtered with added solid  $\text{MgCO}_3$  onto 0.45  $\mu$  Millipore filters and frozen at  $-78^\circ\text{C}$  for later extraction and analysis. Chlorophyll was extracted by grinding or sonication in 90% acetone. Concentrations of chlorophylls *a*, *b*, and *c* and pheophytin *a* were measured by fluorescence analysis (Loftus and Carpenter, 1971). Repetitive samples of natural mixed populations can be analyzed for chlorophyll *a* (ca. 10  $\mu\text{g/liter}$ ) with a coefficient of variation of 10%.

### PLANKTON CONCENTRATIONS

Captured natural samples were examined live and preserved with Lugol Iodine Solution. Identification was limited to dominant species. Blue green algae were counted as strands rather than as individual cells. Unfortunately the taxonomy for the 2-10  $\mu$  nanoplankton is not available; we have used arbitrary size distributions. In the case of phytoplankton cell counts a total of three 0.1 ml aliquots were counted. In any water sample the probability of counting zero of a particular species is  $P(0) = e^{-m}$ , where *m* is the average value of the distribution. The 95% confidence limits for this zero count corresponds to setting  $P(0) = 0.05$ , for which  $m = 3.0$ . Thus, under the present procedure for counting phytoplankton, we can make statistically significant statements about the exclusion of any particular species only when the mean species concentration falls below 10,000/liter. In the case of the zooplankton four samples of 5 ml each were counted. Applying the same

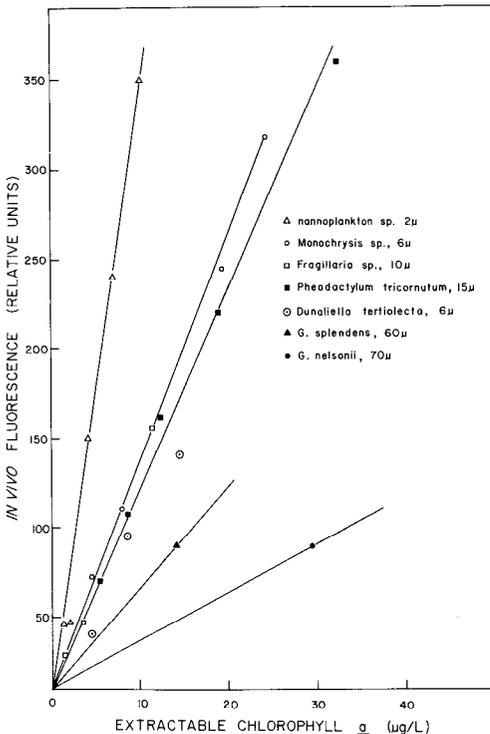


Fig. 1. Species dependent relationship between *in vivo* fluorescence and extractable chlorophyll *a* for several species in unialgal culture. Single point lines for *Gymnodinium splendens* and *G. nelsonii* are average values for each from initial inoculation through stationary phase. The approximate sizes of the various species are given in the figure.

analysis for the 95% confidence limits for the absence of any particular zooplankton species,  $m = 3$  per 20 ml or 150/liter.

## NUTRIENTS

Freshly captured samples were filtered through  $0.45 \mu$  Millipore filters and frozen. Inorganic phosphate was determined according to Murphy and Riley (1962). Nitrite was determined by the method described by Bendshneider and Robinson (1952) and nitrate by that of Morris and Riley (1963).

Ammonia concentrations were determined using the method described by Solórzano (1969). Estimates of total nitrogen and phosphorus were made using the respective inorganic method for phosphate and nitrate referenced above, after a 2 hr oxidation by UV light provided by a 1200 Watt mercury lamp following the methods described in Strickland and Parsons (1968). As several amino acid and ammonium chloride "spikes" showed variable degrees of oxidation for this exposure period in our early work with the lamp, we have termed the estimates of total N and P as "total oxidizable" nitrogen and phosphorus. The values under these headings in Table 2 represent an unknown fraction of the true total but serve to indicate that the totals increased substantially in bloom waters.

## SALINITIES

Salinity was determined using a Beckman conductivity salinometer standardized by chlorinity titration with silver nitrate solutions.

## PRIMARY PRODUCTION

Freshly captured natural samples taken between 1000 h and 1400 h from 0.7 m depth were immediately transferred to light and dark bottles containing tracer C-14 bicarbonate solution and incubated at a depth of 0.3 m for 1 hour in an on-deck polyethylene container containing water from the same area as the original sample in order to retain relatively constant temperature and turbidity conditions. In all cases, a peristaltic pump was used for sample collection, intuitively to avoid the cavitation which occasionally occurs with impeller pumps. In separate checking experiments we have found that this method of sample

collection does not inhibit the subsequently measured photosynthetic carbon uptake as compared with bottle captured water samples. The diel periodicity was not investigated during these surveys. Separate subsamples were taken for chlorophyll and inorganic carbon analyses and for species identification. After 1 hour the samples were filtered onto  $0.45 \mu$  Millipore filters and placed immediately into vials of liquid scintillation counting solutions. All experiments were run in duplicate.

In separate C-14 uptake experiments we have been able to observe uptake proportional to incubation time from 15 minutes to 120 minutes. Carbon-14 uptake can be measured for laboratory cultures at the same chlorophyll *a* concentrations as found in natural samples with a coefficient of variation of *ca.* 4 percent. Inorganic carbon concentrations were measured in a Beckman total carbon analyzer with reference to standard bicarbonate-carbonate solutions. In this range of inorganic carbon concentrations (*ca.* 10 mg/liter) we normally obtain a coefficient of variation of 4 percent.

Data are expressed both as production rate per unit volume  $\text{mgC/m}^3\text{-hour}$  and as assimilation numbers, *Z*,  $\text{mgC/mg chl } a\text{-hour}$ .

## Results and Discussion

### CHLOROPHYLL STANDING CROPS AND SPECIES DISTRIBUTIONS

The area of the bay covered by the *in vivo* chlorophyll fluorescence survey is shown in Fig. 2. The zig-zag lines labelled 1, 2 and 3 represent the three transects along which weekly data are collected on a year-round basis (except for winter icing of the rivers). The overlapping transects for each survey approximate 55 nautical miles over the region from 1 mile north of the Chesapeake Bay Bridge (Sandy Point) to 1 mile south of the West River and from the Severn and South Rivers eastward to the ship channel in the bay. Surveys were made on August 4, 6, 10, 13, 17, 20 and 23, 1971, by which time the blooms were completely dissipated.

The changes in the distributions of chlorophyll *a* standing crops at 0.7 m depth in the Western Bay from August 4 through August 20 are shown in Figures 3 through 8. Lines of equal concentrations of chlorophyll *a* (isocons) are drawn at  $25 \mu\text{g/liter}$  intervals.

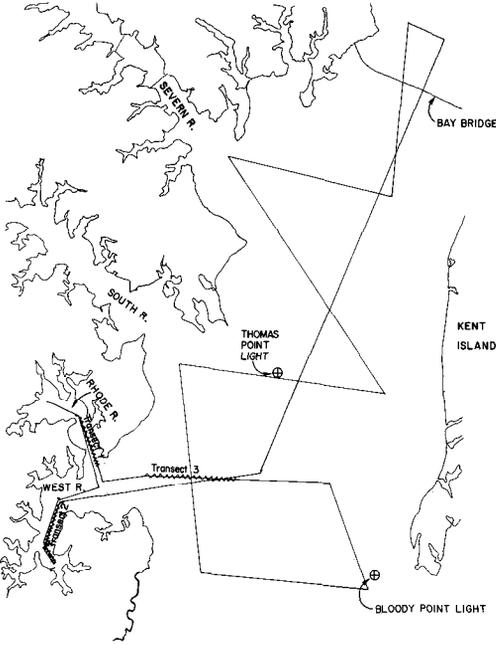


Fig. 2. Solid lines (—) represent transects navigated during the bloom survey. Zig-zag lines (~~~~) are transects navigated in routine plankton survey before, during, and after August 1971.

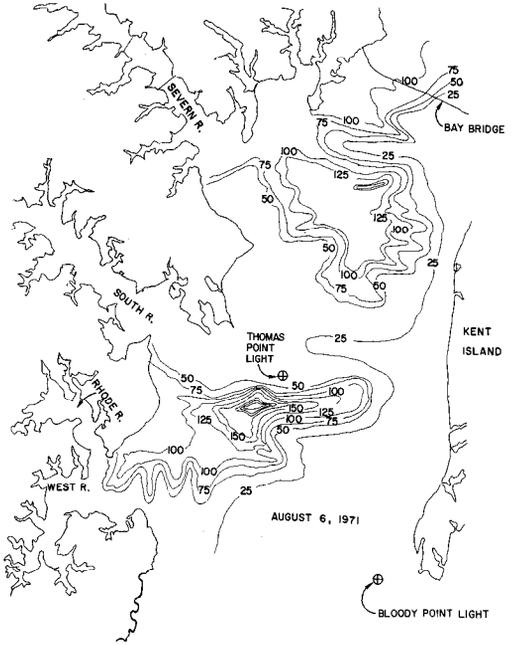


Fig. 4. August 6, 1971 map of chlorophyll *a* isocons (25 µg/L intervals). Isocon location and magnitude determined by interpolation as described in text.

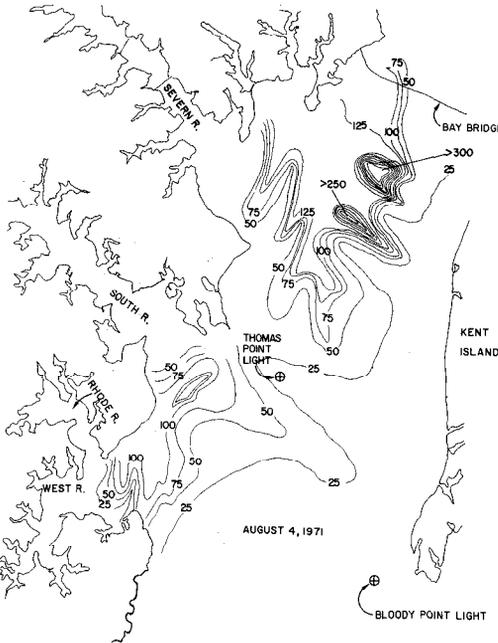


Fig. 3. August 4, 1971 map of chlorophyll *a* isocons (lines of equal concentration). Isocon (25 µg/L interval) location and magnitude determined by interpolation as described in text.

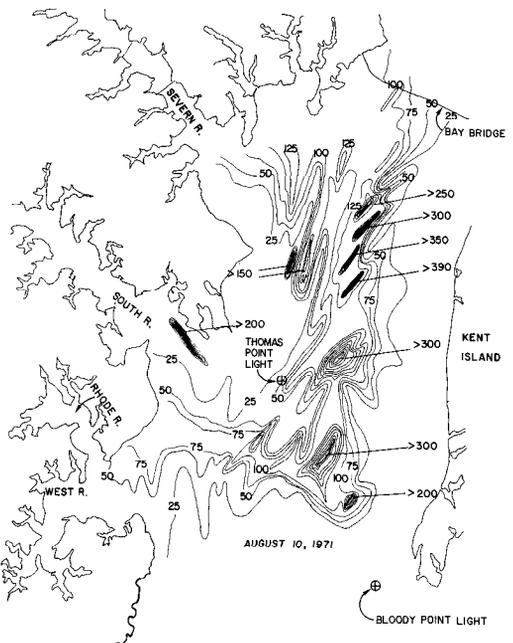


Fig. 5. August 10, 1971 map of chlorophyll *a* isocons (25 µg/L interval). Isocon location and magnitude determined by interpolation as described in text.

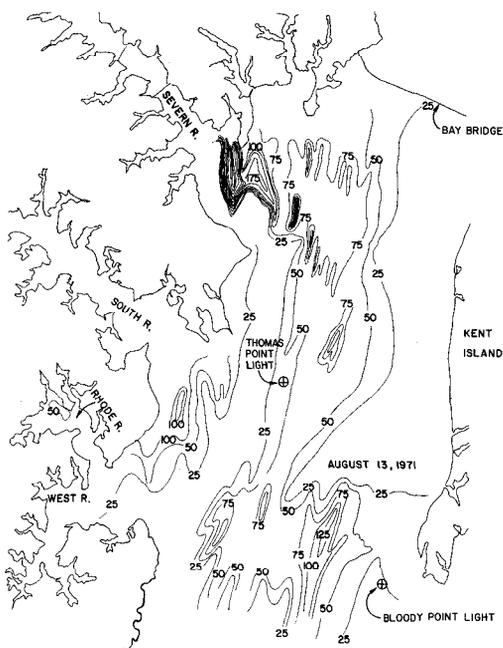


Fig. 6. August 13, 1971 map of chlorophyll *a* isocons (25 µg/L intervals). Isocon location and magnitude determined by interpolation as described in text.

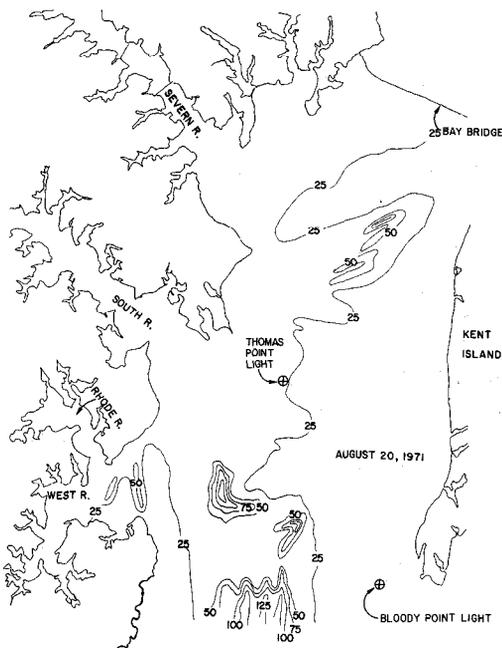


Fig. 8. August 20, 1971 map of chlorophyll *a* isocons (25 µg/L intervals). Isocon location and magnitude determined by interpolation as described in text.

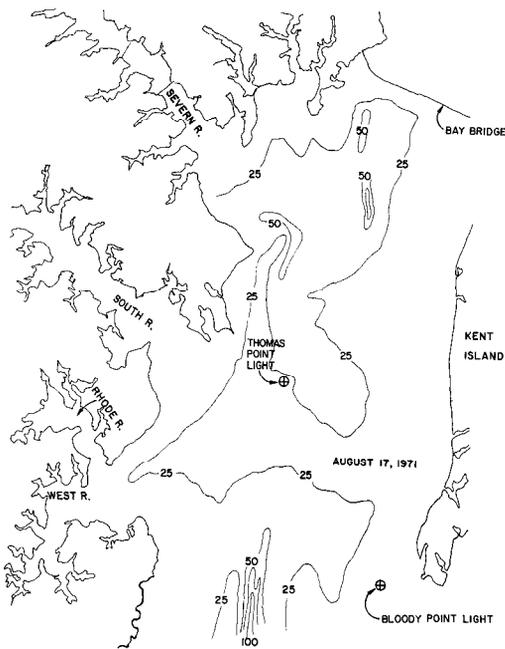


Fig. 7. August 17, 1971 map of chlorophyll *a* isocons (25 µg/L intervals). Isocon location and magnitude determined by interpolation as described in text.

Owing to the finite time required to traverse the transects and the presence of wind and tidal currents, the chlorophyll *a* concentration data for any transect does not have the exact spatial relationship to the other transects that is implicitly assumed in the figures. Therefore, the exact locations of the patches shown in Figures 3 through 8 are uncertain. It would obviously be preferable to obtain these standing crop distributions by an aerial scanning technique, provided the spatial resolution and specificity for chlorophyll *a* could be retained. The data presented in the figures should provide sufficient information relative to the spatial resolution and chlorophyll concentration gradients that are encountered under bloom conditions to establish the criteria for development of the aerial spectral scanning techniques.

However while aerial scanning for chlorophyll would be extremely useful for improving spatial resolution and efficiency in that it might more easily locate bloom areas into which to despatch sampling boats, it can in no way substitute for the physical sampling. In fact, the parallel need for species identification and counting, nutrient assays, measurement of

production and predation rates and for verification of the aerial scanning data by chlorophyll extraction of defined samples almost requires that aerial scanning for chlorophyll be on-line. Photographic techniques without concurrent rapid scanning might be of marginal scientific value although they could be helpful for verification of the boat data.

The relative species composition and therefore the size composition within a dense patch may not be the same as outside the patch. For each survey, samples for calibration of the *in vivo* fluorescence were taken over the range of *in vivo* fluorescence observed. Thus it would be expected that changes in species composition inside and outside of blooms would be evidenced as non-linear slopes of relative fluorescence intensity plotted as functions of extractable chlorophyll *a* per liter. The curves of Fig. 9 show evidence of significant changes in species size distributions between August 4 and August 13. From the calibration curves of Fig. 1 the transition should be from large to small phytoplankton as the major chlorophyll source.

The ratio in mean slopes of a factor of 2 corresponds to a transition from organisms of the size of *Gymnodinium splendens* ( $\sim 60 \mu$ ) to *Monochrysis* sp. ( $6-10 \mu$ ). The data for August 4 and 13 are in good agreement with the average slopes plotted, with coefficients of variation for *R* of 16% and 13%, respectively. The data of August 10 (solid triangles) show two distinct slopes, one at lower chlorophyll *a* concentrations which is essentially the same as the August 4th slope and the second, a steeper slope at high chlorophyll *a* concentrations, within experimental error the same as the August 13th slope. Here is direct fluorometric evidence of a transition period where a smaller species, growing rapidly and defining the bloom areas, has not yet imposed its new and smaller size distribution on the rest of the bay. By August 13, the "new" species was presumed to have become equilibrated with the bay waters. The relative size distributions (species composition) appeared to be the same within and without the blooms, giving rise to a constant slope for *R*.

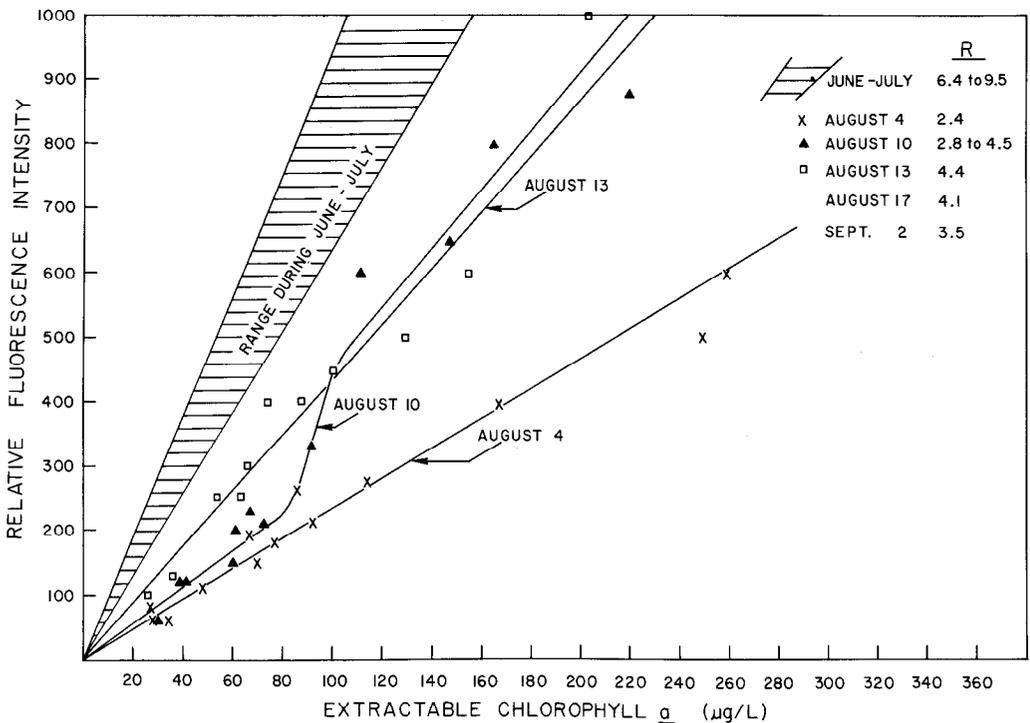


Fig. 9. The calibration relationships between *in situ* fluorescence and extractable chlorophyll *a* from field samples collected on several of the survey dates. The slope *R* is subsequently used to approximate the ambient chlorophyll *a* isocons shown in Figures 3 through 8.

For comparison, the range of R values measured during June and July, 1971, is shown by the cross hatched area. As can be seen from the curves and from the small legend on the figure the phytoplankton species size distributions giving rise to chlorophyll fluorescence during August and even on into September are quite different from those during June and July. It should be emphasized that the June-July data are extrapolated to high chlorophyll concentrations to emphasize visually the differences in slopes; during this period chlorophyll *a* concentrations ranged between 10–20  $\mu\text{g}/\text{liter}$ .

There are several extremely interesting aspects to the observed variations in R with organism size as shown in Fig. 1. The decrease in R with larger phytoplankton sizes has been verified for the *in vivo* fluorescence technique by direct microscopic counting of captured samples and by extraction of chlorophyll. However, in water samples of different turbidities, multiple scattering can increase the effective path length of the exciting light within the fluorescence flow cell, giving rise to an artifact of increased absorption by chlorophyll. Since the fluorescence radiation is essentially isotropic, increased scattering will not affect the efficiency of collection of the fluorescence. This source of error will not be corrected for by measurement of extractable chlorophyll from captured samples and is at present included in the sampling error variance. The results shown in Fig. 1 were obtained with non-turbid laboratory cultures.

It may be that, a) the larger values of R for the smaller phytoplankton are the result of differences in indices of refraction of the cell membranes for the different species, giving rise to different effective cross sections; b) in the small nanoplankton the chlorophyll molecules in the grana or the grana themselves are more efficiently dispersed (much as the manner in which pigment in a photophore cell can be concentrated or dispersed) giving rise to a greater effective absorption of exciting light; c) the efficiency for fluorescence of the nanoplankton chlorophyll is higher than for the larger phytoplankton; d) owing to the greater curvature in the smaller nanoplankton an entering light quantum has a much lower probability of escape and by being multiply reflected is eventually absorbed by the chloro-

phyll within the cell; or 3) the absorption of the blue light by chlorophyll and accessory pigments both of which lead to chlorophyll fluorescence is greater for the smaller sized nanoplankton because of relatively greater amounts of blue-absorbing accessory pigments.

Strickland (1968b) reported that the continuous fluorometric measurement of the depth distribution of chlorophyll *a* concentrations was much more precise in the estimation of standing crops of chlorophyll *a* per unit area than discrete depth sampling, owing to the possibility for dense narrow banding of phytoplankton which could easily be missed by the discrete sampling technique. An example of such a narrow banding vertical profile is shown in Fig. 10 for a station near the Bay Bridge during the August 6th survey. In the figure, the continuous *in vivo* fluorescence is compared with the discrete sampling points. The D.O. data at that time were subject to an instrumental time lag and thus appear to be slightly out of phase with the *in vivo* fluorescence data. Temperatures and salinities on this date were characteristic of a well mixed upper layer. The measurements were carried out to a 3 m depth; the total depth was in excess of 6 m. The horizontal banding, peaking at the 0.7 m depth, in wind mixed water of uniform density, is possibly due to migration to a region of specific light intensity. It is also conceivable that the

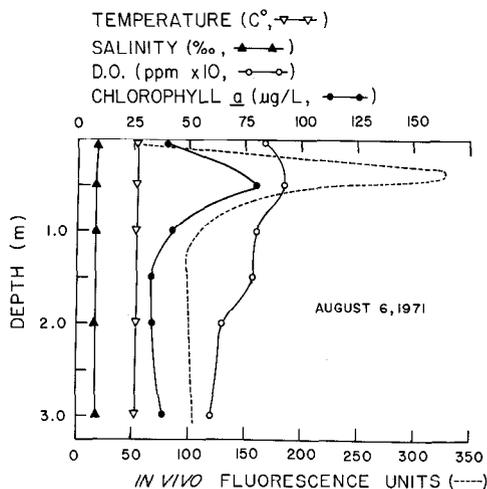


Fig. 10. The vertical profiles of temperature, salinity, dissolved  $\text{O}_2$ , chlorophyll *a*, and *in vivo* fluorescence in the euphotic zone at the Bay Bridge on August 6, 1971.

banding below the surface is due to the steady-state of non-specific positive phototaxis modified by a sinking response of surface organisms to wind produced turbulence, although similar sub-surface banding has been observed in deep tanks in the absence of wind action (Eppley, Holm-Hansen and Strickland, 1968).

The mean salinity in the upper layers on August 6 was 9.0 o/oo, significantly lower than the values of 12 o/oo measured prior to the large local rainfall. Profiles of salinity, temperature and D.O. at various stations in the survey area for August 4, 1971 are shown in Fig. 11. The fresh water influx produced the most marked discontinuity at the Bay Bridge. The waters are reasonably well mixed and of intermediate salinity at Buoy 73 (approximately 15 Km south), and are highest in salinity at the mouth of the Rhode River. The slight effect of Rhode and West River runoff is

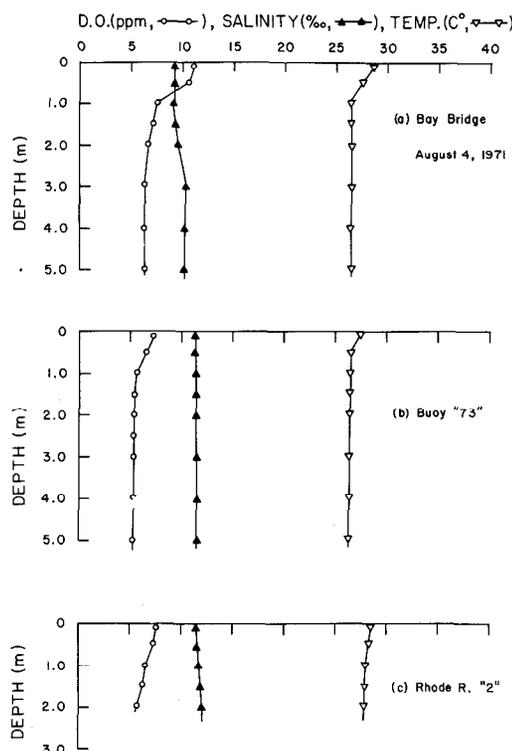


Fig. 11. August 4, 1971 vertical profiles of temperature, salinity, and dissolved  $O_2$ , at (a) Bay Bridge, (b) Buoy "73", and (c) Rhode River buoy "2" showing intrusion of lower density surface waters at the Bay Bridge.

shown by the small positive gradient of salinity with depth. It would therefore appear that the major contributions of fresh water and consequently of nutrients to the bay area opposite the Rhode and West Rivers come from north of these tributaries and that the latter reflect rather than determine the condition of the contiguous bay area.

On day 4 following the rain (August 4, Fig. 3), brown areas up to  $300 \mu\text{g/l}$  chlorophyll *a* were already visible, mainly surrounding the mouth of the Severn River. This early phase of the bloom period was characterized by the mixed nature of the dominant species. Large dinoflagellates (*Gonyaulax* sp., *Gymnodinium nelsonii*, *G. splendens*, *Polykrikos hardmanii* and *Prorocentrum triangulatum*) blue green algae and *Euglena* spp. appeared to be the dominant algal biomass, although  $2\text{--}20 \mu$  nannoplankton (ultraflagellates) were quite numerous ( $>10^8$  per liter). By day 6 (August 6, Fig. 4), the areas of high concentrations had expanded greatly to the south and east but were still to be found surrounding the mouths of both the Severn and the South Rivers. The dinoflagellates, the blue greens and *Euglena* spp. were still quite abundant but now appeared to be overtaken by a smaller dinoflagellate, *Oxytoxum* sp. (*ca.*  $15 \mu$ ). The patches exhibited large density gradients in organism concentrations at their edges. More than an order of magnitude change in chlorophyll *a* concentrations were measured over distances of tens of meters. The remainder of the bay still contained chlorophyll *a* standing crops at preraifall levels (*ca.*  $15 \mu\text{g/liter}$ ).

By day 10 (August 10, Fig. 5), the bloom area had developed into a large number of patches oriented mainly in a north-south direction. The envelope of the bloom had moved southeastward, away from the mouths of the rivers. *Oxytoxum* sp. was now dominant within many of the dense patches.

From the microscopic counting data of Table 1 for August 10, 1972, *Oxytoxum* sp. concentrations as high as  $94 \times 10^6/\text{liter}$  were found inside patches, compared with  $0.19 \times 10^6/\text{liter}$  outside. The larger dinoflagellates and the euglenoids were still present in significant numbers while the blue greens were high in the Severn River. Ciliates, rotifers and tintinnids were approaching high concentrations within the visible blooms.

TABLE 1. Concentrations of phytoplankton and zooplankton at various locations on August 10, 1971. Except where specified as Number/L the concentrations are in millions per liter.

	Mouth of Rhode River	Bay "2"	Bay "73"	Bloom east of Bay "73"	Bloom at Thomas Point					Bay "77"	Severn River Buoy "10"	Bloom South of Bay "78"	Bay "78"	Bay Bridge
					.05m	.5m	1m	2m	3m					
<b>Phytoplankton</b>														
Ultraflagellates	29	(29)	32	43	43	40	29	43	51	61	58	29	54	43
<b>Dinoflagellates</b>														
<i>Gonyaulax</i> sp.	—	.2	.2	3.6	.03	.04	.04	.04	.04	.4	.06	.04	—	.04
<i>Gymnodinium nelsonii</i>	.4	.4	.8	—	.2	.02	.03	.01	—	—	—	1.1	—	—
<i>Gymnodinium splendens</i>	—	—	.01	—	.02	.02	.03	—	.01	0.1	—	—	—	—
<i>Oxytoxum</i> sp.	—	—	12.4	94	47	32	47	40	17	.19	4.8	53	.6	—
<i>Polykrikos hardmanii</i>	—	.4	.02	0.2	.04	.07	.12	.03	—	1.1	.01	1	.6	.6
<i>Prorocentrum triangulatum</i>	.4	2.3	—	.02	—	—	.03	—	—	1.1	.02	.4	.8	.6
<b>Blue Green Algae</b>														
Blue Green Algae	—	.02	.001	.001	—	—	—	—	.002	—	.4	.01	.006	—
<i>Euglena</i> spp. 10-15 $\mu$ long 2-3 $\mu$ diam.	.76	.4	1.1	1.1	2.3	1.1	1.5	1.1	1.5	0.4	1.1	.8	.4	.6
<b>Diatoms</b>														
<i>Coscinosira</i> sp.	—	—	—	—	—	—	—	.02	—	—	—	—	—	—
<i>Cylindrotheca closterium</i>	1.9	.2	.02	—	.4	.01	—	.04	—	—	.02	.2	—	—
<i>Pleurosigma</i> sp.	—	—	—	—	.01	—	—	—	—	—	—	—	—	—
<i>Skeletonema costatum</i>	7.2	.02	.6	.02	—	.4	.02	.01	.08	.03	.04	.04	—	.02
<i>Thalassiothrix frauenfeldii</i>	—	.2	.04	.04	.02	—	—	—	—	—	.02	—	—	—
<b>Total chl a <math>\mu</math>g/L</b>	41.5	61	72	218	109	165	147	111	91	30	68	390	38	60
<b>Zooplankton</b>														
Ciliates	—	—	—	.01	—	—	—	—	—	—	—	.02	—	—
Nauplii	400/L	400/L	—	—	200/L	200/L	—	—	—	—	—	200/L	400/L	400/L
Rotifers	.06	400/L	.01	600/L	.01	200/L	200/L	600/L	200/L	200/L	800/L	.002	—	200/L
Tintinnids	.02	—	—	.01	—	—	—	—	—	400/L	—	—	.01	—

Growth and Dissipation of Phytoplankton in Chesapeake Bay

By day 13 (August 13, Fig. 6), a major southward movement had occurred. The chlorophyll *a* concentrations in the patches had decreased significantly. *Oxytoxum* sp. was still the dominant nanoplankton. Rotifer concentrations up to 100,000/liter were found in patches in the central bay. Only a very dense patch in the mouth of the Severn River was predator-free.

During the first two weeks of August, there were approximately 70 mm of additional rainfall. The continuing run-off into the rivers and into the bay had the effect of extending the period during which the dinoflagellates, blue greens and euglenoids were observed. For example, by day 17 (August 17, Fig. 7) the dense visible patches had mainly dissipated. However euglenoids up to  $1.5 \times 10^6$ /liter and blue greens up to  $.06 \times 10^6$ /liter and dinoflagellates up to  $.8 \times 10^6$ /liter were observed around the mouth of the South River. The receding tail of the chlorophyll *a* distribution was evident southeast of the West River. Rotifers and tintinnids were still abundant.

By day 20 (August 20, Fig. 8), the last day plotted in the series, the bloom had almost completely dissipated. It was only at this late date that the bloom appeared finally to have contributed to the phytoplankton standing crops at the mouths of the Rhode and West rivers. Even this small increase was of short duration. By August 25 the chlorophyll standing crop was uniform throughout the area.

The transition in relative species composition of phytoplankton before and through the bloom period was:

Order of Dominance	July	August 4	August 10	August 25
Major	ultraflagellates <20 $\mu$	dinoflagellates >30 $\mu$	<i>Oxytoxum</i> sp.	ultraflagellates <20 $\mu$
Intermediate		Euglenoids Blue green ultraflagellates <20 $\mu$	ultraflagellates <20 $\mu$	dinoflagellates >30 $\mu$
Minor	dinoflagellates >30 $\mu$			

In accordance with our observations before, during, and after this bloom period, we suggest that the following sequence of chemical, physical and biological interactions occurred, which seems to provide the most plausible explanation for changes in the level of the standing crops and species composition:

1. During early July phytoplankton concentrations in terms of standing crop of chlorophyll *a* were low, around 10-20  $\mu$ g/liter. The major algal biomass as determined by chlorophyll extraction consisted of phytoplankton < 20  $\mu$ . Inorganic nutrient concentrations were also low (Seliger *et al.*, unpublished) and inorganic nitrogen appeared to be limiting.
2. Subsequent to the pulse of rainfall a large volume of lower salinity (density) run-off containing high nutrient levels formed surface layers around the mouths of both Severn and South rivers. The euglenoids and blue green algae normally present in the low salinity waters multiplied rapidly.
3. Of the underlying bay species, only the strongly positively phototactic dinoflagellates were able to migrate into the upper layer in any significant numbers. These organisms, finding themselves in a higher concentration of nutrients, reproduced maximally and became the dominant phytoplankton.
4. The mechanical energy delivered to the system by wind and tide acted to disperse the upper layer containing both the plankton and the nutrients. The continued presence of areas of high phytoplankton concentrations was due to continued run-off and replenishment of the surface layer.
5. The decreasing run-off, and the continuing mechanical dispersion served to make vertical mixing more effective. A smaller but still positively phototactic dinoflagellate (*Oxytoxum* sp.) became dominant. The larger
6. The run-off became normal. Predation and lowered nutrient levels reduced the net rate of reproduction of *Oxytoxum* so that the

dispersion rate became the dominant factor. The pre-rainfall ultraflagellates again became dominant. However, the rapid phytoplankton-zooplankton cycling may have provided some specific nutrient form required by the larger dinoflagellates, since their absolute concentration became greater than during the early summer.

In the absence of sufficient data we cannot yet evaluate the relative importance of the capacity of various species to take up nutrients (Eppley *et al.*, 1969b) and the differential migratory capacity of various species advocated here as one primary factor in determining the succession of species dominance in bloom waters. It should be noted that for resident species there is a correlation between the rate of nutrient supply and the size of the dominant species which is as would be predicted by the trends in  $K_s$  vs. organism size observed by Eppley *et al.*, (1969a).

#### NUTRIENT LEVELS AND GROWTH RATES

Dissolved  $PO_4^{=}$ ,  $NO_2^-$ ,  $NO_3^-$  and approximate total N and total P concentrations corresponding to various chlorophyll *a* standing crop concentrations measured during the bloom period and during early July are shown in Table 2.

During June, July and early September, 1971, the assimilation ratios measured for the surface waters of Transect 3 (Fig. 2) were high.

The mean value and the standard deviation were  $10 \pm 4$  (mgC/mg chl *a* -hr). The standing crop of extractable chlorophyll *a* was ca. 20  $\mu$ g/liter and was primarily due to ultraflagellates  $< 20 \mu$  (Seliger *et al.*, unpublished). For log phase laboratory cultures of *G. nelsonii*, *G. splendens* and blue green algae isolated from bloom areas we have measured carbon/chlorophyll *a* (w/w) ratios of  $112 \pm 12$ ,  $105 \pm 10$  and  $97 \pm 20$ , respectively. These are in agreement with ratios published by Eppley and coworkers (Anon, 1971). An assimilation ratio of 10 corresponds to a doubling rate of  $0.1 \text{ hr}^{-1}$  which roughly translates to a doubling time of 1 day. If we now assume carbon to nitrogen ratios (w/w) of between 6-10 (Anon, 1971), the maintenance of the June-July standing crop of 20  $\mu$ g/liter of chlorophyll *a* required a daily utilization of the pre-bloom phytoplankton of 14-24  $\mu$ g atom/liter of nitrogen. These requirements are significantly higher than the steady-state concentrations of  $NO_2^-$  and  $NO_3^-$  and  $NH_4^+$  shown in Table 2. Whaley *et al.* (1966) have reported  $NH_4^+$  concentrations in this general area of the bay during June and July to range between 0.8-5.9  $\mu$ g atom/liter. Even considering the uncertainties in these approximate calculations it is evident that there must be additional nitrogen sources such as urea (Anon, 1971) which have not been considered, a very rapid replenishment of inorganic nitrogen, or a combination of these.

The highest bloom areas for which assimilation ratios were measured contained 150

TABLE 2. Analytical estimates of ambient concentrations of chlorophyll *a*, ammonia, nitrite, nitrate, total oxidizable nitrogen, inorganic phosphate, and total oxidizable phosphate in non-bloom and bloom waters.

Date	Chl <i>a</i> $\mu$ g/L	$NH_4^+$ $\mu$ g atom/L	$NO_2^-$ $\mu$ g atom/L	$NO_3^-$ $\mu$ g atom/L	Total N $\mu$ g atom/L	$PO_4^{=}$ $\mu$ g atom/L	Total P $\mu$ g atom/L
non-bloom							
7-7-71	13.3	4.3	0.14	1.93	32.5		2.7
7-14-71	20.4	5.0	0.00	0.00	45.0		4.4
7-21-71	25.5	2.6	0.06	1.20	27.0	0.02	1.8
7-29-71	18.3	1.8	0.31	0.00	-	0.41	1.3
8-10-71	30.0	1.5	0.18	0.55	54.0	0.62	2.4
8-16-71	25.0	1.1	0.16	1.88	42.5	0.60	5.1
8-23-71	14.0	2.6	0.12	0.97	37.5	0.10	5.2
Bloom							
8-10-71	72.0	1.93	0.47	2.37	64.0	0.43	4.8
	67.5	0.62	0.16	0.58	60.0	3.53	5.4
	66.0	1.10	0.17	0.99	70.0	3.11	5.7
	386.0	0.42	0.51	0.88	280.0	5.50	11.6
8-20-71	147.0	4.1	-	-	270	-	8.5

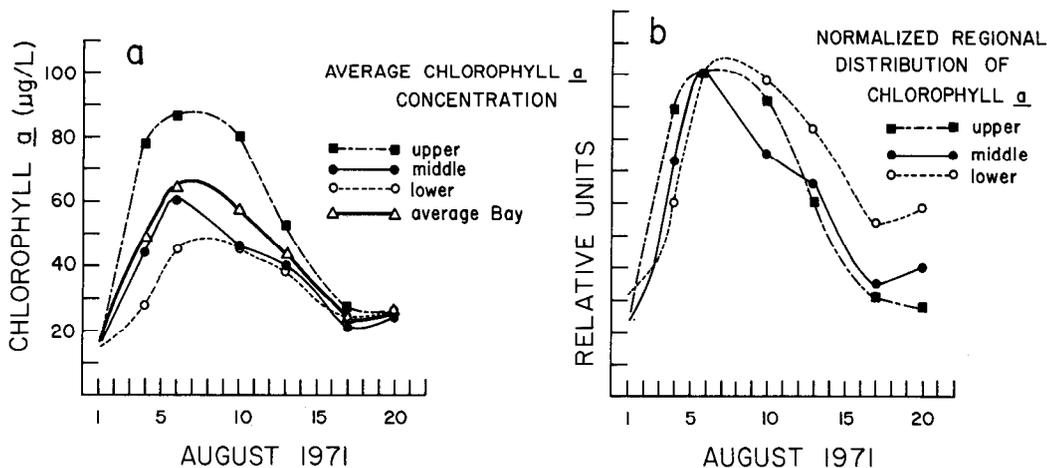


Fig. 12 a. The average concentration of chlorophyll *a* in each of three sub-areas and in the whole area of the survey (Average Bay) for the days of August 1971 (see text). b. The normalized data from Fig. 12a for the three sub-areas of the survey for the days of August 1971.

$\mu\text{g/liter}$  of chlorophyll *a*. The ratios ranged from 4.7–6.0 ( $\text{mgC/mg chl } a \text{ -hr}$ ), not significantly different from the pre-bloom average value of 10. If we consider that surface concentrations of chlorophyll *a* during the bloom became as high as 400  $\mu\text{g/liter}$  and that in Table 2 the inorganic nitrogen concentrations in the dense patches were not much different or even lower than during the pre-bloom measurements, it follows that the turnover time for available nitrogen and/or uptake rate of the phytoplankton can be extremely rapid and variable. The measurement of steady-state concentrations of inorganic phosphorus and nitrogen appears to be of doubtful value in the development of a model, although their time derivatives can show trends of growth or limitation of phytoplankton standing crops.

#### KINETIC ANALYSIS

The isocons of chlorophyll *a* in Figs. 3 through 8, while subject to the spatial uncertainties described previously, can be used for an approximate kinetic analysis of the growth and dissipation of the "excess" phytoplankton during the bloom period. In order to arrive at Fig. 12, we arbitrarily divided the 90  $\text{Km}^2$  area into three areas: an upper third including the Severn River, a middle third including the South River and a lower third including the Rhode and West rivers. For each subarea, we summed the isocons for each survey day according to the following formula:

$$\text{mean chlorophyll } a \text{ } [\mu\text{g/liter}] \text{ for sub area} = \frac{\sum_i [\text{chl } a]_i [\text{area}]_i}{\sum_i [\text{area}]_i} \quad (2)$$

These mean chlorophyll *a* concentrations for each subarea and for each survey day are plotted in Fig. 12(a). The mean values for the entire 90  $\text{Km}^2$  area are shown by the thick solid line ( $\triangle$ — $\triangle$ ). The most rapid rise occurred in the upper third, indicating that the Severn R. is the major contributor of local run-off and nutrients in the region. The rise is slower in the middle sub area (South R.). There is a lag apparent for the Rhode-West River section as though the Severn River runoff required several days to reach this area. Since the division into the three subareas was completely arbitrary, the mean chlorophyll *a* concentrations are subject to some uncertainty. However in Fig. 12(b) where the phytoplankton growth data are normalized to unity as of August 6, the curves are remarkably similar with a half time for dissipation of between 4–5 days and a maximum doubling time of 1 1/2 days.

The areal distributions for various chlorophyll *a* class interval concentrations, plotted in Fig. 13(a) through (f), were calculated from the formula

$$\begin{array}{l} \text{Area of } [\text{chl } a] \\ \text{for the mean} \\ \text{class interval, e.g.} \\ 75 \mu\text{g/liter} \end{array} = \frac{\text{Area of } [\text{chl } a] \geq 50 \mu\text{g/liter}}{\text{Area of } [\text{chl } a] \geq 100 \mu\text{g/liter}} \quad (3)$$

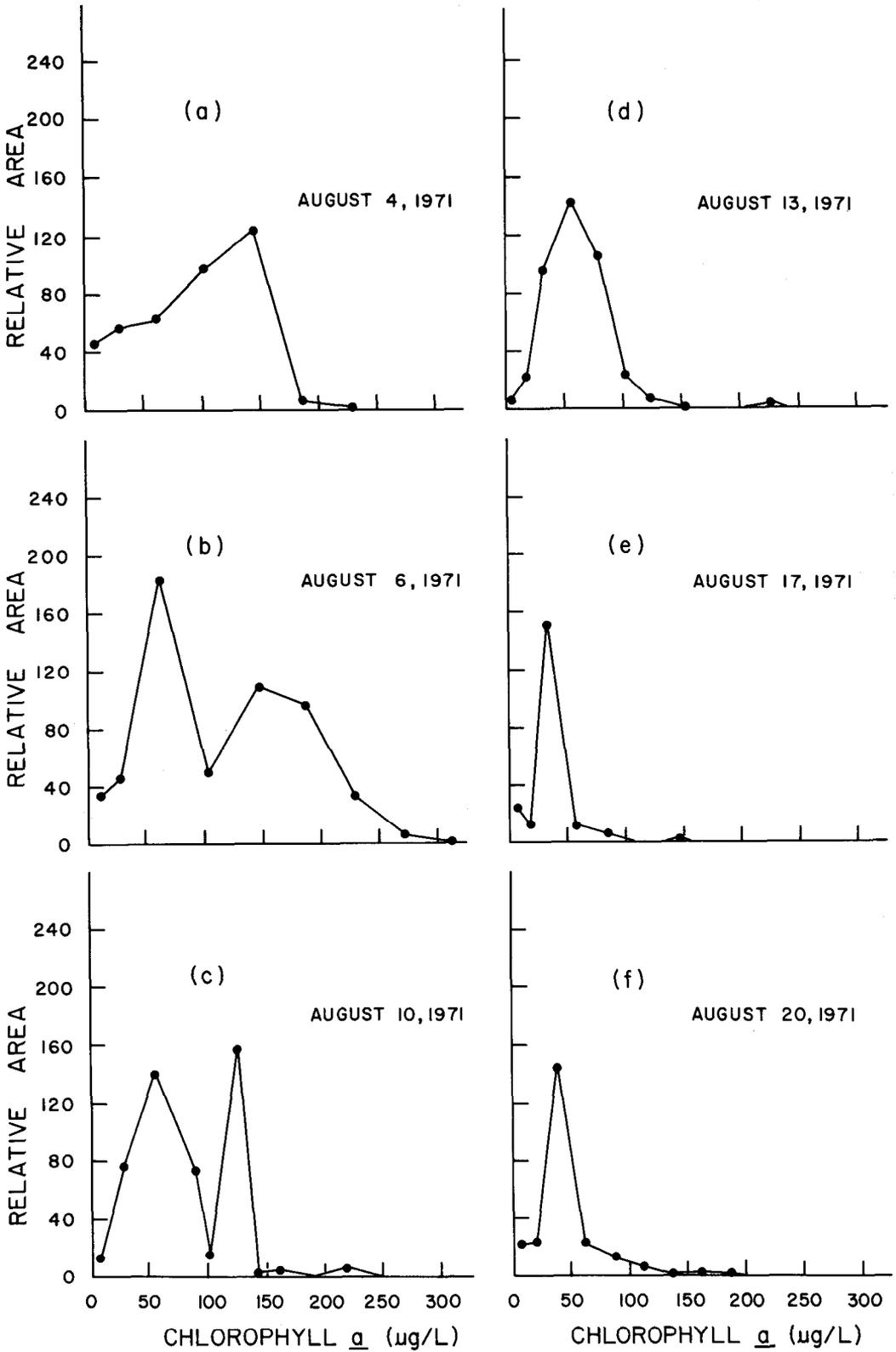


Fig. 13 a-f. The relative area occupied by the mean class interval concentration of chlorophyll *a* (see text for calculation) in excess of the pre-bloom level *ca* 15 µg/liter, for each of the days of the bloom surveys.

obtained for each date from the isocon drawings of Fig. 3 through 8. These frequency distributions show several characteristics: Fig. 13(a)—by August 4 there is a general increase in the area containing higher chlorophyll *a* concentrations; Fig. 13(b)—the surface waters are still not completely mixed. Rapid continued growth in earlier runoff surface layers has produced patches of very high phytoplankton density. Phytoplankton in newer runoff layers are beginning to grow, giving rise to the bimodal distribution of areal concentrations of chlorophyll *a*. Fig. 13(c)—By August 10 predation and dispersion were beginning to overcome the rapid phytoplankton growth rates. The high density patches were becoming diluted and the bimodal distribution was becoming compressed. Fig. 13(d)—by August 13 the area containing high phytoplankton populations was very small and the distribution became essentially singly peaked and rather broad, peaking at 50  $\mu\text{g/liter}$ . Fig. 13(e), (f)—by August 17 and August 20 the high density patches had completely dissipated. The frequency distribution was now sharpened and shifted to lower concentrations more normally encountered in the bay.

These qualitative plots of the kinetics and areal distributions of gross phytoplankton standing crops may have some value as a means of characterizing various areas of the bay according to their responses to large added nutrient pulses. The bimodal areal distributions of chlorophyll *a* concentrations appear to be characteristic of discrete dense patches. It is possible that aerial surveys, despite the large errors in calibration, could be used to obtain more frequent areal distributions for determining the rate constants, the progression and the extent of blooms in the bay.

There are two mechanisms operating to dissipate the phytoplankton standing crops; predation and physical dispersion due to wind and tide action. Predation, especially by the rotifers and tintinnids, appeared to follow the *Oxytoxum* bloom populations rather closely. By day 10 *Oxytoxum* was dominant in the dense patches and by day 13 rotifer populations up to 100,000/liter were found in the *Oxytoxum* patches. These figures are probably magnified as the result of positive phototaxis. To a captured natural sample of 100,000 rotifers/liter (*Euchlanis sp.*) we added 400

$\mu\text{g/liter}$  chlorophyll *a* in the form of *ca.* 2 $\mu$  diameter ultra plankters. Over a 12 hour night period the chlorophyll *a* concentration was reduced to 20  $\mu\text{g/liter}$ . Assuming a constant rate of movement we calculate

$$20 = 400 e^{-k_p P t} \quad (4)$$

where  $k_p$  is the rate constant in terms of  $\text{ml hr}^{-1} \text{rotifer}^{-1}$ ,  $P$  is the density of rotifers per ml and  $t$  is time in hours, from which

$$\begin{aligned} k_p &= 0.0025 \text{ ml hr}^{-1} \text{ rotifer}^{-1} \\ &= 0.06 \text{ ml day}^{-1} \text{ rotifer}^{-1} \end{aligned}$$

Assuming that anything entering the circular plane of the mouth opening (*ca.* 30  $\mu$ ) is consumed, the *Euchlanis sp.* must therefore sweep out a minimum path length  $3.7 \times 10^6 \mu$  per hour, and maintain a mean swimming speed of at least 3.7 m/hour, roughly 10 times the swimming speed of a comparably sized dinoflagellate. Assuming that the frictional force is roughly proportional to the square of the velocity, the rotifer apparently expends 100 times more energy for motility than the dinoflagellate. The predation rate constant calculated above for *Euchlanis* is intermediate between the values of 0.036–0.264 reported by King (1967) for a sister species in laboratory culture.

*Euchlanis* readily consumes *Oxytoxum* but apparently cannot eat 30–40  $\mu$  dinoflagellates. A partial explanation of the observed rise of the larger dinoflagellates in late August might therefore be selective predation on the smaller nannoplankton by the large predator populations generated by the bloom.

Under specified conditions either nitrogen, phosphorus (Ketchum, 1939) or carbon (Myers, 1944) can be the limiting nutrient to phototrophic growth. Ryther and Dunstan (1971) have described a coastal marine environment in which nitrogen may be the limiting nutrient. In general any one of the three inorganic sources (nitrate, nitrite or ammonium) can serve as a sole nitrogen source (Vaccaro, 1965). However, organic nitrogen sources such as urea, uric acid and xanthine have been shown to be equally as effective (Birdsley and Lynch, 1962). Arnou *et al.* (1953) have used arginine, ornithine and citrulline. There already exists a membrane mechanism for active transport of complex organic molecules since absolute requirements

for thiamine and vitamin B<sub>12</sub> by algal species have been recognized for many years (see Droop, 1966). Among the inorganic ions there can be preferential uptake of one form in the presence of the others (Grant *et al.*, 1967; Eppley *et al.*, 1969b; Goering *et al.*, 1970). In coastal and estuarine waters the concentrations of dissolved organic nitrogen sometimes exceed those of dissolved inorganic nitrogen. The sources and turnover rates of both nitrogen and phosphorus are still relatively unknown.

### Conclusions

It would appear therefore that a systems analysis compartmental representation (see Patten, 1971; Kowal, 1971) for the plankton populations in the bay is premature. The number of species involved and their competitive, symbiotic and feedback parameters tend to make the number of adjustable constants in any power series form of solution truly formidable. An even more sobering thought derives from the results of the present study, *i.e.*, as the result of a change in the physical and chemical parameters of the bay the relative species composition may change drastically. Thus, species of normally low concentration or washed out such as the euglenoids and the blue greens would probably not be included among the differential equations describing the "normal" population, although they may become dominant and shape the zooplankton distributions as well.

A promising approach to the relationship of nutrient levels to phytoplankton growth rates has been the analysis of nutrient uptake rates using the Michaelis-Menten concept of half saturation substrate concentrations (Dugdale, 1967; Eppley and Coatsworth, 1968; Eppley *et al.*, 1969a; Eppley and Thomas, 1969; Carpenter and Guillard, 1971; Anon, 1971). These  $K_s$  values appear to be correlated with species requirements in geographical regions with different nutrient levels (MacIsaac and Dugdale, 1969). Multiple nutrient requirements or specific requirements for metabolites that are produced in or delivered to the bay water under special or seasonal conditions may underlie the observed rapid growth peaks of certain plankton species.

The steady-state concentrations of  $\text{NO}_3^-$  and even  $\text{PO}_4^{=}$  were generally higher in the initial

stages of the bloom formation. However, unless the complex utilization rates of inorganic and organic nitrogen by the various algal species and the sources and turnover rates of both phosphorus and nitrogen are understood, determinations of inorganic nitrogen and phosphorus concentrations alone have little quantitative application. The determination of dissolved organic nitrogen and dissolved inorganic nitrogen and their correlation with relative phytoplankton species composition may have some value.

The small and relatively undeveloped Rhode River watershed does not usually contribute significantly to the fresh water or nutrients of this portion of the bay. Since the phytoplankton species compositions in Rhode River are derived from the bay they should be most typical of bay populations which are least modified by river contributions. Conversely, the relative effects of changes in the watershed due to increased agricultural fertilization or sewage or erosion should be greatest in these Rhode River populations. We did not measure silicate concentrations in the bay waters during this survey. However measurements of silicates by Dr. D. Correll at the Chesapeake Bay Center for Environmental Studies indicate that there is a very sharp gradient in silicate concentrations between Muddy Creek, the main tributary of Rhode River, and the saline portion of the river. In the latter the silicate concentrations appear to be unmodified by increased runoff. It might be useful in future surveys to examine this variation for the bay waters as well.

The bay areas contiguous with each river exhibit different kinetics of growth. This may serve to order each individual watershed according to its potential nutrient delivery to the bay.

The development of the bloom shows a bimodal areal distribution for phytoplankton standing crops. It is this aspect which might be most amenable to aerial survey.

The intensive study of plankton growth kinetics following natural perturbation has the advantage that the species successions and the nutrient levels observed can define laboratory culture conditions much more precisely. For some applications the kinetic description might be applied to one particular species.

With proper calibration it is feasible to use the continuous *in vivo* chlorophyll fluorescence

technique for intensive large area assays of chlorophyll *a* standing crops.

Evidence is presented for positive phototaxis, washout of river plankton and selective predation as possible factors in the growth of the larger dinoflagellates over the ultraplankton. Nutritional factors may also be involved.

Mechanisms for supporting diverse phytoplankton populations in a relatively homogeneously dispersed environment have been suggested by Hutchinson (1961).

- a. Existence of symbiosis or commensalism.
- b. Selective predation.
- c. The populations are not really in equilibrium.
  - i. Resultant of washouts from stable chemostatic communities elsewhere in the heterogeneous littoral benthos.
  - ii. Transient state not at equilibrium due to rapidly changing conditions.

Williams (1971) added:

- d. Photoperiodic phasing of cell cycles giving rise to cyclic changes in competitive advantage. Evidence for these diel cycles has already been given by Eppley *et al.* (1971a; 1971b).

Further refinements can be made in terms of the spectral intensity requirements and the effects of vertical mixing, particularly for turbid estuarine environments:

- e. Differences in concentrations of accessory pigments and in morphology can give rise to subtle differences in generation rate constants which are functions of ambient spectral intensity. The mixing in the vertical water column exposes the heterogeneous population to the complete range of spectral intensities, permitting species in turn to experience varying degrees of competitive advantage. Some aspects of this spectral dependence have been looked for (Kiefer and Strickland, 1970).
- f. Differences in phototactic motility and flotation rates due to size tend to segregate species vertically during daylight while more general mixing occurs during the night. This falls under the category of c (above). An extreme of this mechanism occurs in the bay during early fall when, on bright, calm days positively phototactic dinoflagellates form

surface layers of high concentrations. These blooms of visible discoloration are to be distinguished from the blooms reported in this paper. The latter result from a completely new species distribution; the former are the result of a phototactic segregation of the existing species within the water column.

All of these mechanisms appear to be operative in the bay.

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